

STUDIES IN  
Clinical Enzymology

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## INTRODUCTION AND ACKNOWLEDGEMENTS

I have made no attempt in this book to review the whole of clinical enzymology. My object has been to select various clinical topics, and describe the enzyme changes which occur. My original intention was to present this subject in a way which would primarily interest the general physician. However, this monograph in its final form covers in fact a wider field. Although certain enzyme tests are of considerable diagnostic value, there are many other aspects of enzymology which are of general biological interest. Certain sections of this book will also interest the obstetrician, paediatrician, neurologist and those interested in tropical medicine.

It was only possible to complete this book because of the generous help and advice of many friends in Sheffield and elsewhere. In particular I should like to acknowledge the enthusiastic support I have had from Dr. Anthony Ward, Dr. Graham Thorpe, Dr. Timothy Hales, Dr. S. Shah, and Dr. D. Goldberg. I am most grateful to my former chief Dr. C. E. Davies for allowing me to study many of his diabetic cases, the consultant staff of the Royal Hospital, Sheffield, who have freely allowed me access to their patients, and also Dr. Alan Jeanes, Dr. James Liddell and Dr. P. A. Toseland in the department of Clinical Pathology, Guy's Hospital.

Chapter IV deals with the enzyme changes in African heart disease in Nigerians, and I should like to thank Professor A. Brown who allowed us to use data collected in his department.

Chapter V includes data on Nairobi patients suffering from various liver disorders, including hepatic amoebiasis, and I acknowledge the helpful co-operation of the consultant staff of the Kenyatta National Hospital, in particular Dr. Forrester and Dr. Whittaker, who made this investigation possible. Professor C. H. Stuart-Harris also very kindly allowed me to

record data from several patients under his care, including two cases of amoebiasis.

The longest chapter in this monograph is on enzyme changes in pregnancy, and I am most grateful to the consultant staff of the Jessop Hospital for women who kindly allowed me to study their patients. Mrs. R. Taylor collected specimens for me from pregnant mothers; her advice throughout my studies has been invaluable. Dr. F. W. Leigh (general practitioner), and Dr. M. E. Jepson and Dr. M. Flowerday of the Maternity and Child Welfare Centre, Sheffield Public Health Department also very kindly collected specimens from 'normal' pregnant mothers.

Dr. Grainger Muir, Consultant Chemical Pathologist, to the Luton and Dunstable Hospital and the Bedford General Hospital, very kindly wrote Chapter VII which is on the topical subject of enzyme studies in carcinoma of the cervix. This is followed by a section on metabolic and enzyme abnormalities in children by a clinical biochemist, Dr. P. A. Toseland, who has described some of the tests used to unravel certain diagnostic problems in children. Some children were under the care of Dr. J. Black, and we thank him for his co-operation.

I am most grateful to Dr. Davies-Jones who wrote Chapter XI: in this section some interesting cerebrospinal fluid enzyme abnormalities have been recorded in patients with neurological syndromes due to remote carcinoma. A number of these patients were under the care of Dr. J. Carson and Dr. P. Bradshaw, and their co-operation is acknowledged.

My own experiments were carried out in the Department of Chemical Pathology at the Royal Hospital, Sheffield and I should like to thank Dr. Arthur Jordan for his great help, the technical staff for their assistance, and Mrs. Barbara Gibbs for typing most of the text. I should also like to record my thanks to the Board of Governors of the United Sheffield Hospitals for a research award which has allowed me to continue these enzyme studies.

Last, but not least, I should like to thank my wife for her loyal support, especially during moments of real despair.

*June 1968*

D. P. M.

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I am also indebted to the owners of the copyright for permission to use the illustrations and tables from the following works:

*The Journal of Clinical Pathology*, for Tables, 5, 6, 7, 8, 9, 10, and Figures 16, 18, 19.

*Journal of Pediatrics*, published by the C. V. Mosby Co., St. Louis, for Table 22 and Fig. 27.

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TO ANTONIA

## CHAPTER I

## The nature of enzymes and their diagnostic value

Enzymes are biological catalysts which are essential for normal intracellular metabolism. At a normal body temperature, a small concentration of an enzyme will catalyse a specific chemical reaction. Enzymes act by accelerating intracellular metabolism, but in spite of this, they remain unaltered at the end of these various reactions. Some enzymes have been obtained in pure form; thus, in 1926, Sumner was able to crystallize out the enzyme urease. Like other proteins, enzymes are denatured by heat and other agents: for example, after a fixed period of incubation, trichloroacetic acid can be added so as to terminate an enzyme reaction.

Evidence has accumulated that enzymes are proteins, and in some cases their amino-acid sequence has been determined; for instance ribonuclease consists of a single chain of 124 amino acids with four cystine residues cross linking the chain. The protein structure of this enzyme has been investigated employing X-ray diffraction and other methods. Protein models of ribonuclease have been constructed by Avey *et al.* (1967) and Harker *et al.* (1967), but the difference between the two models is considerable, especially as regards the site of the active centre. This emphasizes the importance of duplicating this type of research. Structural analyses have also been made of lysozyme (Blake *et al.*, 1965) and chymotrypsin (Mathews *et al.*, 1967). The tertiary structure of the latter enzyme has recently been discussed in an annotation (*Lancet*, 1967).

### Simple enzyme kinetics

The rate of an enzyme reaction depends on the concentration of enzyme. In the presence of an excess of substrate, a greater concentration of the enzyme will lead to an increase in the rate of disappearance of the substrate and the formation of more product. The relationship between an enzyme and its substrate is often specific. A good analogy is the lock and key idea put forward by Emil Fischer (1894). The temperature and the pH of the reaction mixture are also important. As mentioned, at high temperatures an enzyme will be denatured. Likewise at extremes of pH the reaction rate will fall to zero. An optimal point will be found for both these factors.

The concept of the enzyme-substrate (E.S.) complex is fundamental to the understanding of an enzyme reaction. In 1913 Michaelis and Menten expressed this simply in the following formula:



(Where E = Enzyme, S = Substrate, and P = Product).

If the enzyme concentration is fixed, then increasing the substrate concentration will at first result in a rapid increase in the rate of the reaction. However, beyond point A a further increase in substrate concentration will not result in any further increase in the rate of the reaction (see Fig. 1).

The active sites on the enzyme are fully saturated when there is an excess of substrate and, under these conditions, the enzyme will be working at full capacity.

The facing figure also indicates the  $K_m$ , or Michaelis constant, which is defined as the substrate concentration necessary to produce half the maximum velocity ( $V/2$ ) of the reaction. Thus, if the Michaelis constant ( $K_m$ ) is low, then the enzyme has a high affinity for its substrate. From this it follows that the  $K_m$  determination can be used to compare the activities of an enzyme against different substrates.

### Enzyme units

Considerable chaos has arisen in the past because the activity of an enzyme has been determined by various methods; the

conditions of enzyme assay, including pH, temperature, substrate concentration and length of incubation have varied, and results have been expressed using different arbitrary units.

The main cause for this confusion has been failure to express the velocity of an enzyme reaction in a standard way. To overcome this problem, and compare results from different centres, enzyme activity should now be expressed if possible in International Units. This is in accordance with the recommendations of the International Union of Biochemistry (1964). An international unit is defined as the number of micromoles of substrate transformed per minute under standard conditions.

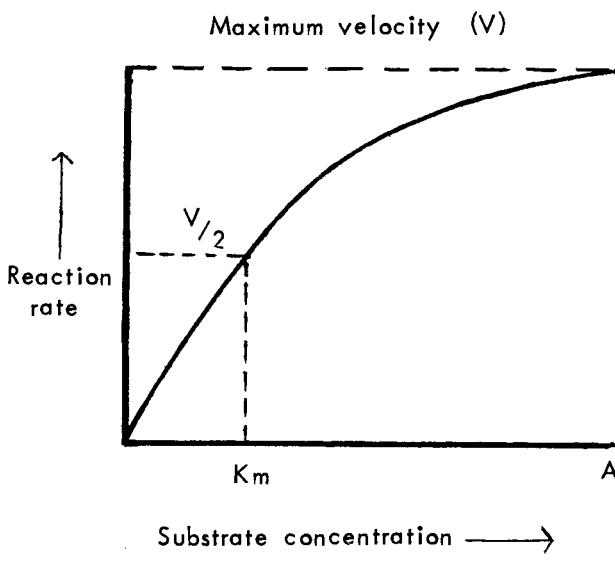


FIG. 1.

The method employed must be specified, and the enzyme concentration should be expressed in units per litre (L) or per ml. It is important that the enzyme activity should be calculated from the initial rate of the reaction; in particular, the substrate concentration should be high so as to saturate all the active sites on the enzyme, and this will allow a zero order type of reaction to occur. On the other hand, the rate of a reaction can fall off with time and this may be due to the

inhibitory effects of the products or a back reaction; in these circumstances, the quantity of the substrate transformed need not be proportional to the concentration of enzyme present.

A few enzymes have been isolated in pure form, and it is then possible to relate the reaction rate to the absolute amount of enzyme present; the specific activity of the enzyme can then be measured in units per mg.

Many of the older units of enzyme activity can now be converted into international units. Furthermore, if the enzyme activity is expressed in international units, it will be possible to compare the relative rates at which an enzyme will act on various substrates. This is clearly explained in a paper by King and Moss (1963) who also give a useful conversion table (see below).

As an example these authors converted both the King-Armstrong and Bodansky unit of Alkaline phosphatase (A.P.) activity into international units. Thus, one King-Armstrong unit is equivalent to the amount of enzyme which will liberate 1 mg. of phenol in 15 minutes by hydrolysis of the substrate phenyl phosphate. i.e. 1 K.A. unit will liberate 1000 µg. phenol in 15 minutes.

$$1 \text{ K.A. unit} = \frac{1000}{94} \times \frac{1}{15} \mu\text{-moles/min.} \quad (\text{where gram molecular weight of phenol} = 94)$$

$$= 0.71 \mu\text{-moles/min.}$$

Similarly, one Bodansky unit of A.P. activity is equivalent to 1 mg. of inorganic phosphorus (P) derived by hydrolysis in one hour from  $\beta$ -glycerophosphate.

$$\text{i.e. } 1 \text{ Bodansky unit} = \frac{1000}{31} \times \frac{1}{60} \mu\text{-moles/min.} \quad (\text{where atomic wt. of P} = 31)$$

$$= 0.535 \mu\text{-moles/min.}$$

Therefore a normal serum

Alkaline phosphatase of 3-13 K.A. units/100 ml. is equivalent to 20-90 µ.moles/min./litre and a normal serum alkaline phosphatase of 1-5 Bodansky units/100 ml. is equivalent to 5.4-27 µ.moles/min./litre.

TABLE I. *Factors for conversion of conventional units per litre for enzymes of current diagnostic significance*

<i>Enzyme</i>	<i>Conventional units</i>	<i>To obtain international units (μ.moles/min./litre) multiply by</i>
Alkaline phosphatase	King-Armstrong (1 unit/100 ml.) (substrate: phenyl phosphate)	7.1
	Bodansky (1 unit/100 ml.) (substrate: β-glycerophosphate)	5.4
Acid phosphatase	King-Armstrong (1 unit/100 ml.) (substrate: phenyl phosphate)	1.8
	Bodansky (1 unit/100 ml.) (substrate: β-glycerophosphate)	5.4
Aldolase	Sibley and Lehninger (1 unit/ml.) Shapira (1 unit/litre)	0.75 16
Phosphohexose isomerase	Bodansky (1 unit/0.04 ml.)	4.6
Reaction with N.A.D. (D.P.N.)		
Transaminases	Optical density (1 unit/ml.)	0.5
Lactic dehydrogenase		
Malic dehydrogenase		
Reactions with N.A.D.P. (T.P.N.)		
Glutathione reductase	Optical density (1 unit/ml.)	0.5
Isocitric dehydrogenase	Wolfson and Williams-Ashman (1 unit/ml.)	0.017
Digestive enzymes		
Amylase	Somogyi (1 unit/100 ml.)	2
Lipase	Bunch and Emerson (1 unit/ml.)	208
Peptic activity	Anson and Mirsky (1 unit/ml.)	550
	Nicotinamide—adenine dinucleotide phosphate (N.A.D.P.)	
	Nicotinamide—adenine dinucleotide (N.A.D.)	

By courtesy of Dr. D. W. Moss and the *Journal of Clinical Pathology*.

In the text, e.g. Chapter II, serum transaminase activity (S.G.O.T., S.G.P.T.), is expressed in King units. If these values are divided by five, activity will then be in international units. (μ.moles/min./litre).

### Classification of enzymes

Many hundreds of enzymes have now been classified into six broad groups (International Union of Biochemistry, Commission on enzymes, 1961).

The enzymes mentioned in the text are placed in the appropriate group, and their other names or abbreviations are given below.

TABLE 2. Classification of enzymes

Number	Systematic name	Recommended name	Other name or abbreviation
<b>1. OXIDOREDUCTASES</b>			
1.1.1.27	L-Lactate: N.A.D. oxidoreductase	Lactate dehydrogenase	L.D.H.
1.1.1.37	L-Malate: N.A.D. oxidoreductase	Malate dehydrogenase	I.C.D.
1.1.1.41	Threo-D <sub>5</sub> -Isocitrate: N.A.D. oxidoreductase (decarboxylating)	Isocitrate dehydrogenase	I.C.D.
1.1.1.43	6-Phospho-D-glucuronate: N.A.D.(P) oxidoreductase	Phosphogluconate dehydrogenase	6P.G.D.
1.1.1.49	D-Glucose-6-phosphate: N.A.D.P. oxidoreductase	Glucose-6-phosphate dehydrogenase	M.A.O.
1.4.3.4	Monamine: oxygen oxidoreductase (deaminating)	Monamine oxidase	D.A.O.
1.4.3.6	Diamine: oxygen oxidoreductase (deaminating)	Diamine oxidase, histaminase	
1.6.4.2	Reduced-N.A.D. (P): oxidized glutathione oxidoreductase	Glutathione reductase	
1.10.3.1	O-Diphenol: oxygen oxidoreductase	O-Diphenol oxidase	Tyrosinase
1.11.1.6	Hydrogen-peroxide: Hydrogen-peroxide oxidoreductase	Catalase	
1.13.1.5	Homogenitase: oxygen oxidoreductase	Homogenitase oxygenase	Homogenitase
1.14.2.2	p-Hydroxyphenylpyruvate ascorbate: oxygen oxidoreductase (hydroxylating)	p-Hydroxyphenyl-pyruvate hydroxylase	
1.14.3.1	L-Phenylalanine, tetrahydropteridine: oxygen oxidoreductase (4-hydroxylating)	Phenylalanine 4-hydroxylase	
<b>2. TRANSFERASES</b>			
2.4.1.17	U.D.P. glucuronyl transferase (acceptor unspecific)	U.D.P. glucuronyl transferase	
2.6.1.1	L-Aspartate: 2-oxoglutarate aminotransferase	Aspartate aminotransferase	
2.6.1.2	L-Alanine: 2-oxoglutarate aminotransferase	Alanine aminotransferase	
2.6.1.5	L-Tyrosine: 2-oxoglutarate aminotransferase	Tyrosine aminotransferase	
2.7.1.2	A.T.P.: D-glucose 6-phototransferase	Glucokinase	
2.7.1.40	A.T.P.: pyruvate phosphotransferase	Pyruvate kinase	
2.7.3.2	A.T.P.: creatine phosphotransferase	Creatine kinase	
2.7.4.3	A.T.P.: A.M.P. phosphotransferase	Adenylate kinase	
2.7.7.10	U.T.P.: $\alpha$ -D-galactose-1-phosphate uridylyltransferase	Galactose-1-phosphate uridylyltransferase	
2.7.7.16	Ribonucleate pyrimidine nucleotide-2'-transferase (cyclizing)	Ribonuclease	R.N.A.ase I

**3. HYDROLASES**

3.1.1.3	Glycerol-ester hydrolase	Lipase	Pseudo-cholinesterase
3.1.1.8	Acylcholine acyl-hydrolase	Cholinesterase	A.P.
3.1.3.1	Orthophosphoric monoester phosphohydrolase	Alkaline phosphatase	
3.1.3.2	Orthophosphoric monoester phosphohydrolase	Acid phosphatase	
3.1.3.5	5'-Ribonucleotide phosphohydrolase	5'-Nucleotidase	
3.1.3.9	D-glucose-6-phosphate phosphohydrolase	Glucose-6-phosphatase	
3.1.4.5	Deoxyribonucleate oligonucleotidol hydrolase	Deoxyribonuclease	D.N.A.ase
3.1.4.6	Deoxyribonucleate 3'-nucleotidolhydrolase	Deoxyribonuclease II	
3.2.1.1	$\alpha$ -1, 4-Glucan 4-glucanohydrolase	$\alpha$ Amylase	
3.2.1.17	Mucopeptide N-acetyl muramyl-hydrolase	Mucopeptide glucohydrolase,	Muramidase
3.2.1.20	$\alpha$ -D-glucoside glucohydrolase	lysosome	
3.2.1.23	$\beta$ -D-galactoside galactohydrolase	$\alpha$ -Glucosidase	Maltase
3.2.1.24	$\alpha$ -D-Mannoside mannohydrolase	$\beta$ -Galactosidase	Lactase
3.2.1.26	$\beta$ -D-Fructofuranoside fructohydrolase	$\alpha$ -Mannosidase	
3.2.1.31	$\beta$ -D-Glucuronide glucuronohydrolase	$\beta$ -Fructofuranosidase	Sucrase
3.4.1.1	L-Leucyl-peptide hydrolase	$\beta$ -Glucuronidase	
3.5.1.5	Urea amidohydrolase	Leucine aminopeptidase	L.A.P.
3.5.4.4	Adenosine amino hydrolase	Cystine aminopeptidase (oxytocinase)	C.A.P.
3.7.1.3	L-Kynurenine hydrolase	Urease	
4. LYASES		Adenosine deaminase	
4.1.2.7	Ketose-1-phosphate aldehyde-lyase	Ketose-1-phosphate aldolase	Aldolases
4.2.1.1	Carbonate hydrolase	Carbonic anhydrase	
5. ISOMERASES		Glucosephosphate isomerase	Phospho-hexose isomerase P.H.I.
5.3.1.9	D-Glucose-6-phosphate ketol-isomerase		
6. LIGASES			

Taken from *Enzyme Nomenclature 1965*. Recommendations 1964 of the International Union of Biochemistry

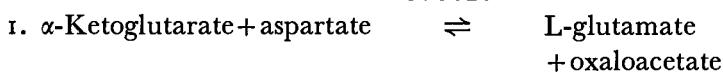
### Activators and co-enzymes

A co-factor may be required to activate an enzyme reaction. This may be a metal, e.g.  $Zn^{++}$ ,  $Mg^{++}$ ,  $Ca^{++}$  and  $Mn^{++}$ . Thus lactate dehydrogenase contains zinc. A number of prosthetic groups and co-enzymes are also activators. In addition, certain pro-enzymes need to be altered before they become active. For instance, pepsinogen is converted to pepsin by hydrochloric acid, and pancreatic trypsinogen is activated by enterokinase derived from the succus entericus.

Co-enzyme I and II (N.A.D. and N.A.D.P.) are extremely important co-factors in the numerous dehydrogenase reactions. Nicotinamide contained in these two co-enzymes readily takes up two atoms of hydrogen and reduced co-enzyme I and II (N.A.D.H.<sub>2</sub> and N.A.D.P.H.<sub>2</sub>) will release hydrogen atoms to an acceptor. The well-known figure below shows that N.A.D.H.<sub>2</sub> (and N.A.D.P.H.<sub>2</sub>) has an absorption band at 340 m $\mu$ , but N.A.D. has no band at this wavelength. Thus the formation or utilization of N.A.D.H.<sub>2</sub> (or N.A.D.P.H.<sub>2</sub>) can be determined using a spectrophotometer.

This spectrophotometric technique has been applied to determine the activity of other enzymes. Thus, in Karmen's method (1955) of measuring serum aspartate aminotransferase, a further coupled enzyme reaction was inserted, which made it possible to measure the rate at which oxaloacetate was produced:

G.O.T.



Malate dehydrogenase



As can be seen, the oxaloacetate formed by the action of G.O.T. is converted to malate with malate dehydrogenase. During this reaction the co-factor N.A.D.H.<sub>2</sub> is oxidized to N.A.D. and this produces a fall in optical density at 340 m $\mu$ . One Karmen unit is equal to a change of 0.001 in optical density per minute per ml. of serum at 340 m $\mu$ . using a 1 cm.

light path. The procedure is carried out at room temperature ( $25^{\circ}\text{ C}.$ ).

### Enzyme inhibition

Inhibitors may be competitive or non-competitive. In the former case the inhibitor competes with the substrate for the active site on the enzyme: thus, the bacteriostatic effect of

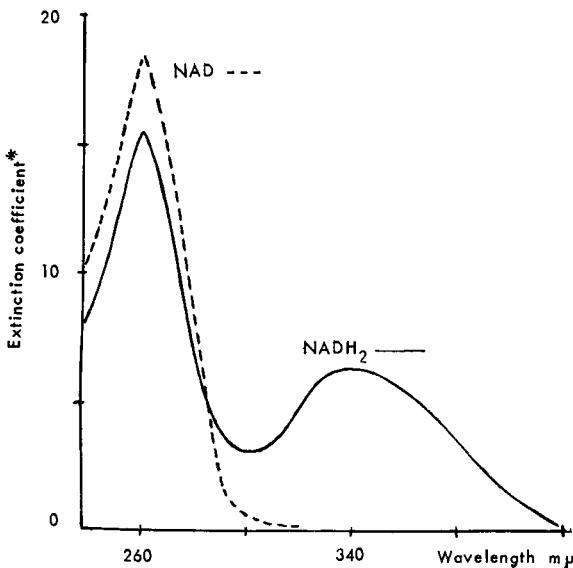


FIG. 2.

\* *Extinction coefficient.* This is a measure of the absorption of light by a dissolved substance. It is expressed by the formula:

$$e = \frac{I}{cd} \cdot \log \frac{I_0}{I}$$

(Where  $I_0$  and  $I$  are the intensities of incident and transmitted light respectively, in a solution  $d$  cm. thick of molar concentration  $c$ .)

sulphonamides is due to the competitive displacement of p-amino-benzoic acid. The latter compound is required for bacterial metabolism.

On the other hand, non-competitive inhibition cannot be reversed by increasing the substrate concentration, and the inhibitor becomes firmly attached to a site on the enzyme.

Alternatively, a stable enzyme-inhibitor compound may be formed. In this way di-isopropylphosphofluoridate (D.F.P.), and the insecticide Parathion act as very potent cholinesterase inhibitors. The other nerve poisons which were produced in Germany during the 1939-45 war also inhibit cholinesterase. Acetylcholine accumulates at nerve endings, and this results in severe symptoms such as vomiting, abdominal colic, twitching, sweating, salivation and pupillary constriction. There are numbers of other poisons which act directly on cellular enzymes. Thus, cyanide and carbon monoxide inactivate cytochrome oxidase causing cellular anoxia.

Another most interesting type of enzyme inhibition has recently been investigated (Arias *et al.*, 1964; Gärtner and Arias, 1966). Gärtner and Arias investigated twenty breast-fed infants with unconjugated hyperbilirubinaemia, which occurred about one week after birth, and they found that milk, but not colostrum, from the mothers of these infants caused marked inhibition of glucuronyl transferase activity *in vitro*. Discontinuation of breast feeding was promptly followed by a fall in the level of serum bilirubin, while resumption of breast feeding lead to an increase in the serum bilirubin. Siblings of these affected infants who had been artificially fed gave no history of neonatal jaundice.

These workers have isolated the abnormal steroid pregnane- $3(\alpha)$ ,  $20(\beta)$ -diol in the milk from four mothers of these jaundiced infants, and in a previous study (Arias and Gärtner, 1964) they produced unconjugated hyperbilirubinaemia by the oral administration of this steroid to two normal new-born infants.

Uridine diphosphate glucuronic acid normally reacts with bilirubin in the presence of glucuronyl transferase to form conjugated bilirubin glucuronide. However, the activity of glucuronyl transferase is normally reduced in neonates, and Gärtner and Arias considered that the ingestion of the steroid pregnane- $3(\alpha)$ ,  $20(\beta)$ -diol could inhibit this enzyme and produce unconjugated hyperbilirubinaemia. A serum inhibitor (in both mother and infant) of glucuronyl transferase has also

been reported in a different syndrome of transient familial neonatal hyperbilinaemia (Lucey *et al.*, 1960), and novobiocin can also inhibit glucuronyl transferase *in vitro* (Lokietz *et al.*, 1963).

## DRUGS AND ENZYMES

The overprescription of a mixture of drugs may lead to a number of adverse reactions. For example, a proprietary preparation containing amphetamine may precipitate a hypertensive crisis in a patient who is already taking a monoamine oxidase inhibitor. It has also been reported that the ingestion of certain rich cheeses in patients treated with tranylcypromine (parnate) can precipitate acute hypertension and occasional fatal cerebral haemorrhage. Asatoor *et al.* (1963) found high concentrations of tyramine in Camembert and Brie cheeses, and they concluded that this amine was the toxic agent which caused hypertension in patients treated with monoamine oxidase inhibitors.

Other problems arising from the interaction of drugs have now been studied (Symposium on interaction between Drugs, R.S.M. 1965), and interest has recently been focused on the factors which alter the metabolism of drugs. In particular, drugs may either increase or inhibit the synthesis of drug metabolizing enzymes in liver microsomes (Brodie *et al.*, 1955; Fouts, 1964; Burns and Conney, 1965). For instance, prior treatment with phenobarbitone enhances the metabolism of bishydroxycoumarin in man with a lowering of the plasma levels of this anticoagulant and a reduction of the prothrombin time (Cucinell *et al.*, 1965). The prothrombin time and the plasma level of bishydroxycoumarin rose again when phenobarbitone was stopped. The addition of phenobarbitone will also result in a fall of plasma levels of phenytoin sodium (epanutin)—Burns and Conney (1965). Likewise, phenobarbitone lowers the blood level of griseofulvin (Busfield *et al.*, 1963). On the other hand, inhibition of drug metabolism may occur; for instance Weiner *et al.* (1965) noted that treatment

with oxyphenbutazone (tanderil) increased the anti-coagulant action of bishydroxycoumarin.

### Function of the cell membrane

Perhaps we should now consider the simple idea that a function of the cell membrane is to contain intracellular enzymes, and that cellular damage will result in leakage of certain enzymes through this membrane. In fact, serum enzyme changes may occur in the absence of actual pathological damage; this is well exemplified in the case of skeletal muscle. Thus, the serum aldolase and transaminases (S.G.O.T. and S.G.P.T.) increase after prolonged physical exercise especially in untrained subjects (Fowler *et al.*, 1962). Likewise, Griffiths (1963) found that the average serum creatine kinase (C.P.K.) level in medical students taking part in the London to Brighton walking race increased seven times after 25 miles and twenty times by the end of the journey (53 miles). Moderate elevations of serum C.P.K. and aldolase levels also occur in tetanus (Mullan and Dubowitz, 1964), see Table 3. In the absence of pathological changes in tetanus (Adams, 1964), severe muscle contractions could lead to increased leakage of enzymes through the cell membrane. Paradoxically, in myxoedemic patients with sluggish muscles, the serum C.P.K. activity is also often increased (Craig and Ross, 1963; Fleischer and McConahey, 1965).

Likewise increased serum C.P.K. activity has also been noted during the treatment of diabetic ketosis (Vélez-Garcia *et al.*, 1966) and in the acute reversible muscular syndrome seen in chronic alcoholics (Perkoff *et al.*, 1966).

Certainly a number of factors influence the permeability of the cell membrane. For instance, it has been shown (Zierler, 1958a and b) that the rate at which aldolase passes out of muscle is dependent on such factors as the oxygen tension, glucose level and pH of the surrounding medium. As pointed out by White (1960), an enzyme does not necessarily tend to leak out of a cell because of its smaller molecular weight. The levels of aldolase and lactate dehydrogenase (L.D.H.) were

reduced in dystrophic muscle, but isocitrate dehydrogenase (I.C.D.) with a relatively lower molecular weight of 65,000 had a higher activity in dystrophic as compared to normal muscle.

TABLE 3. *Enzyme levels in two cases of tetanus. Estimations kindly performed by Mr. A. D. Clarke*

<i>Case no.</i>	<i>Date</i>	<i>Serum-aldolase (units per ml. per hr.) (normal 7-21)</i>	<i>Serum-creatinine-phosphokinase (<math>\mu</math>M per ml. per hr.) (normal 0.3-4.5)</i>	<i>Serum-glutamic-oxaloacetic-transaminase (King units) (normal 0-100)</i>	<i>Serum-glutamic-pyruvic-transaminase (King units) (normal 0-100)</i>
1	June 10*	27.2	16.2	47	47
2	June 18	12.3	5.3	55	35
	June 16†	25.0	18.5	120	not estimated
	June 19	29.8	12.2	95	
	June 24	38.0	16.3	30	
	July 3	18.0	3.8	48	

\* Admitted June 7.

† Admitted June 11.

By courtesy of the *Lancet*.

## DISTRIBUTION OF ENZYMES

Although much is known about the distribution of various enzymes in organs and body fluids, the physiological factors governing their formation and excretion are not fully understood.

Some enzymes are widely distributed; for instance aspartate aminotransferase (G.O.T.) and lactate dehydrogenase (L.D.H.) are found in heart, skeletal muscle and liver, and true cholinesterase is present in both red cells and nervous tissue. Other enzymes are more specifically located, thus, pepsinogen occurs only in the gastric mucosa. Ubiquitous enzymes are less likely to be of diagnostic value to the clinician. However, the study of iso-enzymes may be helpful; for example the fast moving

fraction of lactate dehydrogenase increases acutely after myocardial infarction (see Chapter III).

### **Experimental studies. The mechanism of enzyme increase**

Some interesting experimental work has been done. For instance Dunn *et al.* (1958) made a careful study of the disappearance rate of intravenously injected glutamic oxaloacetic transaminase in dogs. Three-fourths of the injected enzyme disappeared from the blood stream within 6 hours, and synchronous with the fall in blood level, they demonstrated a rise in the G.O.T. level in lymph taken from a cannulated tributary of the thoracic duct; likewise experimental myocardial infarction and hepatic damage due to carbon tetrachloride produced an increase in thoracic duct lymph G.O.T. concentrations in parallel with rising blood levels of this enzyme. These changes suggested that the rapid disappearance of G.O.T. from the blood stream was due to its diffusion into the interstitial fluid. This mechanism was further supported by the absence of G.O.T. in the urine, bile and cerebrospinal fluid after intravenous injection of this enzyme. Furthermore, nephrectomy made no difference to the rate of disappearance of this enzyme.

Another interesting study (Popper and Necheles, 1940) should also be mentioned. These workers produced bile-induced pancreatitis in dogs and found elevation in amylase activity in pancreatic and peripheral venous blood, and in thoracic lymph. However, when the portal vein was occluded, experimental pancreatitis did not give rise to increased amylase activity in peripheral blood or thoracic lymph. On releasing the obstruction of the portal vein the enzyme activity in the peripheral venous blood started to rise. They therefore concluded that the main route by which amylase reached the circulation was via the portal vein.

### **Normal enzyme changes**

The body operates vigorously to maintain a normal 'milieu

interieur'; thus in health, the blood pH and plasma electrolytes remain constant. Likewise there is usually no significant fluctuation in the activity of various enzymes in the body fluids. However, there may be enzyme changes which normally accompany growth and pregnancy. The serum enzyme pattern in the neonate differs from that in the adult. For instance, the serum aldolase is elevated in the new-born. Greatly increased S.H.B.D. values also occur in the early months of life (see Fig. 26, Chapter VIII), and in fact the S.H.B.D. level does not return to the normal 'adult' level until the age of about 14. On the other hand it has been stated that serum amylase is absent from the serum of the neonate; in fact, using a microsaccharogenic method (Ujihira, 1965) it has been possible to demonstrate amylase activity in the serum of the new-born (Berk, 1967). It is of course well known that increased bone growth in children is associated with increased osteoblastic activity and a higher level of serum alkaline phosphatase.

Socio-economic factors are also important. Dunnigan *et al.* (1962) described late rickets in a number of Pakistani children in Glasgow, and later, these workers (Dunnigan and Gardner, 1965) noted that three out of thirty-five white children living in the Gorbals district of Glasgow had levels of serum alkaline phosphatase above 30 K.A. units. Taken with the finding of three subnormal values for inorganic phosphorus, it was suggested that it was possible to discover mild vitamin D deficiency in a poorer area of a large industrial city.

Pregnancy is also of particular interest because of the increasing activity of certain enzymes, e.g. oxytocinase, histaminase and alkaline phosphatase which are probably derived from the placenta.

### **Enzyme changes in disease**

#### *(i) Enzyme changes due to tissue damage*

It is easier to understand those acute enzyme changes which accompany tissue damage. For example, elevation of the serum amylase occurs in patients with pancreatitis, and increased serum enzyme activity (C.P.K., S.G.O.T., S.H.B.D.) occurs

after myocardial infarction. Acute serum enzyme elevation (S.G.O.T., C.P.K.) may be seen in a widespread inflammatory condition such as dermatomyositis. Likewise serum C.P.K. activity is raised in muscle dystrophy, but not in neurogenic wasting. Raised levels of serum leucine aminopeptidase (L.A.P.) have been found in diabetic patients with infected gangrene (see Chapter IX), and this has been associated with an excessive urinary 'spill over' of L.A.P. in the absence of renal disease.

(ii) *The causes of increased phosphatase activity*

Alternatively, there may be a failure of normal excretion of an enzyme; for instance, the serum alkaline phosphatase may be markedly elevated in biliary obstruction. Part of this increased enzyme activity may also be due to increased hepatic synthesis of this enzyme, and in dogs it has been shown (Polin, *et al.*, 1962) that tying one hepatic duct leads to an increase in serum alkaline phosphatase together with an increased loss of this enzyme in the bile.

In 1933 Roberts studied fifty-two cases of jaundice at the Royal Infirmary, Sheffield, and made the now well-known observation that the alkaline phosphatase was invariably high in obstructive jaundice, but only slightly raised in other varieties of jaundice even though the patient may be deeply jaundiced. This led on to the classical work by Armstrong, King and Harris (1934) who showed that ligation of the common bile duct in the dog gave rise to an increase in the level of alkaline phosphatase in the blood. The level of this enzyme then returned to normal on releasing the clamp. During these experiments it was also shown that there was no loss of enzyme in the urine, and the amount of phosphatase activity in the faeces remained high.

Likewise an ampullary carcinoma will cause intermittent biliary obstruction with fluctuating levels of serum alkaline phosphatase. It should be stressed that an increase in serum alkaline phosphatase is a more sensitive index of obstruction than the level of serum bilirubin. The activities of serum

5'-nucleotidase and leucine aminopeptidase (L.A.P.) will also be increased in obstructive hepatobiliary disease (Young, 1958; Harkness *et al.*, 1960). 5'-nucleotidase and L.A.P. activity remain normal in patients with bone disorders such as Paget's disease (Knowles *et al.*, 1961).

On the other hand a raised serum alkaline phosphatase may be due to increased osteoblastic activity. In fact, over 30 years ago Kay (1929, 1930) found raised levels of this enzyme in various bone disorders, including rickets and Paget's disease, and in hyperparathyroidism. Treatment of rickets and adult osteomalacia with vitamin D, or the successful removal of a parathyroid adenoma will result in the gradual return of serum alkaline phosphatase towards a normal level.

Another reason for increased enzyme activity, is abnormal production by neoplastic tissue; this is seen in patients with prostatic carcinoma with bone deposits, who have increased activity of serum formaldehyde stable acid phosphatase. It is also of some interest that Tuchman *et al.* (1959) showed that there was some elevation of the serum acid phosphatase in twelve cases of Gaucher's disease. These abnormal levels were found when phenyl phosphate was used as substrate, but only four out of twelve were elevated when  $\beta$ -glycerophosphate was used. In their patients the raised acid phosphatase was not inhibited by formaldehyde, L-tartrate or copper.

### (iii) *Malignant disease and muscle wasting*

Extensive necrosis of tumour cells could lead to increased serum activity (e.g. aldolase and L.D.H.). However, severe muscle wasting associated with creatinuria may occur in some patients with advanced cancer, and it has been suggested that raised serum enzyme levels (aldolase, L.D.H., G.O.T.) may result from inadequate protein intake and muscle breakdown (White, 1960). This hypothesis was supported by the fact that, in some cancer patients, the administration of intravenous protein hydrolysate or increasing the intake of oral protein, resulted in a prompt decrease of these serum enzyme levels to normal (White, 1958). The latter author also noted that muscle

wasting due to starvation was not associated with elevated serum enzyme levels.

Other changes may be more remote. Thus in the nephrotic syndrome with hypoproteinaemia, the serum pseudocholinesterase is raised (Kunkel and Ward, 1947), and this is due to increased hepatic synthesis of this enzyme.

(iv) *Increased enzyme activity in various biological fluids*

Other biological fluids have been investigated; in cerebral infarction G.O.T., and L.D.H. in the cerebrospinal fluid may be raised. The C.S.F. lactate dehydrogenase (L.D.H.) activity may also be increased in malignant tumours of the central nervous system and in meningitis (Wroblewski, 1959). Patients with cerebrovascular accidents may have elevated cerebrospinal fluid levels of C.P.K. (Acheson *et al.*, 1965) and Nathan, 1967 found that this enzyme was elevated in twelve out of twenty patients with tumours of the central nervous system; the C.S.F. level of C.P.K. was elevated in all four patients with chromophobe adenomas but not in those with benign meningioma. Because of the blood/brain barrier, raised serum enzymes are not reflected by similar changes in the C.S.F.

Enzyme studies have also been carried out in other body fluids. For instance in patients with inflammatory joint disease, including rheumatoid arthritis, pyogenic arthritis and Reiter's syndrome, there is increased activity of various glycolytic and oxidative enzymes in the synovial fluid (West *et al.*, 1963). L.D.H. activity was most marked when there was a high leucocyte count in the joint fluid. Similar enzyme changes have been noted in inflammatory effusions within serous cavities (Brauer *et al.*, 1963). Increased enzyme activity also occurs in malignant effusions; for instance, increased leucine aminopeptidase (L.A.P.) activity has been noted in malignant ascites associated with secondary deposits in the liver. Of course, in such cases, the serum L.A.P. is very high and there is a urinary spill over of this enzyme. Later in this book, other enzyme changes in the urine and vaginal fluid will be discussed.

## SUMMARY

As far as the general physician is concerned the clinical value of enzyme tests vary considerably. First, there are those enzyme estimations which are of great diagnostic help; important examples being marked elevation of serum amylase in acute pancreatitis, and increased serum acid phosphatase activity in prostatic carcinoma. Specific enzyme tests are also very important in paediatrics. Thus in a number of genetically determined disorders, e.g. galactosaemia, it is possible to demonstrate the absence of a particular enzyme (see Chapter VIII).

Other enzyme tests may be of confirmatory value. For instance, a number of enzymes are acutely elevated after myocardial infarction (Chapter III). Serial enzyme levels may also be helpful in following the course of a disease. For instance alanine aminotransferase (S.G.P.T.) determinations will be a useful guide in following a patient with infective hepatitis. In addition it has been found that performing an enzyme and antibody test on the same serum specimen can strongly suggest both the cause of an infection and the organ involved. Thus in hepatic amoebiasis the serum cholinesterase is low and the E.H. antibody titre is elevated (Mullan *et al.*, 1967). It does seem that in this case an accurate 'postal diagnosis' can be made without a clinical examination or radiological help.

Finally, a number of enzyme tests have an initial vogue and then fall into disuse; for instance, an elevated level of serum leucine aminopeptidase (L.A.P.) was thought to be diagnostic of pancreatic carcinoma, but it was later confirmed that elevation of this enzyme occurred in other types of obstructive hepatobiliary disease.

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## CHAPTER II

### Iso-enzymes

#### DEFINITION

It was originally felt that once a pure enzyme was produced all the molecules within this crystalline structure would be similar in composition and have the same substrate specificity. It was later found that enzymes, like other proteins, could be separated by physico-chemical means into various fractions; electrophoretic methods proved to be the most useful way of distinguishing these fractions or iso-enzymes. This situation is analogous to the different naturally occurring isotopes of a single element.

In 1959 Markert and Møller proposed the term isozyme (iso-enzyme) 'to describe the different molecular forms in which proteins may exist with the same enzymatic specificity', and the standing committee on enzymes of the International Union of Biochemistry used the term iso-enzyme to describe the multiple enzyme forms that occur in a single species. Other authors (Wieland and Pfleiderer, 1962) suggested that the term hetero-enzyme should be used to describe those enzymes which have the same action but come from different tissues.

Markert and Møller (1959), using starch gel electrophoresis, showed that the L.D.H. iso-enzyme patterns of beef, sheep, pig, mouse and rabbit heart were all different and the zymogram was unique for each species. In addition, the iso-enzyme patterns of L.D.H. from different tissues of the pig were tissue specific. Furthermore, the L.D.H. iso-enzyme pattern of embryonic tissue was different from the adult pattern.

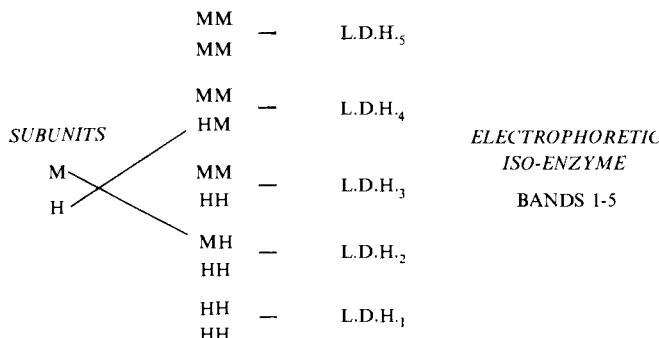
It has also been shown that different molecular forms of an enzyme may be present in different parts of the same cell;

for instance aspartate aminotransferase (G.O.T.) is present in a different form in mitochondria as compared to the supernatant fraction of the cell (Eichel and Bukovsky, 1961).

The definition of iso-enzymes has been broadened still further by Goodfriend (1965) to include all enzymes that catalyse a given reaction, and by this definition acid and alkaline phosphatases can be considered to be iso-enzymes. The phosphatases from prostate, bone and red cells all hydrolyse various phosphate esters, but differ in their pH optima and in their degree of inhibition by certain chemicals. In fact, most authors would reserve the term iso-enzyme to those fractions of acid and alkaline phosphatase that can be separated by electrophoresis.

### Molecular theory

It is likely that two genes are responsible for the two L.D.H. subunits. Skeletal muscle is rich in 'M' subunits and the heart contains mainly 'H' subunits. Using these polypeptide subunits it is possible to build up five tetrameric iso-enzymes:



The above theory was supported by Markert (1963) who showed that, if an equal quantity of L.D.H.<sub>1</sub> and L.D.H.<sub>5</sub> is frozen and thawed, the subunits of L.D.H. dissociate and finally recombine to form all five iso-enzymes fractions demonstrable by electrophoresis. These iso-enzymes were in the proportion 1:4:6:4:1 (L.D.H.<sub>1</sub> to L.D.H.<sub>5</sub>) which is the expected ratio if L.D.H. subunits recombine in a random

manner. In addition, L.D.H.<sub>1</sub> and L.D.H.<sub>5</sub> are immunologically distinct (Cahn *et al.*, 1962).

The alteration of iso-enzymes during life is of considerable interest (Zinkham *et al.*, 1966). L.D.H.<sub>3</sub> is the main iso-enzyme of foetal heart and the adult pattern, with predominance of L.D.H.<sub>1</sub> is reached at the age of 3. On the other hand, in foetal liver L.D.H.<sub>1,2</sub> and L.D.H.<sub>3</sub> are present but tend to decrease considerably after birth, so that L.D.H.<sub>5</sub> remains the main iso-enzyme of the liver in the adult. The latter authors also noted the appearance of a new iso-enzyme between L.D.H.<sub>3</sub> and L.D.H.<sub>4</sub> in the mature testis. This has been called band X which is also the main iso-enzyme of washed sperm. The new iso-enzyme appears in the testes of rabbits at 8 weeks which corresponds to the onset of spermatogenesis (Zinkham *et al.*, 1964).

This molecular theory has been applied to other enzymes. Thus Burger *et al.* (1963, 1964) suggested that creatine kinase (C.P.K.) was dimerous, i.e. consisted of two subunits. There are three types of C.P.K.; the muscle enzyme, the brain enzyme and a hybrid iso-enzyme which appears as an intermediate band on electrophoresis. Dawson and Fine (1967) studied the C.P.K. iso-enzyme pattern of various human tissues. Using starch gel electrophoresis they demonstrated three C.P.K. bands and an additional band between 1 and 2 which proved to be adenylate kinase. The fast moving C.P.K. fraction (band 3) was the only iso-enzyme of brain tissue, but it also occurred in other tissues, i.e. bladder, thyroid and kidney. There is of course no C.P.K. activity in red cells or liver. On the other hand, the last-mentioned authors found that skeletal muscle contained two bands (1 and 2), one slow moving and another intermediate in position.

A number of other enzymes occur in multiple molecular forms. They include various dehydrogenases, aspartate aminotransferase (G.O.T.), cholinesterase, phosphatases, 5'-nucleotidase and leucine aminopeptidase (L.A.P.).

### **Physiological significance**

In anaerobic glycolysis pyruvate is converted to lactate. In the

presence of excess lactate the activity of the fast moving L.D.H. iso-enzymes is decreased, but the slow moving iso-enzymes of L.D.H. continue to operate. In the liver and certain muscles, e.g. the uterus, energy is provided by anaerobic glycolysis and the slow moving iso-enzymes of L.D.H. predominate. On the other hand, in the brain and heart muscle aerobic metabolism occurs and the main iso-enzymes of L.D.H. are of the fast moving type. In the kidney the oxygen tension is higher in the cortex compared to the medulla. Aerobic glycolysis is more marked in cortical tissue, and this correlates with the finding of a greater proportion of fast moving (heart type) L.D.H. iso-enzymes in the cortex, while in the medulla there are more slow moving (muscle type) L.D.H. subunits (Dawson *et al.*, 1964). Furthermore, in experimental work, it has been shown that increasing the oxygen tension in tissue culture of growing muscle causes a reduction in the proportion of slow moving L.D.H. in this tissue (Dawson *et al.*, 1964). In addition, local anoxia can cause changes in concentration of serum L.D.H. iso-enzyme; constricting the upper arm with a cuff for 5 minutes causes an elevation in total serum L.D.H. with a marked increase in the L.D.H.<sub>5</sub> fraction; this change is due to leakage of enzyme from anoxic muscles into the blood stream (Starkweather *et al.*, 1966).

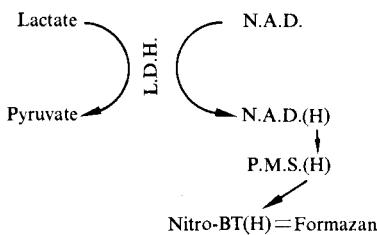
The functional importance of L.D.H. iso-enzymes has also been studied in other species. Of particular interest were the differences of L.D.H. iso-enzymes in the breast muscle of various birds (Wilson *et al.*, 1963). For instance, the Storm Petrel, with its remarkable capacity for prolonged flight, has a high content of L.D.H.<sub>1</sub> in its breast muscle; as a result, lactic acid production is minimal and muscle fatigue is prevented. On the other hand, the domestic fowl only flies short distances, and the predominant iso-enzyme in its breast muscle is L.D.H.<sub>5</sub>.

### Methods

The L.D.H. iso-enzymes have general biological significance and have been extensively investigated. This has been possible

because of the development of sensitive methods of L.D.H. iso-enzyme detection. Starch gel electrophoresis is a particularly valuable method of iso-enzyme separation. A small amount of serum or tissue homogenate is placed into a slot in the gel and the ends of the gel are placed in electrode vessels containing suitable buffer. Starch gel behaves like a molecular sieve and the separation of various protein fractions will depend on both the size and electrical charge of the protein particle. A current is passed through the gel, and the iso-enzymes of L.D.H. migrate as five separate fractions. L.D.H.<sub>1-4</sub> travel towards the anode and L.D.H.<sub>5</sub> migrates towards the cathode.

One method by which enzyme is localized on the cut surface of the gel is clearly described by Zinkham *et al.*, 1966. This is achieved by incubation with a buffered solution containing lactate, N.A.D., phenazine methosulphate (P.M.S.) and nitro-blue tetrazolium (Nitro-B.T.). L.D.H. transfers hydrogen to N.A.D. and hydrogen is then passed on to P.M.S. Finally Nitro-B.T. takes up hydrogen and is converted into a purple insoluble compound (Formazan), which precipitates on the surface of the gel:



In this way the iso-enzymes of L.D.H. can be distinguished and the concentration of each iso-enzyme fraction can be assessed. By altering the incubation mixture this method can also be employed to visualize other enzymes which form N.A.D.H. or N.A.D.P.H.

Methods have been developed for the separation of other iso-enzymes. For instance, the iso-enzymes of leucine aminopeptidase (L.A.P.) have been localized after electrophoresis on cellulose acetate membranes (Meade and Rosalki, 1964). The

L.A.P. bands of tissue homogenates taken from liver, pancreas, kidney and placenta differed in their electrophoretic mobility.

### **Genetically abnormal iso-enzymes. Cholinesterase variants**

Iso-enzyme studies have also been employed to study a genetically determined deficiency or the atypical character of an enzyme. Thus, Brody *et al.* (1965) studied a patient who had a prolonged period of scoline apnoea. The plasma cholinesterase was reduced and low dibucaine and fluoride numbers indicated that the patient was homozygous for the atypical enzyme. After starch gel electrophoresis they found that esterase activity was present in five distinct bands in normal serum. However, the first or fast moving band was absent in the above patient and band 2 was greatly diminished. These bands were identified as cholinesterase because they were inhibited by physostigmine.

The investigation of cholinesterase variants is of considerable genetic interest and the demonstration of a 'silent' cholinesterase gene (Liddell *et al.*, 1962), is noteworthy. These workers recorded the case of a Greek woman of 42 with prolonged scoline apnoea, and complete absence of cholinesterase activity. Homozygotes for the 'silent' gene will have no cholinesterase activity, and this has now been confirmed in a Bantu schoolgirl (Jenkins *et al.*, 1967).

### **Clinical applications**

Isoenzyme studies may be of value in the diagnosis of a number of diseases, and the pragmatic physician will need to know those changes which will be of diagnostic value. First, it should be realized that there may be an increase in the total activity of an enzyme in a wide variety of conditions. This is especially so in the case of an ubiquitous enzyme such as lactate dehydrogenase (L.D.H.). Thus the total serum level of L.D.H. is increased in myocardial infarction, liver disease, megaloblastic anaemia, renal infarction, malignant disease, and in

intravascular haemolysis in patients with prosthetic heart valves (Jorgensen, 1967).

### **Cardiac diseases**

It was only after the development of methods of separation of L.D.H. isoenzymes, that greater diagnostic precision was possible. Thus serum L.D.H.<sub>1</sub> was found to be acutely elevated after myocardial infarction. However, it should be noted that the serum L.D.H.<sub>1</sub> fraction is also elevated in other conditions, e.g. accidental haemolysis of the specimen, rheumatic carditis and pernicious anaemia. On the other hand, serum L.D.H.<sub>4</sub> and <sub>5</sub> isoenzymes are raised in liver disease and pre-icteric hepatitis.

More convenient methods of assessing the relative amounts of fast or slow moving L.D.H. iso-enzymes include the estimation of serum  $\alpha$ -hydroxybutyrate dehydrogenase (S.H.B.D.) (Rosalki and Wilkinson, 1960; Elliott and Wilkinson, 1961) and the heat stability test (Wróblewsky and Gregory, 1961). The latter test depends on heat denaturation of the non-cardiac iso-enzymes of L.D.H. The urea stable lactate dehydrogenase has also been used an index of cardiac iso-enzymes (Lindy and Kouttin, 1967). Non-cardiac iso-enzymes of L.D.H. are denatured by urea, and these authors showed a clear difference in the percentage of urea stable L.D.H. in patients with liver disease (average 40 per cent) as compared to cases of myocardial infarction (average 91 per cent). In the normal control group suppression of L.D.H. activity was intermediate (average 74 per cent).

### **Malignant disease**

Iso-enzyme studies have also been applied to malignant disease. For instance, Starkweather *et al.* (1966) investigated the changes of L.D.H. iso-enzymes during therapy of bronchial carcinoma. Patients with a normal serum L.D.H. iso-enzyme pattern usually had limited disease. On the other hand, a raised serum L.D.H. with an iso-enzyme pattern similar to that of tumour indicated extensive disease. When treatment caused tumour regression then there was a fall in the levels of

L.D.H. iso-enzymes. However, increasing levels of enzyme indicated progressive disease.

It has also been observed that in a number of conditions the total serum L.D.H. may be raised with a normal iso-enzyme distribution (Cohen *et al.*, 1966). This 'iso-morphic' L.D.H. pattern was present in eight cases of polycythaemia rubra vera, three patients with metastatic carcinoma, and in single cases of congenital cyanotic heart disease with erythrocytosis, hypothyroidism, multiple myeloma and reticulum cell sarcoma.

#### **The use of iso-enzymes to determine the source of increased serum enzyme activity in disease (tetanus)**

Iso-enzyme estimations may be most useful in determining the source of an elevated serum enzyme. This is well shown in a paper by Brody and Hatcher (1967). They confirmed significant elevations of serum C.P.K. in three cases of tetanus and found that with recovery the enzyme activity returned to normal. These authors went on to produce experimental tetanus in rabbits, and made the important observation that early elevation of serum C.P.K. activity occurred in these animals before the development of generalized rigidity, and hence serum C.P.K. determination could be useful in the early diagnosis of tetanus in man. In addition, induced flaccid paralysis with scoline failed to lower the serum C.P.K. It was therefore suggested that tetanus toxin produced increased C.P.K. levels by direct injury to muscle tissue.

Using starch gel electrophoresis, Brody and Hatcher (1967) studied C.P.K. and L.D.H. iso-enzymes in rabbit serum, normal 'fast' muscle (*gastrocnemius*), 'slow' muscle (*soleus*), heart muscle and spinal cord. The electrophoretic mobility of serum C.P.K. in rabbits with tetanus corresponded with the main isoenzymes in normal fast, slow and cardiac muscle, but not with the main fast moving C.P.K. iso-enzyme in spinal cord tissue. L.D.H.<sub>1</sub> is present predominantly in heart and 'slow' muscle, while L.D.H.<sub>5</sub> is present mainly in 'fast' muscle (Brody and Engel, 1964). The L.D.H.<sub>5</sub> fraction in serum from rabbits with tetanus was considerably elevated and this con-

firmed that the main source of increased C.P.K. activity in tetanus was from skeletal 'fast' muscle.

### Liver and bone disease

Another common problem confronting the clinical pathologist is the isolated finding of marked elevation of the serum alkaline phosphatase. In this connection, electrophoretic separation of the alkaline phosphatase iso-enzyme has proved to be of some value (Frenkel and van Triet, 1964; Haije and De Yong, 1963). Using agar gel these workers were able to demonstrate three iso-enzymes of alkaline phosphatase: the two faster moving bands were increased in liver disease, and the slowest alkaline phosphatase iso-enzyme band was prominent in patients with increased osteoblastic activity. In fact, it may be easier to measure serum 5'-nucleotidase or leucine aminopeptidase (L.A.P.) activity. These two enzymes are elevated in hepato-biliary disease and in patients with liver secondaries, but in bone disorders, e.g. Paget's disease, serum 5'-nucleotidase and L.A.P. activity is normal.

### Renal disease

Enzyme changes in renal disease will be discussed in Chapter X. A number of urinary enzymes are increased in diseases of the urinary tract. The clinical course of renal diseases may also be followed; for example, in acute tubular necrosis and during exacerbations of the nephrotic syndrome there may be an excessive urinary loss of a fast moving iso-enzyme of alkaline phosphatase (Moss 1964, Butterworth *et al.*, 1965). Finally, iso-enzyme studies will be useful in patients who have had organ transplantation. Thus, it has been shown that increases in serum L.D.H.<sub>1</sub> and L.D.H.<sub>2</sub> occur in patients who have later rejected their kidney homografts (Latner *et al.*, 1965).

## SUMMARY

By employing various methods of separation it has been shown that many enzymes exist in different molecular forms. The

variations in an iso-enzyme pattern in different species, organs and the changes which accompany development are of considerable biological interest. Mention has also been made of the diagnostic value of certain iso-enzymes. The literature on the subject has expanded rapidly, but much of the research has been of academic interest only. Widespread treatment of 'end stage' disease by organ transplantation will encourage the enzymologist to study those changes which herald rejection of the homograft.

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## CHAPTER III

# Enzyme changes in myocardial infarction cardiomyopathies and non-ischaemic heart failure

### INTRODUCTION

This chapter is concerned with the enzyme changes which may be useful in the differential diagnosis of acute chest pain. Some of the more important experimental work in this field is also reviewed. The final two sections in this chapter deal with certain cardiomyopathies and enzyme changes in non-ischaemic heart failure.

There is a tendency nowadays to perform a battery of investigations on patients before making a clinical diagnosis. Laboratory time can be wasted carrying out enzyme tests on patients with undoubted myocardial infarction. If the history of pain is classical, and there are widespread Q waves and ST changes on the electrocardiogram (E.C.G.) these tests need not be done. So often enzyme estimations are performed on a patient's serum when his heart has already been thoroughly examined by the pathologist! In fact, in massive infarction, early death can occur before there is an appreciable rise in the level of serum enzymes.

In certain circumstances, enzyme studies can be useful in the early diagnosis of myocardial infarction. Although the history may be suggestive, E.C.G. evidence of infarction may take several days to develop. Fig. 3 demonstrates serial E.C.G. changes in a 54-year-old man. Twenty-four hours after the onset of ischaemic pain, the E.C.G. (23.5.66) showed little

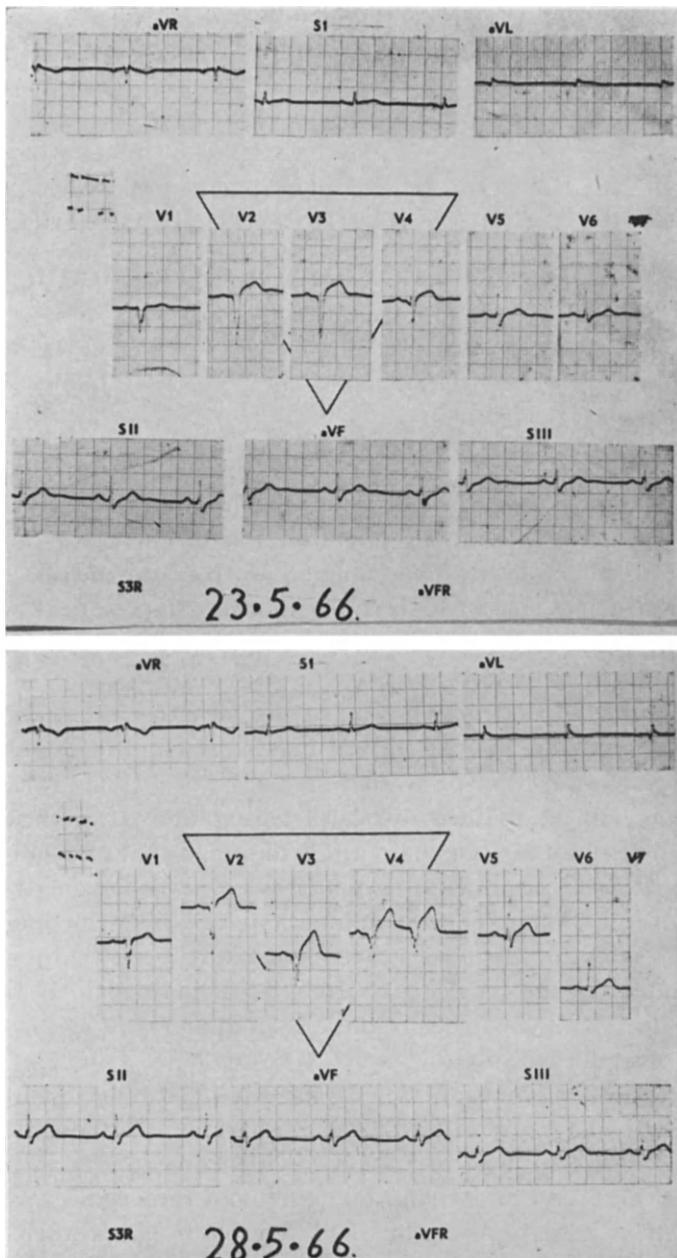


FIG. 3. For description see text. Serial E.C.Gs on 23.5, 28.5 and 4.6.66 in a man aged 54.

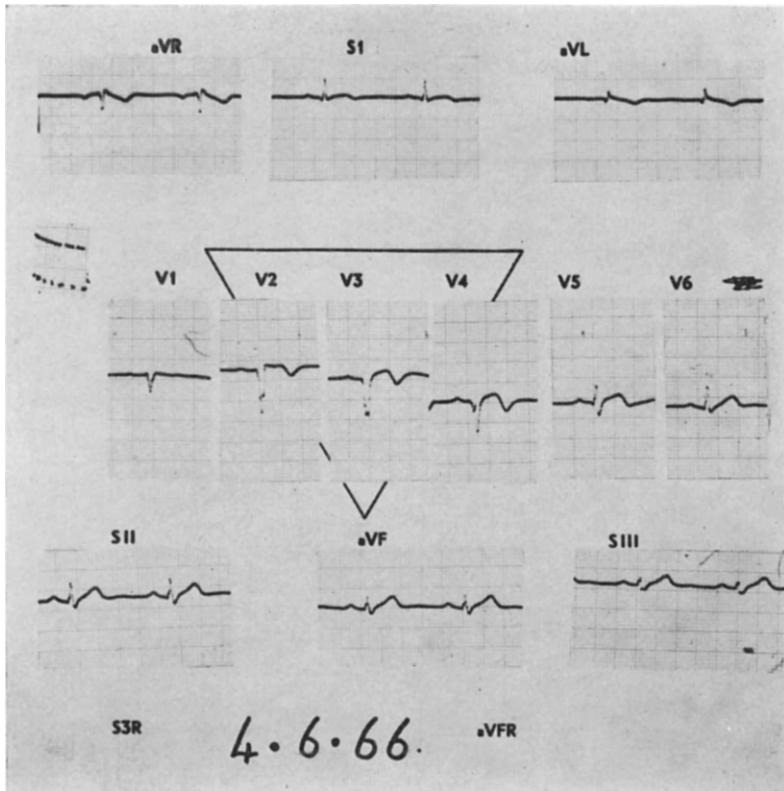


FIG. 3.

change although the serum aspartate aminotransferase (S.G.O.T.) was acutely elevated at 235 King units. The S.G.O.T. levels 2, 3 and 9 days after the onset of pain were 190, 100 and 30 King units respectively, and only the last two E.C.G.s. (28.5.66 and 4.6.66) showed obvious changes.

An early elevation of the serum creatine kinase (C.P.K.) or S.G.O.T. might encourage the enthusiastic physician to start anticoagulant therapy. In fact, it might be more logical to start such treatment at the stage of 'pre-infarction angina'. At this early stage, the history of recent indigestion, or chest pain with typical radiation can be very suggestive. However, the E.C.G. at this early stage may be quite unhelpful (see Fig. 4, which includes a description of the case).

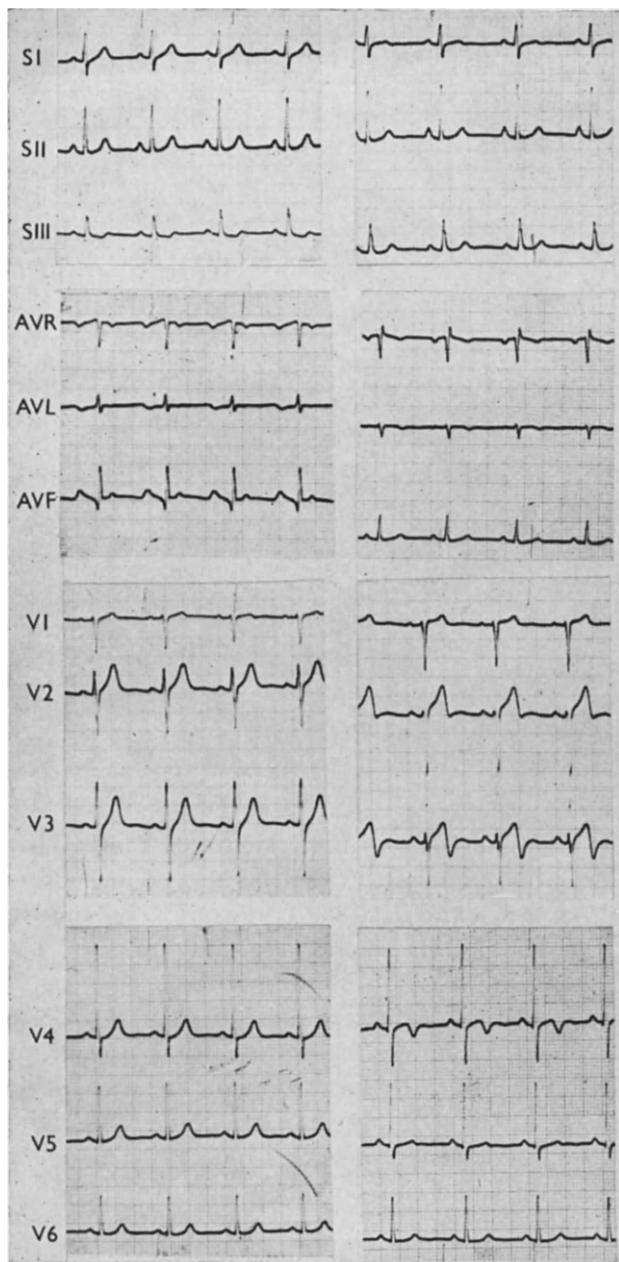


FIG. 4. E.C.Gs on 22.9.66 and 29.9.66 indicating recent infarction.

The E.C.G. tracing on 22.9.66 was taken from a 'fit' gymnast, aged 48. For 4 weeks he had noticed that exertion after meals produced chest pain. When first seen, enzyme studies and an E.C.G. after vigorous exertion were normal; however, one week later (29.9.66) there was clear evidence of an antero-septal infarct. His E.C.G. subsequently returned to normal (see Fig. 5.)

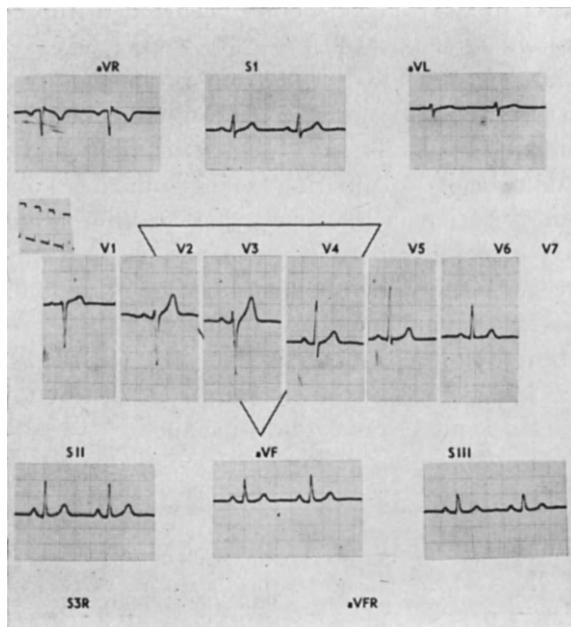


FIG. 5. Three months later the same patient had a completely normal E.C.G. tracing.

It is important to note the time interval between the onset of chest pain and the time when the serum is taken for enzyme estimation. Thus, the S.G.O.T. level will have returned to normal 4 or 5 days after myocardial infarction. This also applies to serum C.P.K. activity which is acutely elevated in the first 1 to 2 days after myocardial infarction. (Hess *et al.*, 1964; Vincent and Rapaport, 1965; Griffiths, 1966).

### Hepatic congestion

Hepatic congestion due to heart failure will lead to a prolongation of the high S.G.O.T. levels with a rise in the S.G.P.T. activity in the serum (Rosin, 1961). It was also suggested (Shields and Shannon, 1958) that high S.G.O.T. titres in myocardial infarction with liver congestion indicated a poor prognosis. Likewise a progressive fall in serum cholinesterase activity after myocardial infarction occurred in those patients who died (Moore *et al.*, 1957).

Liver damage can also result from prolonged hypotension with anoxia, and occasionally certain anticoagulants may have a toxic effect on liver cells. All these factors must be taken into consideration before evaluating the significance of enzyme changes in a patient with myocardial infarction and heart failure.

Enzyme tests may also be helpful when the patient has already had a previous myocardial infarct, in the presence of an arrhythmia, if the E.C.G. is equivocal or shows bundle branch block, or when the ST changes could be due to digitalis therapy, or an acute electrolyte disturbance.

### Differential diagnosis

Cardiac infarction is not the only cause of severe pain in the epigastric 'No Mans Land', and patients with an acute abdomen may occasionally be sent in error to a medical ward. For instance, confusion may arise in acute pancreatitis, when electrolyte loss and shock may be associated with hypokalaemic T wave inversion on the E.C.G. The latter may mimic ischaemia. Furthermore, in acute pancreatitis, the S.G.O.T. level may be raised, but the serum amylase will also be acutely elevated usually to well over 600 Somogyi units/100 ml. The serum amylase may increase after an injection of morphine, and high levels have also been recorded in perforated peptic ulcer, acute biliary disease, intestinal strangulation and uraemia. On the other hand, normal levels of this enzyme exclude acute pancreatitis. This was recently confirmed in a patient who had

possible pancreatitis at laparotomy but with normal levels of serum amylase. Subsequent post-mortem examination revealed an acute perforation of the lower oesophagus.

In acute biliary colic without jaundice the serum alkaline phosphatase and leucine aminopeptidase (L.A.P.) may be elevated. L.A.P. is not elevated in myocardial infarction.

In other conditions, such as pericarditis, the S.G.O.T. level is not usually elevated; in a collected series Agress (1959) noted that only three out of forty-one patients with pericarditis had raised levels of this enzyme. The S.G.O.T. and C.P.K. levels may be elevated in dissecting aneurysm but these tests are not helpful in distinguishing between this condition and myocardial infarction. S.G.O.T. and C.P.K. levels, may also be elevated in rapid arrhythmias (Griffiths, 1966).

### Pulmonary infarction

Pulmonary infarction may be another diagnostic problem. S.G.O.T. elevation, if present, is usually slight and usually occurs after the fourth day in patients with large pulmonary infarctions (Agress, 1959). Wacker *et al.* (1961) suggested that in pulmonary infarction, an elevated serum lactate dehydrogenase (L.D.H.), an increase in serum bilirubin and a normal S.G.O.T. was diagnostic, but Schonnell *et al.* (1966) found that this biochemical triad was not helpful, and did not distinguish between pneumonia and pulmonary infarction. Occasionally there may be slight elevation of C.P.K., and more recently (Stuart, 1965) has noted slight elevation of S.H.B.D. in some cases of pulmonary infarction. Of nineteen patients with probable pulmonary embolus Cohen *et al.* (1966) found that only seven had elevation of total serum L.D.H. Three of these cases had an increase in L.D.H.<sub>1</sub>, three others had an increase in L.D.H.<sub>3,4</sub> and L.D.H.<sub>5</sub> and one had both abnormalities. Four out of twelve patients with normal total serum L.D.H. had an increase in L.D.H.<sub>1</sub>; the remaining eight cases had a normal L.D.H. iso-enzyme pattern. An increase in L.D.H.<sub>3,4</sub> and L.D.H.<sub>5</sub> was due to liver congestion. On the other hand an increase in L.D.H.<sub>1</sub>, in pulmonary infarction could be due to

various factors including haemolysis of red cells or myocardial anoxia due to shock and reduced coronary blood flow.

We therefore again fall back on the clinical picture and, from a practical point of view, pulmonary infarction should be considered probable in the presence of pleuritic pain and dyspnoea without infected sputum, basal 'pneumonia' with haemoptyses, syncope and thrombo-phlebitis. The E.C.G. need not be typical, but radiological changes with basal collapse and some elevation of the diaphragm are strongly suggestive. Massive pulmonary embolism is certainly common in healthy people (Fleming and Bailey, 1966) and anticoagulant therapy is effective and should be started early. A more specific enzyme test for pulmonary infarction is not yet available (Trujillo *et al.*, 1967).

### **Value of S.G.O.T. in diagnosis of myocardial infarction**

Interest in the enzyme confirmation of myocardial infarction was stimulated in 1954 when La Due, Wróblewski and Karmen demonstrated that following myocardial infarction there was an acute increase in the level of serum S.G.O.T. Other workers (Baron, 1958; Agress, 1959) have confirmed their results. The latter author in a review of 1255 proven cases of myocardial infarction found that almost 97 per cent had an elevated S.G.O.T. If the infarct is small there may be a small transient rise of this enzyme within the normal range.

### **Experimental studies**

Careful experimental work (Nydick, Wróblewski and La Due, 1955) on the dog showed that tying a coronary artery with resultant infarction caused an acute elevation of S.G.O.T. Furthermore, the concentration of this enzyme in the infarcted area was much lower than in healthy heart muscle in the same animal. An acute leakage of enzyme from heart muscle in the blood stream was suggested. It was therefore not surprising to find that other enzymes present in high concentrations in heart muscle such as lactate and malate dehydrogenase, creatine kinase (C.P.K.), aldolase, and phospho-hexose isomerase

were acutely elevated following myocardial infarction (West *et al.*, 1966).

On the other hand, although isocitrate dehydrogenase (I.C.D.) is present in high concentration in heart muscle, this enzyme is not apparently elevated after myocardial infarction (Sterkel, 1958). This interesting anomaly has been investigated by White (1960) who found that experimental myocardial infarction in dogs resulted in a transient increase in I.C.D. activity of blood taken from the coronary sinus and inferior vena cava. In addition the I.C.D. content of the dog's infarcted cardiac muscle was reduced. It was also shown by Strandford, Thomas and White (1959) that injected I.C.D. in dogs rapidly disappeared from the circulation while injected L.D.H. was cleared at a slower rate. The main I.C.D. iso-enzyme of heart muscle is unstable (Campbell and Moss, 1962) and the activity of this iso-enzyme falls off rapidly. This could account for the failure to detect raised I.C.D. blood levels after myocardial infarction in man.

### **Enzymes in acute coronary insufficiency**

Serum enzymes are rarely elevated in coronary insufficiency or angina (West *et al.*, 1966). These authors studied about 200 patients and found the incidence of abnormal levels to be small for most enzymes (G.O.T. 5 per cent; G.P.T. 2 per cent; L.D.H. 15 per cent and I.C.D. 2 per cent). However, phospho-hexose isomerase (P.H.I.) was increased in 33 per cent and serum aldolase was above normal in 30 per cent of these cases. They felt that this was due to the easier escape of these two enzymes from anoxic heart muscle. Modest elevations of serum C.P.K. were also noted on the first or second day in some patients with acute coronary insufficiency, and more recently Hedworth-Whitty *et al.* (1967) showed increased activity of serum  $\gamma$ -glutamyl transpeptidase in seven out of ten patients with severe angina. Other authors (Goble and O'Brien, 1958) had previously carried out serial determinations and found fluctuating levels of S.G.O.T. in cases of acute coronary insufficiency. We may therefore conclude that in some patients

coronary insufficiency can lead to episodes of myocardial necrosis.

### **Caeruloplasmin and serum metals in myocardial infarction**

In parallel with these enzyme studies, other workers have found acute changes in various serum metals following acute myocardial infarction. The serum caeruloplasmin and copper increases after myocardial infarction (Vallee 1952) and during pregnancy (Adelstein *et al.*, 1956). The gradual increase in serum oxidase activity after myocardial infarction was confirmed by Rowell and Smith (1959) but these authors also found increased oxidase activity in a patient with cor pulmonale without any evidence of myocardial infarction. Walker *et al.* (1956) also showed that serum zinc was lowered significantly after acute myocardial infarction. The serum manganese level is also elevated in both myocardial and pulmonary infarction (Hedge *et al.*, 1961), but in the latter condition the serum aluminium was elevated.

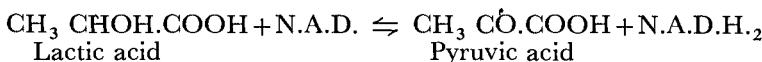
### **L.D.H. iso-enzymes in myocardial infarction**

In 1960 Wróblewski, Ross and Gregory studied the 'iso-enzymes' of lactic dehydrogenase (L.D.H.) in myocardial infarction. In plasma there were five iso-enzymes which were separated electrophoretically. The fast moving fraction L.D.H.<sub>1</sub> was present in greater concentration in heart muscle, and these authors showed that following infarction there was an acute elevation of L.D.H.<sub>1</sub> and some increase in L.D.H.<sub>2</sub>. Serial iso-enzyme determinations in one case showed a significant elevation of L.D.H.<sub>1</sub> 11 days after the onset of pain and in another case of subendocardial infarction L.D.H.<sub>1</sub> was raised in spite of a normal S.G.O.T. On the other hand the chief iso-enzyme of liver is the slow moving fraction L.D.H.<sub>5</sub> and it is this component which is acutely elevated in liver damage secondary to cardiac infarction (Aber *et al.*, 1966) and acute liver disease such as infective hepatitis.

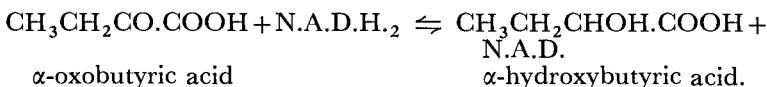
L.D.H. iso-enzymes can be separated using the vertical

starch gel electrophoretic method of Latner and Skillen (1961) or fairly simply on a cellulose acetate strip (Barnett, 1964). Using the latter method Handa (1967) confirmed an elevation of L.D.H.<sub>1</sub> in all twenty-six patients with myocardial infarction. There was also a shift of the L.D.H.<sub>1</sub>:L.D.H.<sub>2</sub> ratio to more than unity. These changes persisted for at least 12 days. In normal individuals the ratio of L.D.H.<sub>1</sub> to L.D.H.<sub>2</sub> was nearly always less than one. Cohen *et al.* (1966) had previously shown similar changes in twenty-four proven cases of myocardial infarction. Using an agar gel electrophoretic method they also carried out L.D.H. iso-enzyme studies in patients with ventricular fibrillation who had been resuscitated with external cardiac massage and electrical defibrillation. They demonstrated either an increase in L.D.H.<sub>1</sub>, or an increase in L.D.H.<sub>1,3,4</sub> and <sub>5</sub>. The former change was presumably due to myocardial damage, and the latter iso-enzyme increase could have been due to anoxia and shock affecting other tissues, e.g. muscle, skin or liver. In normal individuals the concentration of each L.D.H. isoenzyme may vary considerably from person to person. In disease repeat estimations are therefore desirable to detect any change in any particular fraction.

Lactate dehydrogenase (L.D.H.) reversibly converts lactate to pyruvate in the presence of nicotinamide-adenine dinucleotide (N.A.D.).



The serum iso-enzymes of L.D.H. also catalyse the reduction of  $\alpha$ -oxobutyrate:



Rosalki and Wilkinson (1960) showed that with increasing electrophoretic mobility of L.D.H. there is a gradually increasing activity against  $\alpha$ -oxobutyrate. The component active against  $\alpha$ -oxobutyrate has been designated ' $\alpha$ -hydroxybutyrate

dehydrogenase' (S.H.B.D.) (Wilkinson, 1962). Increased activity of S.H.B.D. has been found to be of considerable value in the diagnosis of myocardial infarction (Elliott, Jepson and Wilkinson, 1962; Preston *et al.*, 1964; Stuart *et al.*, 1965).

### S.H.B.D. compared with other enzymes in myocardial infarction

Fig. 6 shows that S.H.B.D. remains elevated up to 10 days or more after myocardial infarction, i.e. when the other enzymes have returned to normal. Another advantage of S.H.B.D. is that it is not elevated after a surgical operation (Preston, 1964). Furthermore S.H.B.D. activity may now also be determined colorimetrically (Rosalki, 1962). It is important to note that S.G.O.T., L.D.H. and S.H.B.D. activity is increased in a haemolysed specimen. On the other hand C.P.K., which is almost absent in red cells, is not increased by haemolysis. Creatine kinase (C.P.K.) determination is more laborious and has to be done within a few hours of collection: if serum from a patient with a myocardial infarct is left overnight, the raised activity of this enzyme drops by about a half (Hess *et al.*, 1964). However, the addition of cysteine prior to incubation will activate the enzyme.

It is clear from Fig. 6 that during the first 3 days after myocardial infarction the present routine use of serial S.G.O.T. levels provides useful confirmation of the diagnosis in the majority of patients. If admission is delayed by a week or more the raised S.H.B.D. would be diagnostic. On the other hand, by this time E.C.G. changes have usually occurred. In research studies the serial estimation of serum C.P.K. may also be useful in the younger patient who requires monitoring after myocardial infarction. Of course, post-infarction arrhythmias of all types are extremely common and may give rise to some increase in enzyme activity. There is no doubt that with the proliferation of intensive care units, the work load of the clinical enzymologist will increase. However, the current tendency to measure as many 'parameters' as possible should be resisted.

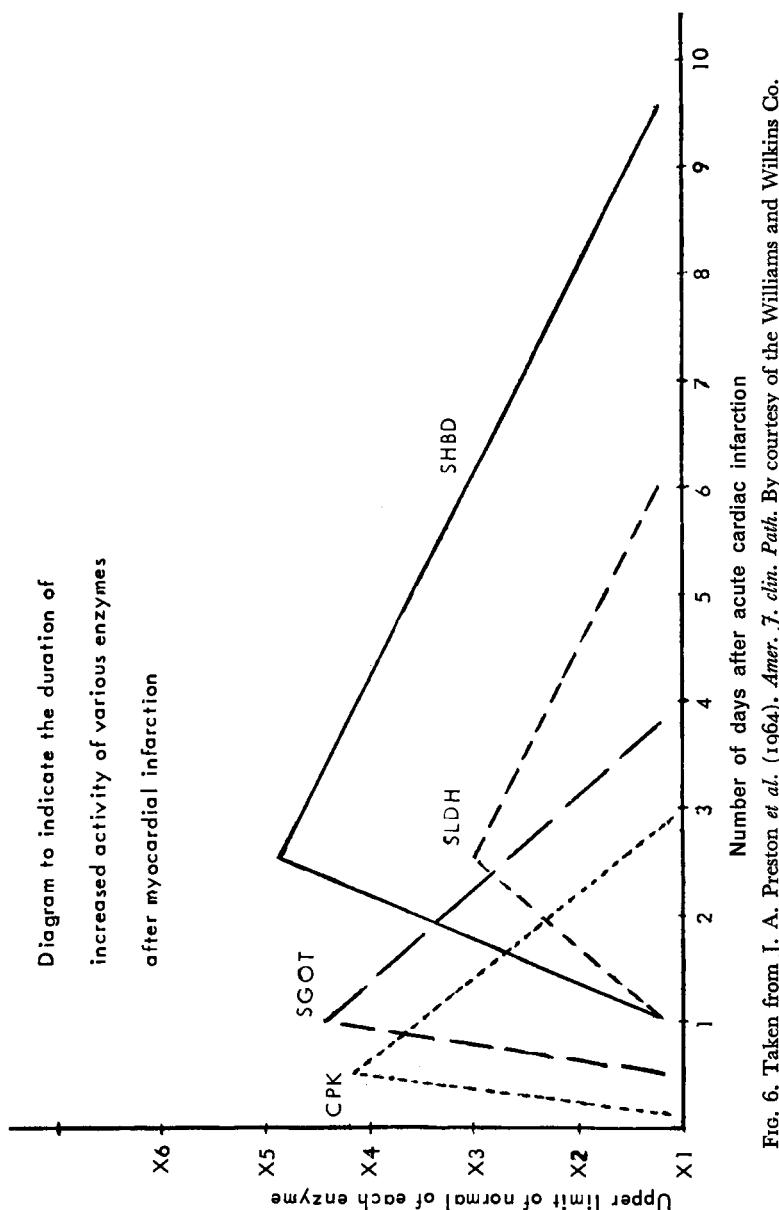


Fig. 6. Taken from J. A. Preston *et al.* (1964). *Amer. J. clin. Path.* By courtesy of the Williams and Wilkins Co.

### Cardiomyopathies

Cardiomyopathy may complicate a generalized disease, e.g. viral infection, collagen disease or amyloidosis. The aetiology is usually obscure but recently Braimbridge *et al.* (1967) have suggested the possibility of a new infective agent in congestive cardiomyopathy.

A useful method of classifying the cardiomyopathies was suggested by Goodwin *et al.* (1961). Thus, patients may present with evidence of: (a) Unexplained congestive cardiac failure and atrioventricular incompetence. A good example of this is acute heart muscle disease in the African, which in the acute phase is associated with some transient hypertension (Parry and Ikeme, 1966). Another example is congestive heart failure in alcoholics with thiamine deficiency (Alexander, 1966). (b) Alternatively, patients may present with features suggestive of constrictive pericarditis. (c) Finally, patients may have evidence of hypertrophic obstruction to ventricular outflow. In the left ventricular type there may be marked hypertrophy of the septum (Teare, 1958). On the other hand, excessive contraction of heart muscle during systole may lead to 'functional obstruction of the left ventricle' (Brock, 1957). The symptoms are similar to those of true aortic stenosis, but treatment with beta-blocking drugs may be beneficial.

Pearse (1964) carried out detailed studies of the histochemistry and electron microscopy of obstructive cardiomyopathy. He confirmed the presence of marked mitochondriosis, together with the disappearance of hypertrophic myofibrils, and increased glycogen deposition. Oxidative mitochondrial and monoamine oxidase (M.A.O.) activity was also increased. Of particular interest was the demonstration of a greater number of sympathetic nerves in this abnormal muscle, together with an increase in nor-adrenaline content. The interesting suggestion was made that obstructive cardiomyopathy could be due to a developmental anomaly of the sympathetic nervous system.

In acute heart muscle disease in the African moderate elevation of S.H.B.D. was found in eight out of thirteen cases

(Chapter IV). Further serum enzyme studies (C.P.K., G.O.T., S.H.B.D.) would be of interest in patients with other cardiomyopathies, and the investigation of those with a positive family history could well be rewarding.

### **Enzyme changes in congestive heart failure not due to myocardial infarction**

It is not surprising that serum enzyme changes should accompany heart failure. The release of liver enzymes could be due to actual liver cell necrosis or severe anoxia and correction of the heart failure should result in a return of these enzyme changes towards normal.

In this connection, Richman *et al.* (1961) studied 175 patients with acute and chronic heart failure due to a number of conditions including hypertension, arteriosclerotic heart disease, rheumatic heart disease, cor pulmonale and other miscellaneous conditions. The serum bilirubin and S.G.O.T. levels could be acutely elevated in right-sided heart failure, especially when there was underlying acute centrilobular hepatic necrosis. However, the elevation of serum bilirubin in right-sided heart failure can occur without any evidence of pulmonary infarction. In addition, elevation of the serum bilirubin or S.G.O.T. was found to be unusual in acute left ventricular failure, pulmonary oedema or hypotension. The above authors also found that the serum cholinesterase activity was reduced in 48 per cent of their cases, but in 90 per cent of their patients the serum alkaline phosphatase was normal. Some patients with congestive heart failure have a raised serum alkaline phosphatase. In these cases, having treated the heart failure, it will be important to exclude biliary obstruction by gall stones, for example.

More recently, McEwen and Harrison (1965) have found increased levels of serum monoamine oxidase in chronic congestive heart failure. This enzyme is distinct from caeruloplasmin and diamine oxidase (D.A.O.) in human serum. These workers used a spectrophotometric method to measure the conversion of the substrate benzylamine to benzaldehyde.

They studied seventy-nine patients, fifty-seven of whom had rheumatic heart disease, while the remainder had various forms of congenital heart disease. Irrespective of the type of heart disease, it was found that the serum monoamine oxidase level increased in parallel with increasing grades of severity of heart failure. Operative treatment, e.g. aortic valve replacement in a patient with aortic stenosis and heart failure, resulted in a fall of serum monoamine oxidase which coincided with clinical improvement and weight loss. Monoamine oxidase activity could not be correlated with levels of S.G.O.T. and S.G.P.T., and therefore increased activity of this enzyme could not be attributed to acute hepatocellular necrosis. Furthermore, normal levels of the 'enzyme' were found in cases of acute hepatocellular damage, renal failure and in myocardial infarction not complicated by heart failure. Although it is known that this enzyme is present in many tissues the reason for increased serum activity in heart failure remains uncertain.

### SUMMARY

The enzyme changes which follow myocardial infarction have been described and some of the experimental work in this field has been noted. Elevation of S.G.O.T., S.H.B.D. or serum C.P.K. activity will confirm the diagnosis of myocardial necrosis. However, maximum activity of these three enzymes occurs on different days after infarction (see Fig. 6). The cardiomyopathies and the enzyme changes secondary to different types of heart failure are discussed in the last two sections of the chapter.

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## CHAPTER IV

## Enzyme studies in African heart disease

A. M. WARD, G. J. THORPE AND D. P. MULLAN

## INTRODUCTION

In the previous chapter serum enzyme changes following acute myocardial infarction were described. In Africa entirely different types of heart disease occur, and ischaemic heart disease is uncommon (see Table 4). Enzyme studies in various

TABLE 4. *The diagnosis of 260 cases registered in the Cardiac Department at University College Hospital, Ibadan, Nigeria*

	No. of cases	%
Heart muscle disease (H.M.D.)	93	35.8
Rheumatic heart disease (Rh.C.)	40	15.4
Hypertensive heart disease	31	11.9
Endomyocardial fibrosis (E.M.F.)	29	11.2
Unclassified mitral incompetence		
with pulmonary hypertension	25	9.6
Cor pulmonale	12	4.6
Puerperal cardiomyopathy	11	4.2
Congenital heart disease	6	2.3
Constrictive pericarditis	4	1.5
Annular subvalvar left ventricular aneurysm (L.V.A.)	3	1.2
Ischaemic heart disease	2	0.8
Other	4	1.5
Total	260	100

By courtesy of Professor A. Brown, Department of Medicine, University College Hospital, Ibadan.

forms of African heart disease have been few, chiefly because of lack of time and equipment. In a sense, too, such studies are

only of theoretical interest; thus increased enzyme activity may reflect heart muscle damage, but the cause of the particular heart condition may remain obscure. However, a different pattern of enzyme change may be useful in distinguishing between two heart diseases. The African patient usually presents with gross heart failure, and account must therefore be taken of alteration of serum enzyme activity due to acute hepatic congestion. In terms of discovering the aetiology of these idiopathic African heart diseases, immunological studies may be more helpful. For instance, a higher incidence of increased filarial antibody titre in endomyocardial fibrosis (E.M.F.) may be significant (Ive *et al.*, 1967).

The cardiac conditions studied included heart muscle disease (H.M.D.—thirteen cases), endomyocardial fibrosis (E.M.F.—eight cases), and rheumatic heart disease (Rh.C—ten cases). Serum enzyme estimations were also carried out in patients with annular subvalvular left ventricular aneurysm (L.V.A.—five cases). Angiographic confirmation was obtained in all cases of E.M.F. and three patients with L.V.A. and autopsy was performed on two patients with L.V.A. and one with E.M.F. The anti-o-streptolysin titre exceeded 1/125 in five cases of acute rheumatic carditis.

The clinical features of these conditions have been well described elsewhere (*Bull. Wld. Hlth. Org.*, 1965, Parry and Ikeme, 1966). As can be seen (Table 4), heart muscle disease, endomyocardial fibrosis and rheumatic heart disease are common. On the other hand, subvalvular left ventricular aneurysm is rare, and is found almost exclusively in and around Ibadan.

Serum specimens\* were sent to the United Kingdom by air, kept at  $-20^{\circ}$  C., and thawed out in batches just prior to enzyme estimation. The serum enzymes which were measured included serum aspartate and alanine aminotransferase (S.G.O.T. and S.G.P.T.),  $\alpha$ -hydroxybutyrate dehydrogenase (S.H.B.D.), isocitrate dehydrogenase (I.C.D.), cholinesterase, leucine

\* The blood was collected into glass containers. Blood collected into plastic containers can give higher S.H.B.D. values (Rosalki, 1967).

aminopeptidase (L.A.P.) and the serum caeruloplasmin. Liver function tests and enzyme estimations were also performed on serum from thirty-three normal Africans.

Provided an enzyme remains relatively stable, it should be possible to carry out enzyme studies (and other immunological tests) at 'long range'. In fact, it has been shown (Clarke, 1963) that the enzyme activity of aspartate transaminase (S.G.O.T.) and S.H.B.D. in sera kept at  $-20^{\circ}\text{ C}.$ , remains relatively constant for up to 6 weeks. Likewise, using frozen serum sent by air from East Africa, changes were found in enzyme activity of the transaminases, L.A.P., cholinesterase and alkaline phosphatase which correctly matched the clinical diagnosis (Mullan *et al.*, 1967).

## Results

The results of the enzyme estimations are demonstrated graphically in Figs. 7 to 11. Each figure also shows the normal range in the African for each enzyme. As can be seen, increased S.H.B.D. activity occurred in the majority of patients in all four disease groups; fewer patients had raised S.G.O.T. levels. It is interesting to note that although thirty-three of thirty-six patients were in gross heart failure with liver congestion, the alanine aminotransferase levels (S.G.P.T.) were practically all normal; the only very high S.G.P.T. value was found in a girl of 10 who died shortly afterwards with severe rheumatic heart disease.

The serum caeruloplasmin levels were all strikingly elevated in patients with gross congestive cardiac failure. The exceptions were three cases with left ventricular aneurysms who were not in heart failure. The origin of this increased copper protein is not clear but may be related to increased hepatic synthesis or decreased breakdown of this enzyme. Chronic parasitic infection could be another possible reason for increased serum caeruloplasmin activity. In a small series of eighteen Sheffield patients with gross heart failure due to old ischaemic heart disease, chronic rheumatic heart disease, cor pulmonale and hypertension there was surprisingly no increase in serum

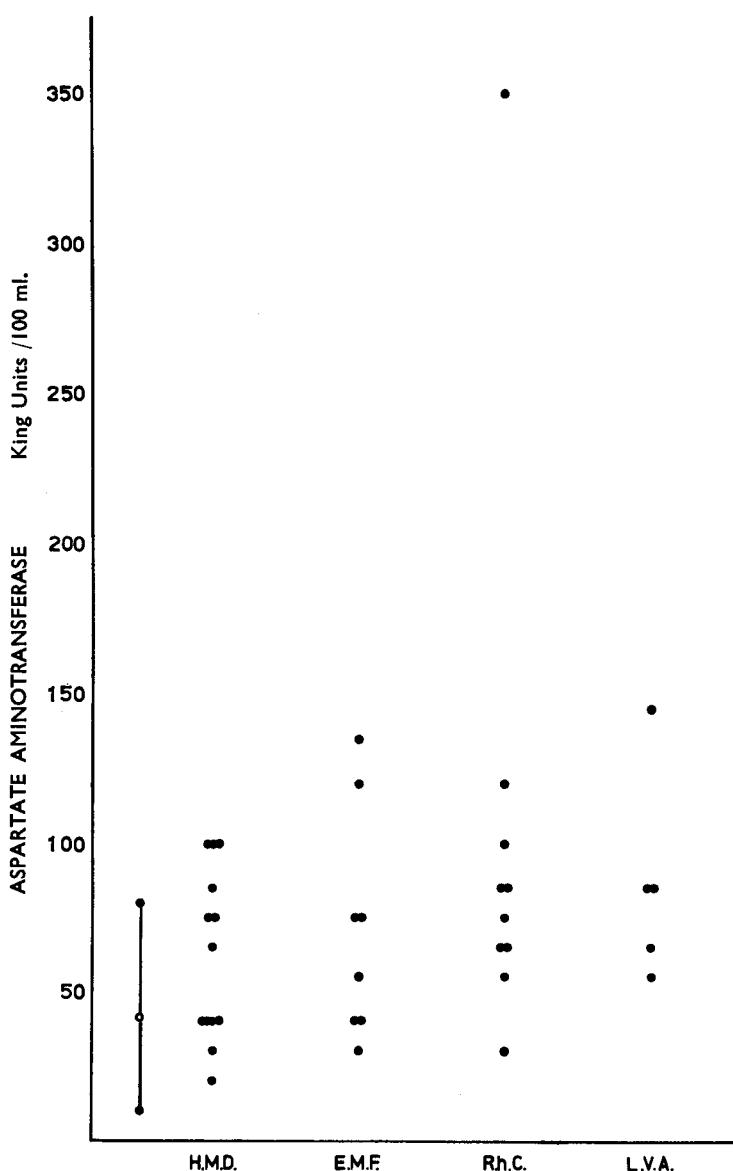


FIG. 7. Serum aspartate aminotransferase activity in normal Nigerians and in the four disease groups.

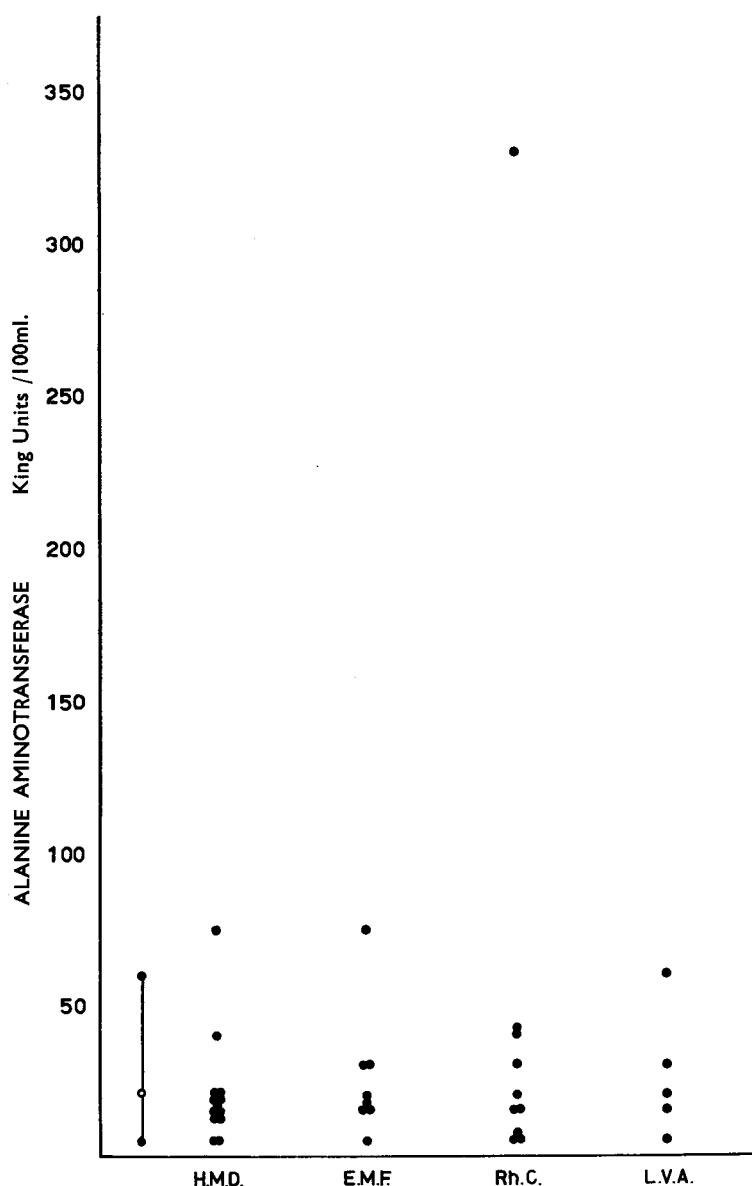


FIG. 8. Serum alanine aminotransferase activity in normal Nigerians and in the four disease groups.

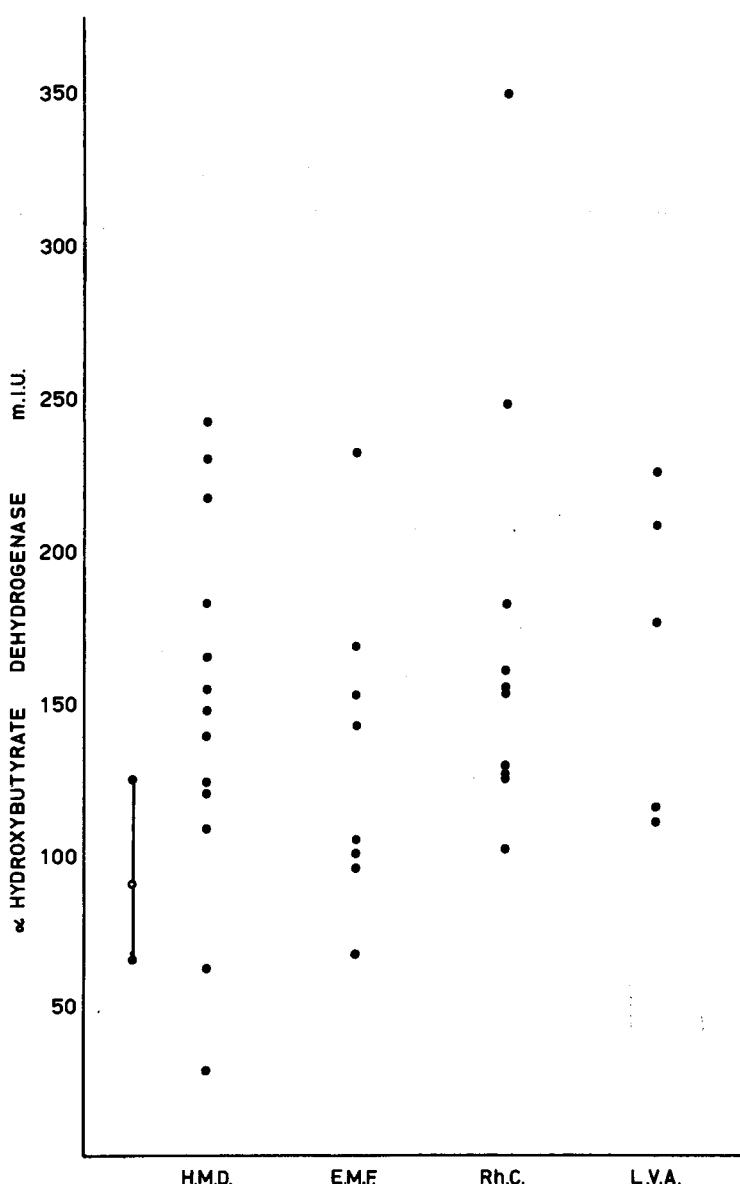


FIG. 9. Serum  $\alpha$ -hydroxybutyrate dehydrogenase activity in normal Nigerians and in the four disease groups.

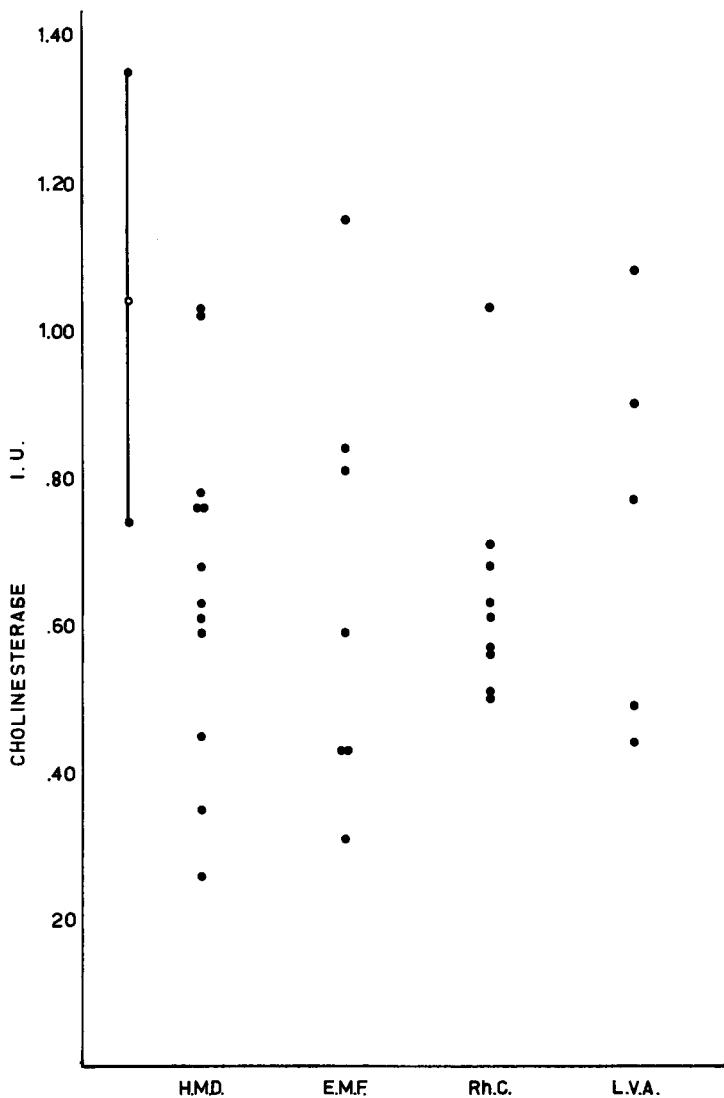


FIG. 10. Serum cholinesterase activity in normal Nigerians and in the four disease groups.

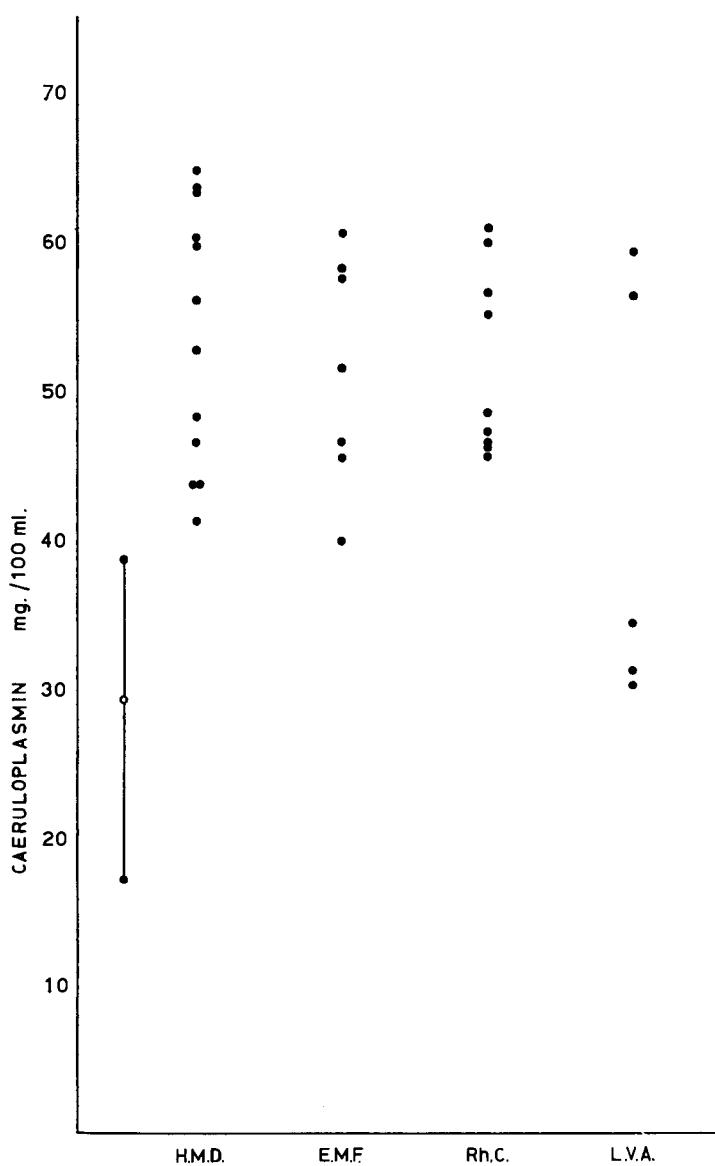


FIG. 11. Serum caeruloplasmin levels in normal Nigerians and in the four disease groups.

caeruloplasmin activity. The only exception was a woman with heart failure due to thyrotoxicosis.

The other observation which seemed fairly clear was depression of serum cholinesterase activity in most African patients with heart failure and liver congestion. This applied to all four cardiac diseases which were studied. The combination of increased serum caeruloplasmin and depression of serum cholinesterase activity was very typical of African patients with heart failure and liver congestion. On the other hand, measurement of serum I.C.D. and L.A.P. levels were not helpful in the assessment of the severity of heart failure in the African.

The standard liver function tests, including serum bilirubin, thymol turbidity and flocculation, and alkaline phosphatase, showed no difference between the four disease groups. As anticipated, the blood cholesterol level was low in normal controls and also in all the patients with heart disease. The changes in serum proteins were unhelpful, patients with heart failure tended to have lower serum albumin levels and rather higher  $\gamma$ -globulin values compared to normal African controls.

## DISCUSSION

Heart muscle disease is the commonest cause of heart failure in Ibadan. During the acute stage of this congestive cardiomyopathy it was expected there might well be a leakage of enzymes from damaged myocardial cells. A slight increase in S.G.O.T. activity occurred in only four cases, but a greater proportion, i.e. eight out of thirteen patients, had increased S.H.B.D. levels.

The levels of these enzymes were elevated in some patients with endomyocardial fibrosis (E.M.F.) and this is of interest since all cases had established disease with endocardial fibrosis in the ventricles. The enzyme results, however, could indicate continuing myocardial damage, and lend some support to the concept of 'active disease' in E.M.F. (Parry and Abrahams, 1965).

Normal levels of S.G.O.T. have been found in cases of established E.M.F. (Campbell and Somers, 1960) and in early E.M.F. (Parry and Abrahams, 1965). Our results for S.G.O.T. activity in E.M.F. are more abnormal. This discrepancy is probably due to the fact that the upper limit of normal for S.G.O.T. activity in the normal Nigerian was relatively lower than that indicated by Campbell and Somers. It was also

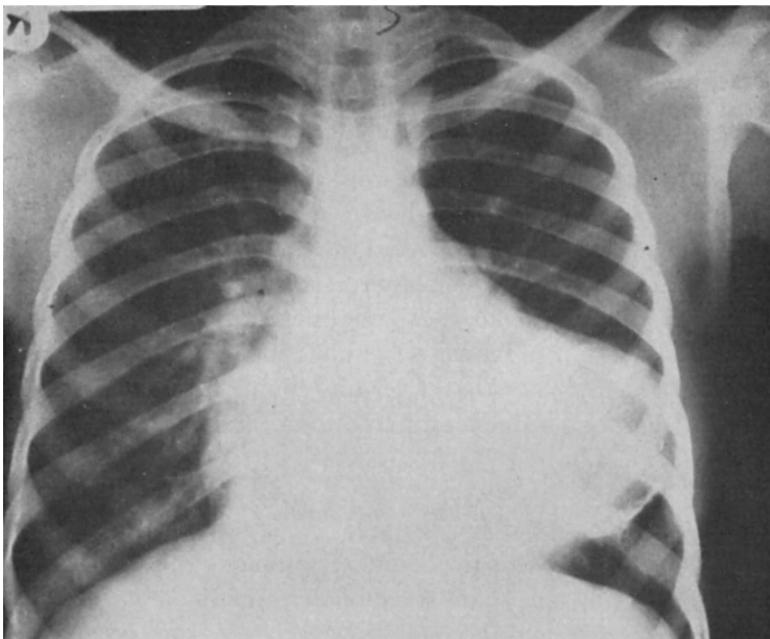


FIG. 12. Radiograph showing a calcified left ventricular aneurysm.  
By courtesy of Professor W. P. Cockshott, Department of Radiology,  
University College Hospital, Ibadan.

noted that the presence of either left- or predominantly right-sided E.M.F. made no appreciable difference to the enzyme pattern. Furthermore, normal S.G.P.T. activity, in spite of liver congestion, was an unexpected finding both in E.M.F. and in the other cardiac groups studied.

Elevation of serum G.O.T. and L.D.H. may occur in active rheumatic carditis (Megahed and Yassin, 1965), and Fig. 9 shows elevated S.H.B.D. values in six out of ten cases. Megahed

and Yassin also found that when the carditis resolved the serum enzymes returned to normal. Furthermore, the other parameters of activity in rheumatic carditis, e.g. E.S.R., fever and pulse rate returned to normal before the S.G.O.T. and L.D.H. had declined to normal levels. It was also noted that of the African patients studied the two cases with chronic rheumatic heart disease had only slightly raised S.G.O.T. and S.H.B.D. activity.

The raised levels of S.H.B.D. in three cases of L.V.A. could reflect the myocardial damage which occurs during the formation of the aneurysm. Acute stretching of a coronary artery (circumflex branch) over the aneurysm, could also result in myocardial ischaemia and increased S.H.B.D. activity. Alternatively, the raised S.H.B.D. levels may be the result of breakdown of blood clot within the multilocular aneurysm. It is also interesting to note that the two patients with normal S.H.B.D. levels were not in heart failure. Elevation of S.H.B.D. (and L.D.H.) may occur in megaloblastic anaemia due to  $B_{12}$  or folic acid deficiency but there was no evidence of this in the cases studied.

## SUMMARY

Heart muscle disease, rheumatic heart disease, endomyocardial fibrosis and left ventricular aneurysm may all present with left ventricular failure and mitral incompetence. It was hoped that serum enzyme studies might contribute to the differentiation of these disorders. Serum enzyme estimations were interesting but proved to be of no great diagnostic value. In future studies of tropical heart diseases, the estimation of serum creatine kinase (C.P.K.) activity could well prove to be a more reliable test of heart damage. In particular, C.P.K. activity should not be affected by liver congestion. It would also be of interest to perform serial enzyme estimations before and after diuretic therapy.

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## CHAPTER V

## Imported tropical disease, amoebiasis and other liver disorders

Imported tropical disease has become a significant problem (*Brit. med. J.*, 1967), and there have been a number of papers indicating the importance in this country of such diseases as malaria (Jackson and Woodruff, 1962; Seaton, 1967), trypanosomiasis, (Willett, 1965), amoebiasis (Mullan and Williams, 1965; Wright, 1966; Allen, 1966; Weaver, 1967), leprosy (Jopling, 1963), hookworm infection (Salem and Truelove, 1964) and helminth infestations in immigrant children (Archer, Bamford and Lees, 1965). Schistosomiasis is not uncommon in Adenese immigrants in Sheffield; these patients have splenomegaly with portal hypertension and may present with bleeding from oesophageal varices. Yaws has also been described in this city (Daly and Morton, 1963), and two cases of 'Bejel' have been reported (Wray, 1966). Though not strictly a tropical disease, active pulmonary tuberculosis remains common in immigrants from Pakistan; alternatively, these patients may present with florid glandular tuberculosis.

The admission of a patient with an acute tropical disease certainly does much to enliven medical interest in a ward which may be otherwise largely geriatric. A number of tropical conditions have recently been observed in this city, and they have included patients suffering from malaria, loa loa, amoebiasis and schistosomiasis. One death from cerebral malaria has been recorded. Acute abdominal pain occurred in one patient with a sickle cell crisis, and in another case severe biliary colic was due to a fluke within the common bile duct.

The environmental health and infectious diseases of immigrants in Sheffield has recently been reviewed (Parry, 1966). The total coloured population in the United Kingdom in 1965 was about 800,000, and in this city of nearly half a million inhabitants, there are 8000 coloured immigrants. Clearly some knowledge of the diseases likely to occur in immigrants is essential, especially when it is realized that the combined population in this country of Indians, Pakistanis and West



FIG. 13. The abscess cavity from below, demonstrating its rough shaggy wall.

Indians is likely to reach 3½ million by the year 2000. In addition, rapid air travel in a shrinking world will continue to make the importation of infectious diseases a real hazard.

### **Amoebiasis**

'La dysenterie amibienne est une maladie grave, qui présente, dans les pays chauds, une mortalité élevée. Tant que les

Amibes ou leurs kystes persistent dans les selles, le pronostic doit être réservé, car le *malade est toujours sous la menace d'une rechute* ou d'une des complications dont nous avons parlé plus haut.\*

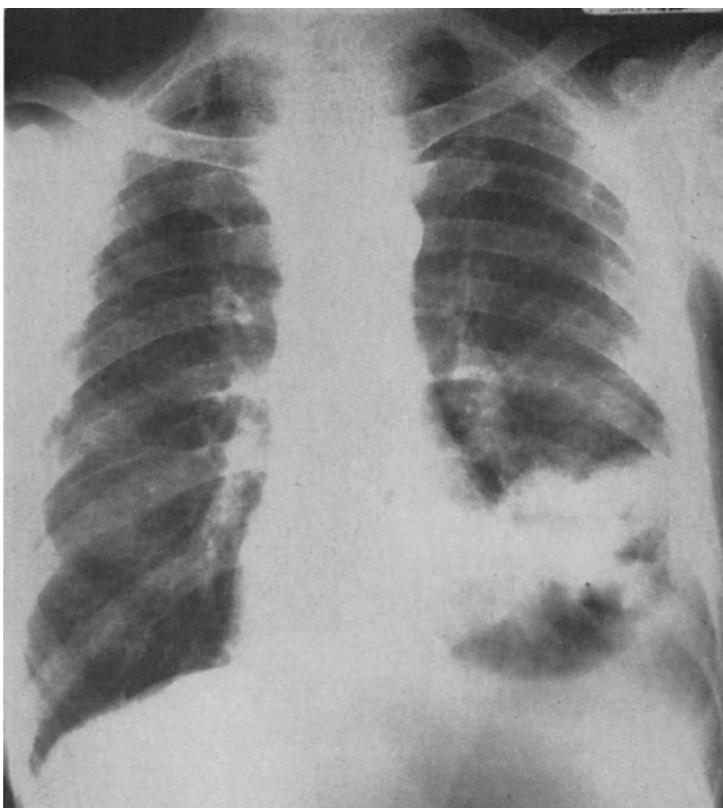


FIG. 14. Posterior anterior radiograph showing a gas filled cavity

in the left lower zone. Amoebic abscess of the left lobe of the liver.

By courtesy of Dr. T. Lodge, Department of Radiology, Royal Hospital, Sheffield, and the British Medical Journal.

Research into this subject was stimulated by the death at the Royal Hospital, Sheffield, of a man aged 73. He originally contracted amoebic hepatitis 48 years previously in Mesopotamia. Autopsy revealed an amoebic abscess of the left lobe of

\* Brumpt, E. (1922). *Précis de parasitologie*, première section, p. 119, Troisième Edition. Paris. Masson et Cie, Éditeurs.

the liver which had ruptured through the left dome of the diaphragm causing pulmonary cavitation and pericarditis (Mullan and Williams, 1965). Actively motile amoebae,

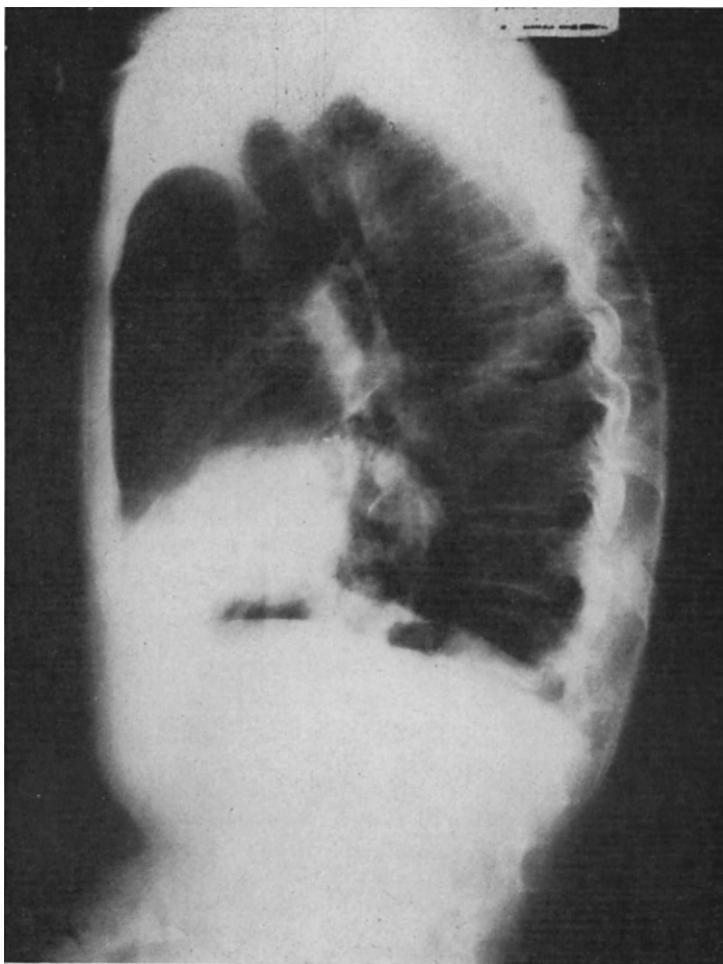


FIG. 15. Corresponding left lateral chest radiograph.  
By courtesy of Dr. T. Lodge.

containing red cells, were visible in a wet film which was taken from the pulmonary cavity. Figs. 14 and 15 show posterior-anterior and left lateral X-ray films of this patient taken just

prior to death, and Fig. 13 is a photograph of the abscess cavity from below, demonstrating its rough shaggy wall.

Following bronchoscopy for suspected bronchial carcinoma this patient developed prolonged scoline apnoea, which was shown to be due to a marked deficiency of serum cholinesterase. This enzyme is produced by the liver and it normally rapidly destroys injected suxamethonium (scoline). Low serum cholinesterase levels may be genetically determined (Harris *et al.*, 1960). Low levels of this enzyme have also been found in chronic liver disease (Lehmann and Ryan, 1956) and in bronchial carcinoma with myasthenia (Anderson, Churchill-Davidson and Richardson, 1953). Furthermore, in an interesting paper, Magill and Killough (1958), in a study of 101 patients with amoebiasis showed that the plasma cholinesterase was the most useful test in indicating liver involvement. Four of their cases with proven amoebic liver abscess had very low levels of cholinesterase.

### Cases of amoebiasis studied

Table 5 gives details of five patients with amoebic liver disease. They were all male and their ages ranged from 13 to 59. The predominance of hepatic amoebiasis in males compared to females is well documented (DeBakey and Ochshner, 1951). One patient (case 45) had a laparotomy for suspected appendicitis. The diagnosis of amoebic typhlitis in this case was confirmed by finding actively motile forms of *Entamoeba histolytica* in material aspirated from the caecal lumen. In addition, this patient had a small amoebic liver abscess. In two other cases the diagnosis of amoebiasis was confirmed by aspirating large amounts of 'anchovy sauce' from the liver. The remaining two patients (cases 41 and 42) responded very well to specific anti amoebic therapy.

### Liver function and enzyme changes in hepatic amoebiasis

In five patients with hepatic amoebiasis (Table 5) the serum bilirubin was less than 0.6 mg./100 ml. Jaundice is unusual

TABLE 5. *Amoebiasis*

Case No.	Age	Sex	Diagnosis duration (N.K.=not known)	Electrophoresis												
				Serum bilirubin mg./100 ml.	Direct van deen Bergh bilirubin	Tyrosol trituration macrogan units	Tyrosol trituration floculation units	Serum alkaline phosphatase Ring units	Serum pseudocholinesterase Normal 30-90 units/ml.	Aspartate aminotransferase S.G.O.T. Ring units	Globulin S.G.P.T. Ring units	Serum L.A.P. Normal < 200 units	Serum albumin g./100 ml.	Serum globulin g./100 ml.	Electrophoresis Increased $\alpha_2$ and $\gamma$	
41	13	M	Amoebic liver abscess N.K.	-ve	0.1	5	+	18	55	80	55	95	185	3.5	3.3	
42	25	M	Amoebic liver abscess	$\frac{4}{5}^{\text{v}}$	-ve	0.3	3	-ve	8	75	80	145	200	203	3.6	
43	18	M	Amoebic liver abscess N.K. (Anchovy sauce (Spec. 1) aspirated) (Spec. 2)	-ve	0.4	10	++	28	11.5	80	100	95	306	2.3	3.4	
44	59	M	Amoebic liver abscess (2.2 litres 'anchovy sauce' aspirated) (Spec. 1)	-ve	0.4	1	-ve	19	14	87	135	40	221	2.4	4.1	
				+ve	0.4	2	-ve	16	24	88	75	15	230	2.2	4.0	
				(Spec. 2)												
45	22	M	Amoebic typhlitis and small liver abscess		$\frac{2}{1}^{\text{v}}$	-ve	0.5	1	-ve	6	52	84	85	75	126	4.7
															1.3	
															Normal	

By courtesy of the *Journal of Clinical Pathology*.

in amoebic liver disease; in an analysis of 263 cases 12.9 per cent were jaundiced (DeBakey and Ochshner, 1951). Lamont and Pooler (1958) found the serum bilirubin to be over 0.8 mg./100 ml. in nineteen out of 187 cases, and the prognosis was poor in those patients with marked jaundice.

As can be seen, there was some increase in the serum alkaline phosphatase activity in three cases, and the serum leucine aminopeptidase (L.A.P.) levels were raised in three patients. In one case both the S.G.O.T. and S.G.P.T. were elevated. There was very marked depression of serum cholinesterase activity in two patients (cases 43 and 44) who had large amoebic liver abscesses, and in both these cases the serum albumin levels were low, i.e. 2.3 and 2.4 g./100 ml. On recovery the serum cholinesterase levels tended to return to normal (Tables 5 and 6). The fact that a patient (case 45) with a single small amoebic liver abscess of only 30 c.c. had some depression of his serum cholinesterase was of interest, and this is in agreement with Magill and Killough (1958), who stated that depression of serum cholinesterase activity was a sensitive index of hepatic involvement in amoebiasis. Serum protein electrophoresis was of no great diagnostic value in these five cases; a variable increase in the  $\gamma$ -globulin fractions was noted and similar changes have been observed by other workers (Santhanagopalan *et al.*, 1964).

Previous authors (Sodeman and Lewis, 1945) have indicated that data on the liver function tests in patients with hepatic amoebiasis is both fragmentary and inadequate, and Zavala and Hamilton (1952) state that even with an advanced liver abscess abnormalities of the liver function tests may be slight.

Brem (1955) found that the serum alkaline phosphatase was raised in five out of eight cases with amoebic liver abscess, and increased bromsulphthalein retention occurred in five patients.

More recently, Viranuvatti *et al.* (1963) studied 274 cases of hepatic amoebiasis in Thailand. These authors found that a positive iodine test, a raised alkaline phosphatase level, and increased bromsulphthalein retention were the most sensitive tests in patients with amoebic liver abscess. The alkaline

phosphatase level was in fact elevated in sixty-two out of a total of seventy-five cases of amoebic liver disease (83 per cent). On the other hand, the S.G.O.T. level was raised in ten out of twenty-four patients (41 per cent) and the S.G.P.T. was elevated in only four cases. The serum amylase was increased in three out of twenty-eight patients with hepatic amoebiasis.

### Differential diagnosis

In the tropics, amoebic liver abscess has to be differentiated from a primary malignant hepatoma. Both conditions may give rise to painful enlargement of the liver. Alternatively, the patient may have hepatomegaly due to some other cause, associated with a basal pneumonia and some elevation of the diaphragm. In this country, the diagnosis of amoebic liver disease may not even be considered and a patient may be wrongly diagnosed as having malignant liver deposits or pyogenic liver abscesses. Furthermore, it is well known that patients with amoebic liver disease often have no history of preceding colitis. It is in this type of case that additional evidence, such as a raised antibody titre against *Entamoeba histolytica* (E.H.), would be of great diagnostic value (see below).

### Amoebic antibody studies\*

Table 6 shows the E.H. antibody titres in normal individuals, patients with a variety of liver diseases and in four cases of amoebic liver abscess. Amoebic antibody levels were determined using an indirect fluorescent antibody technique (Jeanes, 1966). The latter author has found that E.H. antibody titres of 1:64 or above are very suggestive of amoebic infection.

As can be seen, there was a significant elevation of E.H. antibody titres, ranging from 1:128 to 1:512 in all the patients with hepatic amoebiasis. One patient (case 31) had cysts of *Entamoeba histolytica* in the stools but the E.H. antibody was not elevated. In fact this patient failed to respond to a course of emetine and the deterioration in her liver function tests made

\* Kindly carried out by Dr. A. Jeanes in the department of Clinical Pathology, Guy's Hospital.

TABLE 6. *Indirect fluorescent antibody titres against Entamoeba histolytica*

A. NORMALS							C. AMOEBIASIS						
Case No.	Age	Sex	Antibody titre	Case No.	Age	Sex	Diagnosis	Antibody titre	Pseudocholinesterase (units/ml.)	Antibody titre	Antibody titre	Antibody titre	
1	20	M	<1:8										
5	19	M	1:16	41	13	M	Amoebic liver abscess						5
19	18	M	<1:8	43	18	M	Amoebic liver abscess						
20	20	M	<1:8				Before treatment						1:512
22	19	M	<1:8				After treatment						1:16
23	19	M	<1:8	44	59	M	Amoebic liver abscess						52
—	20	F	<1:8 (Normal L.F.T.s)				Before treatment						1:256
				45	22	M	Amoebic typhilitis and small liver abscess (30 cc.)						14
B. ABNORMALS							Tender over liver and in R.I.F.						24
26	13	M	<1:8	<i>Infective hepatitis</i>									
30	38	M	<1:8	<i>Infective hepatitis</i>									
33	10	M	<1:32	<i>Cirrhosis</i> / <i>Schistosomiasis</i>									
34	35	M	<1:8	<i>Cirrhosis</i> / <i>Schistosomiasis</i>							1:10.65		52
36	50	F	<1:8	<i>Cirrhosis</i>							16.10.65		61
37	10	M	<1:8	<i>Kala azar</i>							18.10.65	Laparotomy	
38	12	M	<1:8	<i>Kala azar</i>							20.10.65		
40	27	M	<1:8	<i>Filariais (Loa loa)</i>							30.10.65		
31	Adult	F	<1:8	? <i>Infective hepatitis</i>							12.11.65	E.H. on caecal aspiration	88

(Case numbers as in Tables 5, 7 and 8)

By courtesy of the *Journal of Clinical Pathology*.

the diagnosis of severe hepatitis very likely. The other point of interest was that after specific therapy and aspiration of the liver abscesses (cases 43 and 44) there was a striking fall in the E.H. antibody levels. The indirect fluorescent antibody test may therefore be of value in diagnosing a recurrence of amoebic disease.

Another not uncommon clinical problem is the patient with ulcerative colitis who also passes amoebic cysts in the stools. Such a case, who failed to respond to a therapeutic trial of emetine, was recently seen in Sheffield. The E.H. antibody titre was not elevated and the subsequent course in this man was typical of ulcerative colitis. On the other hand, patients may be mistakenly diagnosed as having ulcerative colitis (Paulley, 1961). Wright (1965) recently described a patient who had never been to the tropics, and who died following a total colectomy. Severe amoebic colitis was later confirmed by the pathologist.

To conclude, some of the important clinical features of amoebiasis have here been deliberately stressed. Enzyme changes, in particular depression of the serum cholinesterase, will indicate liver involvement. If there is also a significant elevation of the E.H. antibody titre then a 'postal diagnosis' of hepatic amoebiasis can be made.

#### **Enzyme studies in East Africans**

Frozen serum was rapidly transported by air from Nairobi to the United Kingdom. These serum specimens were taken both from normal African blood donors and from other patients with a variety of tropical and liver diseases. A number of enzyme and liver function tests were then carried out (Mullan *et al.*, 1967).

Table 7 gives the results in twenty-four young healthy males. As can be seen, all the enzyme tests were normal except for one patient who had the highest alkaline phosphatase (A.P.) and leucine aminopeptidase (L.A.P.) levels. These normal male Africans were all well nourished and, as expected, the mean serum albumin was satisfactory at 4.2 g./100 ml. In

ten cases the  $\gamma$  globulin was elevated. It has been stated that chronic infections in the African cause a raised  $\gamma$  globulin. However, elevation of the  $\gamma$  globulin in the normal Negro may in fact be racially determined (Edozien, 1957; Siegel *et al.*, 1965).

Patients with various liver and tropical diseases have been studied. Table 8 includes six patients with *infective hepatitis*.

TABLE 7. *Normals*

Cases 1-24 All male Ages 17-23 years	Range	Mean	S.D.
Direct van den Bergh	all -ve		
Serum bilirubin	0.1-0.9*	0.4	$\pm$ .18
	normal < 1.0 mgm/100 ml.		
Thymol turbidity	1-5		
	normal 0-4 Maclagan units		
Thymol flocculation	(24 -ve ( 1 +ve		
Serum alkaline phosphatase	5-20*	11	$\pm$ 4.0
	normal 3-13 King units		
Serum Pseudocholinesterase	44-82	60	$\pm$ 11
	normal 30-90 units/ml.		
Dibucaine number	74-90		
Aspartate aminotransferase (S.G.O.T.)	20-100	45	$\pm$ 22.5
	normal 29-116 King units		
Alanine aminotransferase (S.G.P.T.)	15-95	56	$\pm$ 25
	normal 26-93 King units		
Leucine aminopeptidase (L.A.P.)	117-288*	156	$\pm$ 37
	normal 84-200 units		
Serum albumin g./100 ml.	3.8-4.8	4.2	$\pm$ .28
Serum globulin g./100 ml.	1.5-3.9	2.8	$\pm$ .50

\* Case 7 had the highest serum bilirubin, alkaline phosphatase, and leucine aminopeptidase.

By courtesy of the *Journal of Clinical Pathology*.

These Africans were all jaundiced and the typical biochemical changes included marked elevation of the transaminases, some depression of the serum cholinesterase and abnormal thymol turbidities. The serum albumin was normal in these cases but the  $\alpha_1$  and  $\alpha_2$  globulins were depressed (Sunderman and Sunderman, 1957). Because of poor sanitation, infective

hepatitis remains extremely common in Africa; some of the more severe cases can in fact present in coma, but the prognosis in these patients seems to be good. Other causes of jaundice, such as malaria, must be excluded. Serum transaminase determinations are most useful in following the course of infective hepatitis, and in detecting anicteric cases.

Primary *malignant hepatoma* is a very common type of malignancy in Africa (*Lancet*, 1967), the patient (case 32) with a biopsy proven tumour had interesting enzyme changes: the serum alkaline phosphatase and leucine aminopeptidase levels were both elevated, but the serum cholinesterase activity was reduced. Serum aldolase activity and transaminases levels were normal in this case. When the tumour starts infiltrating the liver, the level of alkaline phosphatase will increase still further. In this connection, it has been noted that the level of alkaline phosphatase is usually higher in patients with malignant hepatoma than in those with amoebic liver abscess (Powell, 1959) and, in fact, in the five cases with amoebic liver abscess (cases 41 to 45) the highest serum alkaline phosphatase was only 28 King units.

As can be seen the patients with *cirrhosis* and ascites (cases 35 and 36) had very low levels of serum albumin, increased  $\gamma$  globulin, and reduced serum cholinesterase activity. Serum albumin is synthesized in the liver (Madden and Whipple, 1940; Whipple, 1942) and in the presence of liver disease the synthesis of cholinesterase will also be impaired (Farber, 1943). It has been confirmed that the capacity to regenerate cholinesterase after an injection of di-isopropylfluorophosphate (D.F.P.) is significantly less in patients with liver disease as compared with normal individuals (Kunkel and Ward, 1947; Vorhaus, Skudamore and Kark, 1950).

The normal levels of alkaline phosphatase in the patients with cirrhosis (cases 33 to 36) are of interest. Considerable increase in serum alkaline phosphatase activity may occasionally occur in patients with alcoholic cirrhosis, but the level of this enzyme is more likely to be increased if the cirrhotic patient is also jaundiced (Popper and Schaffner, 1957).

TABLE 8. *Abnormals*

Case No.	Age	Sex	Diagnosis duration	Spec. 1	Spec. 2	dein Bergh direct van den Bergh	Serum bilirubin mg./100 ml.	Thermal turbidity MacCollagran units	Thrombocytopenia	Serum alkalin phosphatase Klüg units	Serum pseudocholinesterase Normal 30-90 units/ml.	Serum G.O.T. Klüg units	Aspartate aminotransferase S.G.O.T. Klüg units	Serum L.A.P. Klüg units	Serum normal 200 units	Serum albumin g./100 ml.	Serum globulin g./100 ml.	Electrophoresis
25-30	13-38	5M 1F	Infective hepatitis (N.K. = not known)	+ or 2-7 ++ 11.1	5- 16 + +	2 -ve 10 + +	10 68	83	30	75	180	4-3	2.7	Increased $\alpha_2$				
31	Adult	F	? Infective hepatitis N.K. (Spec. 1) (Spec. 2)	-ve ++ 6.0	2 -ve 17 57	10 68	83	30	435	297	3-7	4-3	Increased $\gamma$					
32*	Adult	F	Hepatoma	-ve ++ 6.0	3 -ve 8 +ve	52 20	78	65	20	302	3-0	3-9	Increased $\alpha_2$ and $\gamma$					
33	10	M	Cirrhosis proven $\frac{6}{12}$	-ve 0.6	8 +ve 9	30 84	75	80	72	2.8	3-3	Increased $\gamma$						
34	35	M	Cirrhosis schistosomiasis N.K.	-ve 1.0	1 -ve 9	52 67	67	55	95	180	5-0	2-0	Normal					
35	Adult	F	Cirrhosis asities N.K.	-ve 1.2	12 + +	11 22	81	75	50	185	2.1	3.6	Increased $\gamma$					
36	50	F	(2 cases)	-ve 0.3	14 + +	13 27	85	100	70	176	2.2	3-7	Increased $\gamma$					
37	10	M	Kala azar N.K.	-ve 0.6	8 +	20 56	90	120	70	135	3-3	3-8	Intense increase $\gamma_2$					
38	12	M	(3 cases)	-ve 0.4	18 + +	7 38	71	190	125	86	2.9	6.1	Intense increase $\gamma_2$					
39	10	F		-ve 0.4	20 + +	36 54	78	85	160	203	3-1	5-4	Intense increase $\gamma_2$					
40	27	M	Filariasis N.K.	-ve 0.2	1 -ve	7 Not done	40	30	Not done	40	4-9	2.0	Normal					done

\* serum aldolase = 14 units/ml./hr.

The enzyme tests in three patients with *kala azar* showed some increase in the transaminases. The alkaline phosphatase levels were 20 and 36 King units in two patients who were both aged 10. Increased osteoblastic activity in growing children will normally give higher levels of serum alkaline phosphatase. This view is confirmed by finding normal L.A.P. values in these two cases. As expected, the Sia test was positive in all three patients, and serum electrophoresis revealed an intense increase in the  $\gamma_2$  globulin.

Other authors have studied serum enzyme changes in protein malnutrition. In *kwashiorkor* there is fatty change in the liver and atrophy of acinar cells in the pancreas (Sherlock, 1963). The serum albumin level is very low, and the synthesis of certain enzymes by the liver is also impaired. Thus, in kwashiorkor, the serum alkaline phosphatase is low (Schwartz, 1956) and the activity of serum cholinesterase and amylase is reduced. In an interesting paper Baron (1960) demonstrated an increase in the serum transaminase and isocitric dehydrogenase levels in patients with kwashiorkor. Baron suggested that the alteration in serum enzymes in protein malnutrition was due to release of these enzymes from liver cells. He also showed that enzyme activity returned to normal when kwashiorkor had been treated.

## SUMMARY

In this chapter the importance of imported tropical disease has been stressed. In particular a fairly detailed account is given of the liver function and antibody tests which proved useful in confirming the diagnosis of hepatic amoebiasis. Serum enzyme tests were also carried out in other tropical and liver disease, and the changes in liver function were typical of the conditions studied. The rapid transport by air of frozen serum from Africa to the United Kingdom should make diagnosis at 'long range' relatively simple.

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## CHAPTER VI

## Enzyme studies in pregnancy

## INTRODUCTION

In this chapter enzyme changes in pregnancy will be reviewed. Particular attention will be paid to those serum enzymes which increase during pregnancy, i.e. oxytocinase (cystine aminopeptidase, C.A.P.), Leucine aminopeptidase (L.A.P.) and alkaline phosphatase (A.P.). The placental origin of certain enzymes will also be discussed.

The use of enzyme tests in the diagnosis of certain complications of pregnancy will be considered and, where possible, those enzyme changes which may be a practical guide in the management of clinical problems will be stressed; for example, Babuna and Yenen (1966a) suggested that decreasing serum levels of oxytocinase in toxæmic patients indicated placental insufficiency and impending foetal death. Provided foetal size was adequate, the obstetrician could then decide to terminate pregnancy.

It has been found that low levels of serum oxytocinase (C.A.P.) occur in cases of placental insufficiency (Mullan, 1967—see Fig. 21). Patients with impaired placental function excrete reduced amounts of pregnanediol in their urine (Russell, Dewhurst and Blakey, 1960). To avoid 24-hour urine collections, it may be more convenient to estimate rapidly serum C.A.P. activity in patients with suspected placental insufficiency.

Using a rapid method for serum oxytocinase (C.A.P.) estimation (Babuna and Yenen, 1966a), it has also been possible to confirm low levels of this enzyme in patients with missed abortion. For example, a woman who was 22 weeks

pregnant by her dates, had a uterine bleed 1 month previously. A pregnancy test on her urine was negative, and her serum C.A.P. level was in the non-pregnant range. She subsequently aborted spontaneously; the placenta was infarcted and the pathologist concluded that the foetus was approximately 13 weeks old by size.

The placenta is a crucible of chemical activity, and infarction could well cause acute elevation of certain serum enzymes. Thus, Dawkins *et al.* (1959) suggested that, in the absence of liver disease, an increase in serum isocitrate dehydrogenase (I.C.D.) indicated active placental degeneration. Serum I.C.D. levels rose in some patients with pre-eclamptic toxæmia and this could indicate diminishing placental function.

In the course of this chapter the changes in serum proteins during pregnancy will be discussed. In addition, there is a section dealing with ovulation and the menstrual cycle.

Mention will be made of the use of liver function tests in the diagnosis of jaundice complicating pregnancy, and also the alteration in hepatic function that can occur in women taking the contraceptive pill.

Toxæmia of pregnancy remains a 'disease of theories' (Jeffcoate, 1966) and, as this author noted, it is significant that one of the shields on the walls of the Chicago Lying-in Hospital remains blank—waiting for the day when the name of the discoverer of the cause of eclampsia is known. Later in this chapter there is a fairly detailed section dealing with some current views on the aetiology of pre-eclamptic toxæmia. The possible role of placental monoamine oxidase (M.A.O.) in the aetiology of hypertension is discussed. In addition, changes in serum oxytocinase (C.A.P.), S.G.O.T., S.G.P.T., and the urinary leak of aminopeptidases in toxæmia of pregnancy will be described.

### **Changes in serum proteins during pregnancy**

Enzyme changes in pregnant patients cannot be considered in isolation. The altered hormonal milieu of pregnancy is associated with other important changes in serum proteins

such as the appearance of a new  $\alpha_2$  globulin (Smithies, 1955), an increase in the serum caeruloplasmin (Sheinberg *et al.*, 1954), an elevation of thyroxine-binding globulin (Dowling *et al.*, 1956) and protein-bound iodine, and also an increase in transcortin (Slaunwhite *et al.*, 1959).

Using starch gel electrophoresis Alfonso and de Alvarez (1964) found that the  $\alpha_2$  globulin of pregnancy did not appear before 9 weeks gestation but was present in 80 per cent of patients in the last trimester of pregnancy. They showed that this new globulin was non-foetal in origin and disappeared after delivery. Furthermore the same  $\alpha_2$  globulin appeared in the serum of non-pregnant women treated with Enovid.

Robinson *et al.* (1966b), confirmed that the  $\alpha_2$  globulin was not specific for pregnancy but was also present in patients receiving Enovid and in other women with trophoblastic disease. The specific enzymes of normal pregnancy included the pregnancy associated phosphatase and two cystine aminopeptidase bands (C.A.P.<sub>1</sub> and C.A.P.<sub>2</sub>); these components were not present in patients on the 'pill' or others with active trophoblastic disease. The serum caeruloplasmin is also elevated in women taking the contraceptive pill (Carruthers *et al.*, 1966).

### Ovulation and the menstrual cycle

Much more information is needed on the enzyme changes during the normal menstrual cycle. Some enzymes may be hormone dependent; thus, using a biological method, Page in 1947, showed that the plasma pitocinase (oxytocinase) fluctuated during the normal menstrual cycle and was higher at the time of ovulation. There is scanty information in the literature on this important subject. However, it has been noted that in some patients there may be an acute elevation of serum isocitrate dehydrogenase (I.C.D.), phosphohexose isomerase (P.H.I.), and lactate dehydrogenase (L.D.H.) activity at the time of ovulation (Dr. Grainger Muir—personal communication, 1966). It would be interesting to see if suppression of ovulation with the contraceptive pill altered these enzyme changes.

Of interest were the changes of the endometrial isozymes of lactic dehydrogenase, during the normal menstrual cycle, that have been reported by Roddick *et al.* (1966). In the early oestrogen-stimulated proliferative phase of the cycle the main endometrial iso-enzymes were L.D.H.<sub>1, 2</sub> and <sub>3</sub> while in the late secretory phase, in pregnancy, and in women on progesterone, there was a shift in the L.D.H. iso-enzyme pattern so that the predominant fractions were L.D.H.<sub>3, 4</sub> and <sub>5</sub>. The above study emphasizes the important relationship between enzyme and hormonal changes within the body. Likewise,  $\beta$ -glucuronidase in uterine endometrium varies during the menstrual cycle (Odell and Fishman, 1950), and Callard and DeMerre (1966) demonstrated that large amounts of this enzyme were lost in menstrual blood.

Certain other chemical changes occur during the normal menstrual cycle (Hartman, 1965). Thus Shorr *et al.* (1942), showed that the lowest urinary levels of citric acid occurred during menstruation, while the highest levels were present at about the middle of the cycle. In some of the patients studied there was a brief acute fall in urinary citric acid excretion at the time of ovulation. Edwards *et al.* (1950) recorded similar citric acid cycles but they found no correlation between urinary citric acid and oestrone-oestradiol levels in the urine.

Recently a whole book has been devoted to the endocrine aspects of ovulation\*. Women with ovulatory menstrual cycles have a mid-cycle increase in urinary luteinising hormone (L.H.) activity but this increase in L.H. excretion is suppressed when the patient takes oral contraceptives (Brown *et al.*, 1964; Stevens *et al.*, 1965; Taymor, 1964; Bermes *et al.*, 1966). Using a new sensitive method for radio-immuno-assay of plasma L.H. in man, Ross *et al.* (1966) observed a sharp peak in plasma L.H. activity near the mid-cycle in each subject. No mid-cycle rise in plasma L.H. occurred when the patient was taking the contraceptive pill.

These hormone studies are clearly important but are too

\* *Human Ovulation* (1965). Edited by Chester S. Keefer, M.D. J. & A. Churchill Ltd., London.

complicated for every day use. Neither is it always possible to rely on the acute temperature shift at the time of ovulation; thus Bermes *et al.* (1966), in a study of 105 temperature charts, showed that in seventeen cases there was no significant temperature shift at mid-cycle. The usual teaching is that the post-ovulatory phase is fairly constant at 14 days. However, the last mentioned authors found that this phase varied considerably from 6 to 19 days. What is urgently required is a simple chemical test, preferably of the urine, which will reliably signal ovulation. This would be of great help to those wishing to avoid further pregnancies.

### Serum enzymes in pregnancy

Broadly speaking serum enzyme changes in pregnancy can be classified as follows:

1. *No alteration* in serum enzyme activity during pregnancy: e.g. serum amylase (Burt and McAlister, 1966). These authors found the activity of this enzyme in cord blood was 20 per cent of the maternal serum values.
2. *Decreasing* serum enzyme activity. Various authors (Wetstone *et al.*, 1958; Pritchard, 1955; Friedman and Lapan, 1961) found decreasing levels of serum cholinesterase throughout pregnancy and this could in part be explained by haemodilution. On the other hand, Meade and Rosalki (1965) showed that the cholinesterase values remained within the normal range throughout pregnancy, labour and the post-partum period. Cord blood activity of this enzyme was also normal. However, other authors (Lehmann *et al.*, 1957) found subnormal activity of cholinesterase in many samples of cord blood tested. Shnider (1965) noted that 60 per cent of his patients had abnormally low cholinesterase levels 3 days post partum. He also described a patient who, after Caesarean section, developed scoline apnoea due to deficiency of this enzyme. A similar complication has been reported by Bingham (1957).

In an interesting paper, Fleisher *et al.* (1965) showed that the hypermetabolic states of both pregnancy and thyrotoxicosis, were associated with reduced levels of serum C.P.K. activity. In addition, the level of this enzyme fell with advancing pregnancy.

3. *Enzyme activity normal throughout pregnancy but increased during labour.* Serum transaminases (S.G.O.T., S.G.P.T.) isocitrate dehydrogenase (I.C.D.), lactate dehydrogenase (L.D.H.) and  $\alpha$ -hydroxy-butyrate dehydrogenase (S.H.B.D.) behaved in this way (Meade and Rosalki, 1963). Many authors agree that transaminase and L.D.H. values are normal during uncomplicated pregnancy (Brody, 1957; West and Zimmerman, 1958; Crisp *et al.*, 1959; Horwitz and Rossano, 1966).
4. *Other enzyme changes in cord blood.* Higher values for I.C.D., L.D.H. and S.H.B.D., were found in cord blood as compared to maternal serum during labour (Meade and Rosalki, 1963). West and Zimmerman (1958) previously noted elevation of cord blood L.D.H. in eighteen out of nineteen cases. Serum aldolase is also high in cord blood but even higher in the neonate (Friedman and Lapan, 1958), and increased phosphocreatine kinase (C.P.K.) activity has been found in cord blood as compared to maternal serum, especially when the mother has had toxæmia of pregnancy (Chadd *et al.*, 1966). An increased cord blood C.P.K. is therefore of no use in the early diagnosis of a familial muscular dystrophy.
5. *Enzyme activity increasing during pregnancy.* This group includes serum alkaline phosphatase (A.P.) and oxytocinase (cystine aminopeptidase, C.A.P.). Serum leucine aminopeptidase (L.A.P.) activity also increases during pregnancy; however the substrate used to measure 'L.A.P.' activity is hydrolysed by oxytocinase (Page *et al.*, 1961; Glendening *et al.*, 1965), and this accounts for the increased 'L.A.P.' activity in the serum of pregnant women.

The blood *histaminase* (D.A.O.) in pregnancy is also of great interest; Ahlmark, in 1944, using a biological method, demonstrated a rapidly increasing level of activity of this enzyme from about the seventh week after the previous menstruation. Subnormal levels of blood histaminase were found in a case of imminent abortion, and in another patient with an ectopic pregnancy.

It was also noted that human placenta contained a considerable amount of histaminase. Man and other animals (guinea-pigs and rats) with increased placental histaminase also had raised serum activity of this enzyme during pregnancy. On the other hand, cats and rabbits had low histaminase activity in both placenta and blood.

Plasma *diamine oxidase* (D.A.O.) activity rises in pregnancy in response to the increased production of an amine, possibly histamine, which is derived from either the foetus (Kahlson, 1962) or the placenta. Using a sensitive radio-assay method with C<sup>14</sup>-putrescine as substrate, Southren *et al.* (1966) showed that the plasma D.A.O. normally rises rapidly in the first 20 weeks of pregnancy. Subnormal levels of D.A.O. occurred in missed and some threatened abortions and in some patients with a poor obstetric history. Low levels of this enzyme were also recorded in patients who gave a history of frequent past abortions; in spite of this, all seven patients in this group had live births. Subnormal levels of D.A.O. were also found in mid-trimester in patients with the incompetent os syndrome; thus what appears to be a simple mechanical fault may be associated with a biochemical defect.

### Serum alkaline phosphatase

Elevation of serum alkaline phosphatase in late pregnancy was first noted over 30 years ago (Coryn, 1934). Many authors have confirmed that the activity of this enzyme increases especially in the last trimester of pregnancy (Cayla and Fabre, 1935; Meranze, 1937; Speert *et al.*, 1950; McMaster *et al.*, 1964). Boyer (1961) showed that a new pregnancy phosphatase appeared between the fifteenth and twenty-eighth week of

pregnancy, and Robinson *et al.* (1966a), established a separate identity for the pregnancy phosphatase and the  $\alpha_2$  globulin.

Using horizontal starch gel electrophoresis the last-mentioned authors showed that some individuals had one phosphatase component (Zone A) and others had two components, Zone A and a more slowly migrating component (Zone B). The mobility of Zone A enzyme was decreased by incubation of serum with neuramidase prior to electrophoresis, but the mobility of Zone B enzyme was not affected by this procedure. After using the neuramidase technique, a more slowly migrating pregnancy phosphatase band could be distinguished from the Zone A component.

Various theories have been put forward to explain the elevation of this enzyme; thus Kerleau and Cayla (1939) suggested that the high level in the mother was due to transmission of foetal osteoblastic alkaline phosphatase across the placenta into the maternal circulation. However, Speert *et al.* (1950) found no correlation between the levels of alkaline phosphatase in maternal serum and cord blood, and in most cases the maternal alkaline phosphatase exceeded the foetal values. These authors also noted slightly higher puerperal levels of this enzyme in lactating women. Ramsay (1938) suggested that raised alkaline phosphatase activity could be due to increased maternal osteoblastic activity.

Preliminary studies have also shown that the serum 5'-nucleotidase activity is not increased in single or twin pregnancy (A. Belfield—personal communication, 1967). This suggests that the raised serum alkaline phosphatase in pregnancy is not derived from maternal liver but comes from some other source.

Most recent workers have favoured the placental origin of this enzyme, and it has been shown that maternal serum alkaline phosphatase resembles placental alkaline phosphatase in a number of ways including migration on starch gel (Boyer, 1961), substrate specificity (Sadovsky and Zuckerman, 1965), and heat stability of this enzyme (McMaster *et al.*, 1964). The last authors showed that the raised total alkaline phosphatase

in late pregnancy was due to an increase in the heat-stable fraction; the cord blood total alkaline phosphatase was also elevated but the heat-stable (or placental) fraction was almost absent. Heat resistance is a special feature of placental alkaline phosphatase as opposed to other human alkaline phosphatases (Neale *et al.*, 1965).

Immunological studies have now been carried out by Birkett *et al.* (1966), and it has been shown that maternal serum alkaline phosphatase at term is partly inactivated by an anti-human placental alkaline phosphatase antibody. The placental origin of this enzyme is further supported by histochemical studies (Wislocki and Dempsey, 1946; Cursen, 1964; McKay *et al.*, 1958; Jacock *et al.*, 1963) who detected a rise in placental alkaline phosphatase with advancing pregnancy. The last-named authors also demonstrated that, in cases of toxæmia, the placental alkaline phosphatase at this stage of 33–36 weeks gestation was greater than in normal placentæ at the same stage of gestation. This favoured the concept of premature ageing of the toxæmic placenta.

Serum alkaline phosphatase levels have been studied throughout normal pregnancy, in severe toxæmia and in patients with pre-existing hypertension (Curzon and Morris, 1965). However, these authors could find no significant differences in enzyme levels in these three groups. More recently Watson *et al.* (1965) in a small series noted that three patients with pre-eclamptic toxæmia had higher than normal levels of plasma heat-stable alkaline phosphatase (H.S.A.P.). They also made the interesting observation that both the total alkaline phosphatase and the H.S.A.P. were lower in pregnant diabetics, and they felt that this could be the result of proliferative vascular lesions in the placentæ of diabetics (Burstein *et al.* 1965). Spasmodic fluctuations of H.S.A.P. were noted in terminal pregnancy, and it was felt that the slow disappearance of H.S.A.P. after intra-uterine death made this test unhelpful in the diagnosis of placental insufficiency. Significant elevation of the serum alkaline phosphatase occurs only in the last trimester of pregnancy, and therefore serial estimations of this

enzyme will not be helpful in the diagnosis of the early complications of pregnancy.

### Leucine aminopeptidase (L.A.P.) in pregnancy

It is well known that serum L.A.P. activity rises during pregnancy (Green *et al.*, 1955; Arst, Manning and Delp, 1959; Siegel, 1959; Bressler and Forsyth, 1959; Lewis, 1962; Mullan, 1967) and the serum level of this enzyme returns to normal 6 to 8 weeks post-partum.

Using the method of Goldbarg, Pineda and Ruterburg (1959), similar results were obtained (see Fig. 16). In Figs. 16, 17, 18 and 19, serum L.A.P. activity is expressed in units as defined by Harkness *et al.* (1960). A serum L.A.P. unit is the number of microgrammes of  $\beta$ -naphthylamines liberated by 1 ml. of a 2 per cent dilution of the serum. These authors found a normal range in seventy-three non-pregnant females of 4.5–19.3 (mean 10.3) L.A.P. units. Likewise, Rutenburg (1958) found a range of 6.7–17.5 (mean 10.8) L.A.P. units in sixty non-pregnant females. In figures 16, 17, 18 and 19, the total 24-hour urinary L.A.P. excretion is recorded, and this represents the number of milligrammes of  $\beta$ -naphthylamine liberated from the entire 24-hour collection of urine. Goldbarg *et al.* (1959) found that the mean total 24-hour urinary L.A.P. activity (10 N.V.) in fifty non-pregnant women was 43 (S.D. 12) with an upper limit of normal of 79 (i.e. mean + S.D.  $\times 3$ ) mg.  $\beta$ -naphthylamine.

As can be seen (Fig. 16), the urinary excretion of this enzyme remained relatively low throughout normal pregnancy, i.e. below 120 mg.  $\beta$ -naphthylamine per 24 hours. In four patients with multiple pregnancy the urinary excretion of L.A.P. towards term was higher than in single pregnancy (see Fig. 17). In this connection, it has already been noted that the activities of serum cystine aminopeptidase (C.A.P.) and L.A.P. tend to be higher in twin as compared to single pregnancies (Miller *et al.*, 1964). In the patient (case C), with a twin pregnancy, the pre-delivery urinary L.A.P. value was not elevated and it is of interest that the second twin in this case had a meningocele,

spina bifida and exomphalos and died soon after a breech delivery.

'High risk' patients were also studied (Table 9). As can be seen (Fig. 18), one of these patients (case 7) had a high urinary L.A.P. excretion throughout her pregnancy. Post partum the urinary L.A.P. returned to normal and at no stage was there any evidence of underlying renal disease.

LEUCINE AMINO-PEPTIDASE (L.A.P.) ACTIVITY IN NORMAL SINGLE PREGNANCY (Cases 1 to 6)

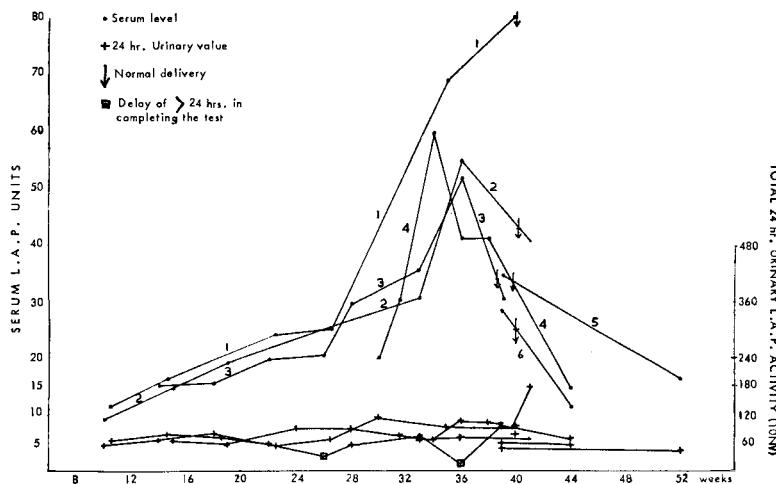


FIG. 16. By courtesy of the *Journal of Clinical Pathology*.

The substrate used for the L.A.P. test is L-leucyl-beta-naphthylamide, and this compound is also hydrolysed by oxytocinase (Page *et al.*, 1961; Glendening *et al.*, 1965). It is therefore possible that the raised urinary 'L.A.P.' in case 7 could in fact be due to excessive loss of oxytocinase in the urine. It may be that some 'high risk' patients are 'oxytocinase leakers' and it may be possible to show that such patients excrete too much of this urinary enzyme in successive pregnancies.

Urinary L.A.P. excretion was also measured in patients with mild, moderate and severe toxæmia and also hypertension (Table 10, groups 4 to 7). In three patients with essential

familial hypertension without proteinuria, the urinary L.A.P. values were elevated. Patients with toxæmia and proteinuria (groups 6 and 7) had even higher levels of urinary L.A.P. and it was found that when frank proteinuria was present the urinary L.A.P. value was over 120 mg.  $\beta$ -naphthylamine per

#### LEUCINE AMINO-PEPTIDASE (L.A.P.) IN MULTIPLE PREGNANCY

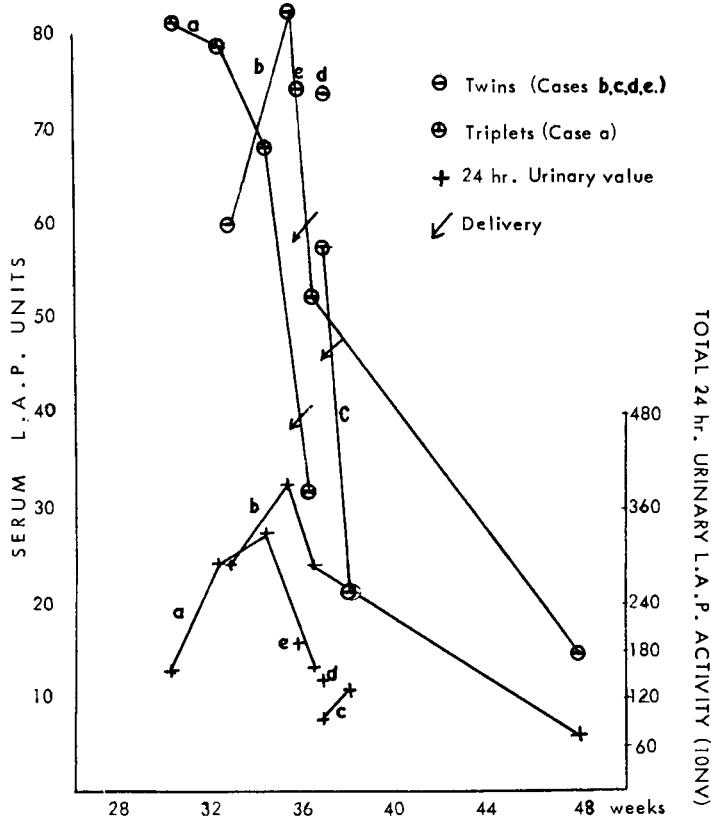


FIG. 17.

24 hours. There was no significant difference between the average maximum pre-delivery 24-hour urinary L.A.P. value in groups 6 and 7, and therefore this value could not be used to predict foetal survival in severe cases of toxæmia. It was also noted that if intra-uterine death occurred and foetal delivery

TABLE 9. 'High risk' patients

<i>Case No.</i>	<i>Age</i>	<i>Gravida</i>	<i>Past history</i>	<i>Outcome</i>	<i>Birth wt.</i>	<i>Placental wt.</i>
7	22	IV	3 Abortions Previous I.U.D. at full term	N.D. at 36/52 N.D. at 40/52	6 lb. 7 oz. 7 lb. 14 oz.	1 lb. 7 oz. 1 lb. 7 oz.
8	20	II				
9	22	III	Stillbirth at 31/52	N.D. at 36/52	8 lb. 3 oz.	1 lb. 8 oz.
10	23	II	Abortion at 12/52 Prem. at 34/52 (3 lb. 4 oz.)	N.D. at 36/52	5 lb. 2 oz.	1 lb.
11	22	II	Stillbirth at 36/52	N.D. at 38/52	6 lb. 10 oz.	1 lb. 3 oz.

By courtesy of the *Journal of Clinical Pathology*.

was delayed, then the urinary L.A.P. excretion fell gradually. The urinary L.A.P. values were noted to be low in two cases of intra-uterine death without toxæmia (group 8).

Finally, Fig. 19 demonstrates the massive 'enzyme diuresis' that can occur in the immediate post-partum period.

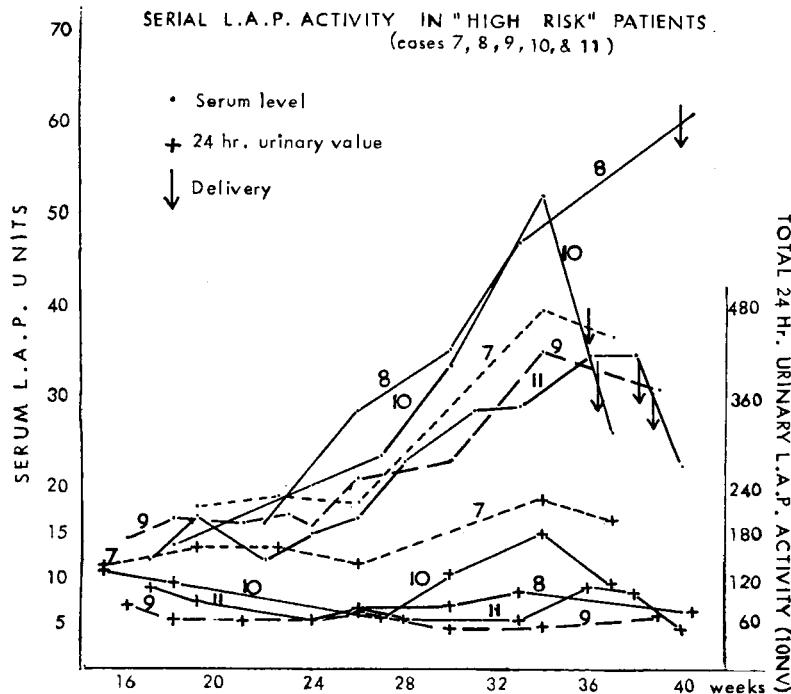


FIG. 18. By courtesy of the *Journal of Clinical Pathology*.

The toxæmic patients in this study were followed up. Repeat urinary L.A.P. estimations were normal, and urine culture was sterile in each case. Urinary L.A.P., like other enzymes can be raised in patients with renal disease (Bergmann and Scheler, 1964). It was felt that if, post-partum, the urinary L.A.P. failed to return to a normal low level, then underlying pyelonephritis should be considered.

#### Oxytocinase (cystine aminopeptidase C.A.P.)

Using a biological method Fekete (1930) first showed that

TABLE 10. *Predelivery 24 hour urinary L.A.P.*

		No. of cases	Milligrammes of $\beta$ -naphthylamine	Time when L.A.P. estimation performed
Group 1	Normal single pregnancy	6	< 120 mg.	Throughout pregnancy
Group 2	Multiple pregnancy	5	All > 138 mg.	Between 30 and 37 weeks
Group 3	'High risk' cases	5	3 Cases < 120 mg. 1 Case > 138 mg.	Throughout pregnancy Throughout pregnancy
			<i>An. maximum predelivery 24 hr.</i>	
Group 4	Mild toxæmia no proteinuria	3	52 mg.	Range urinary L.A.P.
Group 5	Essential hypertension	3	163 mg.	42-60
Group 6	Toxæmia, proteinuria and foetal survival	7	202 mg.	Between 38 and 39 weeks
Group 7	Severe toxæmia proteinuria and I.U.D.	6	207 mg.	Between 34 and 38 weeks
Group 8	I.U.D. and no toxæmia	2	42 and 36 mg.	Between 32 and 38 weeks
		—	At 32 and 33 weeks	37

By courtesy of the *Journal of Clinical Pathology*.

pregnancy plasma inactivated oxytocin, and Page (1947) employing a bioassay technique suggested that at the early stage of 4 weeks gestation, there was an elevation of serum

### POST PARTUM "ENZYME DIURESIS"

(Cases 14, 20)

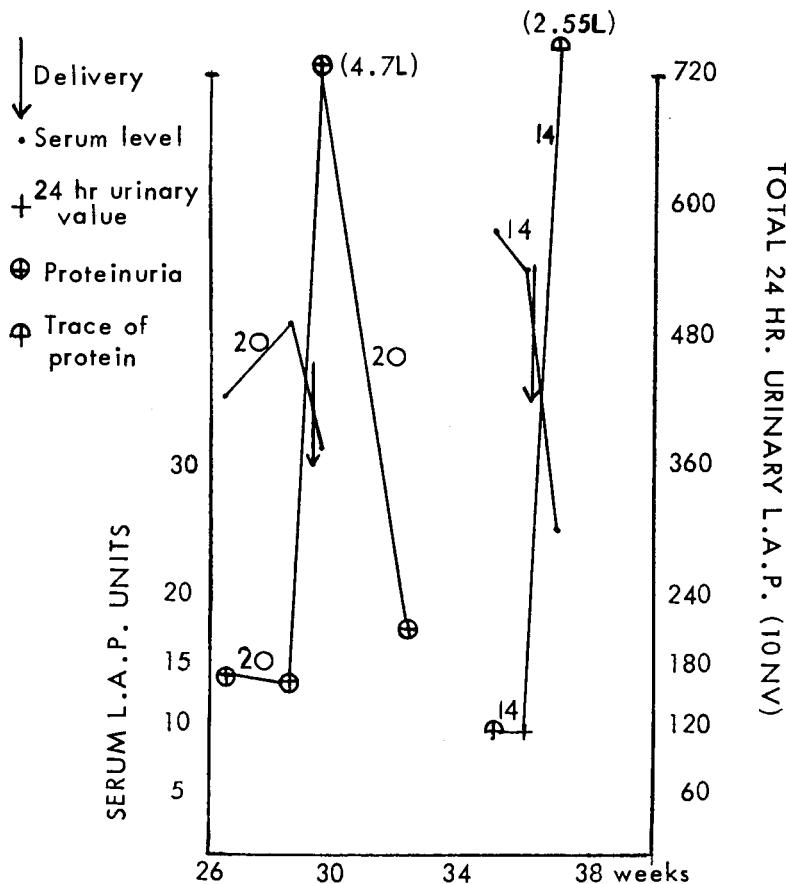


FIG. 19. By courtesy of the *Journal of Clinical Pathology*.

oxytocinase. However, Dicker and Whaley (1959) employing an isolated rat uterus preparation failed to find significant inactivation of synthetic oxytocin by plasma until after the thirteenth week of pregnancy. Later, using a chemical method,

it was shown that significant elevation of serum oxytocinase (cystine aminopeptidase, C.A.P.) activity started to occur about the fourteenth week of pregnancy (Titus *et al.*, 1960; Melander, 1965; Babuna and Yenen, 1966a) and increased progressively up to term. In addition, Page *et al.* (1961), using starch gel electrophoresis, demonstrated two C.A.P. bands in pregnant serum. C.A.P.<sub>1</sub> appeared earlier than C.A.P.<sub>2</sub> but the L.A.P. band remained constant throughout pregnancy.

### **Species variations**

There are some interesting species variations; although oxytocinase is present in the plasma of pregnant anthropoid apes (Werle *et al.*, 1950), it is absent in pregnancy plasma of other mammals including rats, rabbits and dogs. The myometrial content of oxytocinase varies in different species. Thus Sawyer (1954) reported that the myometrial content of this enzyme in the rat increased during pregnancy but suddenly fell at the time of delivery. On the other hand, there was no significant difference in healthy women of myometrial oxytocinase content throughout pregnancy, or active labour (Melander 1965); this suggests that, in women, myometrial oxytocinase is not important in preventing premature uterine activity.

### **The origin of oxytocinase**

There is good evidence that oxytocinase is derived from the placenta (Hooper and Jessup, 1959; Page *et al.*, 1961; Riad, 1962; Melander, 1965; Ryden, 1966). Only low oxytocinase activity is found in foetal blood (Page *et al.*, 1961) and this implies that the foetus is not the source of this enzyme. Particularly high values for C.A.P. (and L.A.P.) are found in patients with multiple pregnancy (Miller *et al.*, 1964; Ichaliotis and Lambrinopoulos, 1965; Babuna and Yenen, 1966b) and this is probably due to the increased placental mass in these cases. On the other hand low levels of serum oxytocinase occur in patients with placental insufficiency (Mullan, 1967; see Fig. 21).

### Parturition

It has been clearly shown that there is no acute decline in plasma oxytocinase (C.A.P.) activity during labour, parturition or in the early puerperium (Titus *et al.*, 1960). Hence spontaneous labour is not due to a sudden reduction of plasma activity of this enzyme. However, Hashimoto (1961) has suggested that the onset of labour occurred earlier in those patients whose serum oxytocinase prior to delivery showed the greatest rate of increase. Significantly higher levels of serum oxytocinase were also found in patients whose pregnancy was 'over term' (Lambrinopoulos, 1964), and it was suggested that the high oxytocinase levels in prolonged pregnancy could be important aetiologically in the form of uterine inertia. Likewise increased serum oxytocinase activity was present in primary uterine inertia at term (Babuna and Yenen, 1966b).

It has been suggested that oxytocinase, by inactivating oxytocin during pregnancy, keeps the uterus at rest and prevents premature labour. This theory is a little too facile. In fact there are a number of other ways in which oxytocin can be eliminated from the circulation. By measuring the half life of injected oxytocin in pregnant women at term, it was shown that the blood level fell rapidly (Gonzalez-Panizza *et al.*, 1961). Likewise, injected oxytocin is rapidly removed from the circulation in the human male (Fitzpatrick, 1961).

### The structure of oxytocin, and methods of determining oxytocinase (C.A.P.) activity

The chemical structure of oxytocin was established by Tuppy (1953) and also by Du Vigneaud *et al.* (1953). As can be seen (Fig. 20) it is an octapeptide amide. It was later shown that pregnancy plasma inactivated synthetic oxytocin by splitting the ring between cystine and tyrosine (Tuppy and Nesvadba, 1957); they proved this by adding performic acid to the inactivated product which resulted in a split of the disulphide linkage and the liberation of cysteine. These authors therefore

concluded that the enzyme in pregnancy plasma which inactivated oxytocin was a cystine aminopeptidase. Accordingly they introduced a chemical method for determining enzyme activity; L-cystine-di-beta-naphthylamide was used as the substrate (Fig. 20) and enzymic action resulted in the liberation of  $\beta$ -naphthylamine. The free  $\beta$ -naphthylamine was diazotized and finally coupled with N-(1-naphthyl) ethylenediamine to

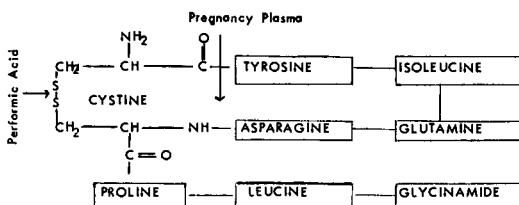
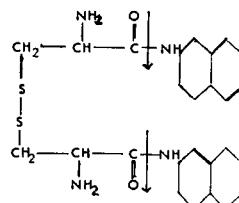


FIG. 20. (Above) Showing the structure of oxytocin and indicating the site at which the cystine—tyrosine linkage is split by pregnancy plasma. (Below) Showing structure of substrate, L-cystine-di-beta-naphthylamide and indicating linkages split by cystine aminopeptidase.



yield a stable blue dye. The optical density of this dye was then measured. Most workers have employed the lengthy chemical method of Tuppy and Nesvadba to determine plasma oxytocinase (C.A.P.) activity (Titus *et al.*, 1960; Riad, 1962; Ichaliotis and Lambrinopoulos, 1964; Mellander, 1965), but Babuna and Yenen (1966a) have now introduced a modified method with only a 2-hour incubation. Another rapid method has been suggested by Hardy and Ritchie (1966): a chromogen, diazotized 3-chloro-4-nitraniline is added directly to the enzymically liberated  $\beta$ -naphthylamine and the product, which is a red dye, is then measured.

### Clinical applications

The clinical value of serum oxytocinase (C.A.P.) estimation has not yet been fully established. However, Fig. 21 demonstrates simply the value of estimating serum oxytocinase in confirming the diagnosis of placental insufficiency. Mrs. S. C. was 'small for dates', and her predelivery levels of C.A.P. were

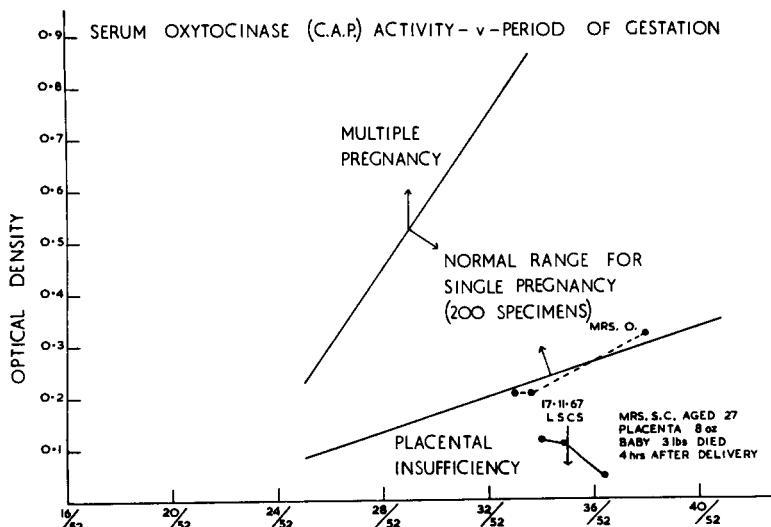


FIG. 21. Indicating the normal wide range of serum C.A.P. activity in single pregnancy. Values below the lower line are subnormal.

low. She was delivered by lower segment Caesarean section, but the baby died shortly after birth. Her placenta (Fig. 22) was small in size but no infarcts were visible. There was no obvious explanation for placental insufficiency, in particular, there was no history of threatened abortion or hypertension. In contrast, Mrs. O. was 'small for dates' at 33 weeks, but foetal growth accelerated and at 38 weeks the serum C.A.P. level had risen to within the normal range. Babuna and Yenen (1966b) have made other interesting observations; thus in intra-uterine death, when the serum C.A.P. levels remained high, labour was delayed. On the other hand, a rapid fall in serum C.A.P. heralded early spontaneous labour. These

authors also found very low levels of this enzyme in missed abortions indicating greatly diminished placental function. However, normal levels of serum C.A.P. activity were found in cases of ectopic pregnancy and in patients with foetal abnormalities. The changes of serum and urinary oxytocinase in pregnancy toxæmia will be discussed in detail in the next section of this chapter.

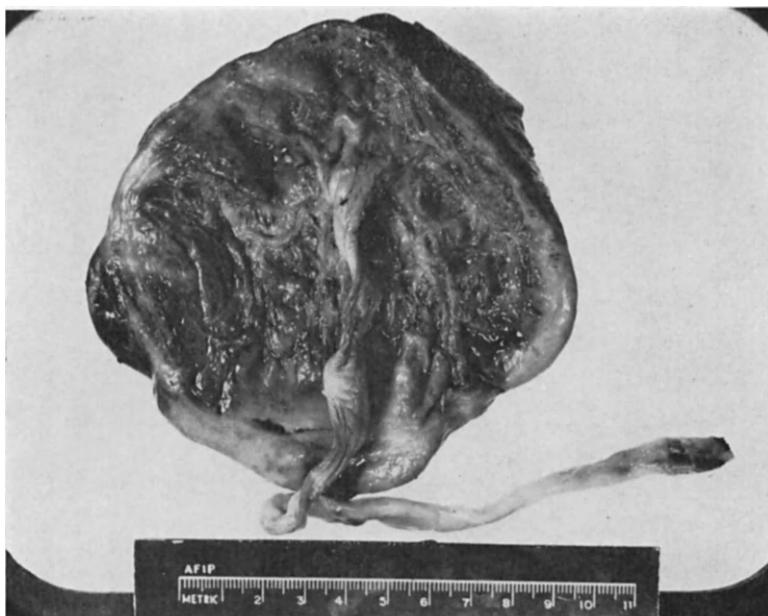


FIG. 22. Photograph of placenta (Mrs. S. C.). By kind permission of Mr. R. Lunt of the Northern General Hospital, Sheffield.

### Influence of liver disease on C.A.P. activity

The serum C.A.P. activity in non-pregnant patients with hepatocellular or obstructive jaundice may be just above the low level found in non-pregnant females (Mullan, 1967). For instance, one interesting patient with 'morning sickness' had some elevation of serum C.A.P. activity when she was only 8 weeks pregnant. A very early diagnosis of twins was considered, but in fact the S.G.P.T. level was elevated at 400 King units which favoured the diagnosis of anicteric hepatitis, complicating

early pregnancy. One month later the patient's serum transaminase levels (S.G.O.T. and S.G.P.T.) and liver function tests had returned to normal.

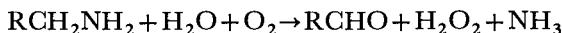
It is interesting to measure serum C.A.P. activity at different pH values. Non-pregnant women and men have some serum C.A.P. activity at pH 6.3, while jaundiced patients have quite marked C.A.P. and L.A.P. activity at this pH. On the other hand, pregnancy is associated with the appearance of increased 'true' oxytocinase activity at pH 7.8. This affords additional proof that C.A.P. and L.A.P. are different enzymes (Mullan, 1967).

### Toxaemia of pregnancy

Research on this subject is important because this complication of pregnancy is now the chief cause of maternal morbidity and neonatal death (*Brit. med. J.*, 1964; Butler and Bonham, 1963). Pre-eclamptic toxæmia is characterized by the development of hypertension with or without oedema and proteinuria in the last trimester of pregnancy. The early appearance of heavy proteinuria or onset of eclamptic fits or coma is especially grave.

The cause of pregnancy toxæmia remains uncertain (*Brit. med. J.*, 1964; Browne, J. C. McC., Morris, N. and Sophian, J., 1957). One theory to account for hypertension is the elaboration of a renin-like substance in response to placental anoxia. The 'Goldblatt placenta' concept is analogous to experimental hypertension produced by the anoxic kidney (Goldblatt *et al.*, 1934). However, though plasma renin is raised in normal pregnancy, increased levels are not found in pre-eclampsia (Brown *et al.*, 1965).

High concentrations of monoamine oxidase were found in human placenta by Luschinsky and Singher (1948) in America and in London by Thompson and Tickner (1949). Monoamine oxidase catalyses the following reaction:



By using various substrates, e.g. tyramine, the oxygen uptake of homogenized placenta, or the amount of one of the reaction

products, e.g. ammonia, can be measured. It was later confirmed that monoamine oxidase (M.A.O.) was present in lower concentrations in placental tissue of toxæmic patients (Sandler and Coveney, 1962; DeMaria and See, 1966). Monoamine oxidase is very oxygen sensitive (Kohn 1937) and placental anoxia, due to diminished utero-placental blood flow in toxæmic patients (Assali *et al.*, 1954; Browne and Veall, 1953; Morris *et al.*, 1955), will result in local accumulation of amines. These amines could then produce a 'vicious circle' with further vasoconstriction, placental anoxia and hypertension.

Recent evidence suggests that water retention unaccompanied by comparable sodium retention occurs in toxæmia (Davey *et al.*, 1961). Furthermore, Pollak and Nettles (1960), in careful renal biopsy studies, showed that oedema of glomerular endothelial and epithelial cells was characteristic of toxæmic kidneys. They also made the interesting observation that elevation of the serum uric acid was characteristic of pre-eclampsia and that hyperuricaemia was most marked in severe cases.

In the presence of these pathological changes it is not surprising that there is also an excessive excretion of certain urinary enzymes in patients with toxæmia and proteinuria (e.g. urinary leucine aminopeptidase—see Table 10). In fact, Page *et al.* (1961) have demonstrated L.A.P., C.A.P.<sub>1</sub> and C.A.P.<sub>2</sub> enzyme bands in the urine of patients with pre-eclampsia. Clearly these aminopeptidases leak through the glomeruli and can be detected in the urine together with other proteins. A method of measuring 24-hour urinary excretion of oxytocinase C.A.P., needs to be established, and this could be used to assess the severity and prognosis of individual cases of toxæmia.

It has been shown that serum oxytocinase tends to be decreased in pre-eclampsia (Ichaliotis and Lambrinopoulos, 1964). However, Babuna and Yenen (1966b), in estimating serum C.A.P. activity in thirty-three patients with pre-eclamptic toxæmia or eclampsia, found values which were scattered

above and below the normal range. On close inspection of their results eleven patients out of a total of fifteen with '3 plus' proteinuria, had a reduced serum level of oxytocinase, and this could be due to impaired production or an excessive urinary leak of this enzyme. In the remaining four cases the C.A.P. level was raised above the control value; this might well be explained by placental overproduction of this enzyme. Current views on 'hyperplacentosis' in toxæmia (Jeffcoate, 1966) would favour this thesis.

Babuna and Yenen (1966a) also demonstrated that decreasing levels of serum oxytocinase in severe toxæmia indicated diminishing placental function and impending foetal death. This information could be most useful to the obstetrician and would encourage him to perform an urgent Caesarean section.

In severe toxæmia there is generalized arteriolar constriction, and post-mortem examinations of fatal cases of eclampsia have revealed extensive small subcapsular and intrahepatic haemorrhages, together with scattered areas of periportal necrosis of liver cells (Sherlock, 1963). The crude liver function tests are usually normal, but the serum transaminase levels are often raised. Dass and Bhagwanani (1964) found that in six out of nineteen cases of severe toxæmia and eleven out of twelve patients with eclampsia the S.G.P.T. levels were elevated; in the latter group, peak activity occurred about 5 to 7 days after the onset of convulsions. The elevation of S.G.O.T. activity occurred earlier, and this is similar to the pattern seen in infective hepatitis (Rosalki, 1960). Dass and Bhagwanani also noted that the S.G.P.T. levels were particularly high in patients presenting in coma, and in those with liver enlargement and heavy proteinuria. Likewise, Crisp *et al.* (1959) found the highest S.G.O.T. levels in those toxæmic patients with marked proteinuria, and these authors suggested that the S.G.O.T. level remained elevated in those patients who responded poorly to treatment. They also found that the S.G.O.T. level was normal in patients with essential hypertension without superimposed toxæmia, and in cases with chronic renal disease.

### **Jaundice**

Jaundice does not often complicate pregnancy (Sheehan, 1961), and most authors agree that viral hepatitis accounts for most patients with this complication. There may be a history of contact with another affected person or the virus may be transmitted by a syringe or needle. The symptoms of anorexia, vomiting and weakness followed by jaundice are typical, and the helpful biochemical tests include a marked increase in the S.G.P.T. and positive flocculation tests. The alkaline phosphatase is only moderately elevated, but higher levels will be found if hepatitis complicates later pregnancy because normally the serum alkaline phosphatase is raised towards term.

Cholestatic hepatosis, or recurrent jaundice of pregnancy is of special interest. This type of jaundice occurs in the last trimester of pregnancy and is preceded by pruritus and abdominal symptoms. However, there is no fever. Severe itching and moderate jaundice may complicate subsequent pregnancies but the prognosis is excellent (Thorling, 1955; Svanborg and Ohlsson, 1959). Liver biopsy shows evidence of biliary stasis and biochemical tests include moderate elevation of the serum bilirubin and negative flocculation tests. The serum alkaline phosphatase activity is increased and exceeds that of normal pregnancy (Thorling, 1955). As in other types of obstructive jaundice the prothrombin time may be prolonged but this is corrected by parenteral vitamin K. A similar type of cholestatic jaundice may be due to chlorpromazine and methyl testosterone. The other causes of jaundice in pregnancy are unusual. They include gall stones, sepsis and obstetric acute yellow atrophy. Haemolytic jaundice can occur in some cases of eclampsia, in incompatible blood transfusions and some abortions.

### **The pill**

Steroids with an alkylated group at the C<sub>17</sub> position (e.g. methyl testosterone) can be hepatotoxic. Likewise, impaired liver function can occur in women taking the contraceptive

pill (e.g. Lyndiol, 17  $\alpha$  ethynodiol). A number of reports have indicated that oral contraceptives may have a hepatotoxic effect; these include papers from Chile (Lucchini *et al.*, 1965), Finland (Eisalo *et al.*, 1964; Palva and Mustala, 1964), Scandinavian countries (Thulin and Nerman, 1966), but also the U.K. (Lucey, 1967). On the other hand, Swyer and Little (1965) studied twelve pre-menopausal women, on oral contraceptives from 3 to 6 years, and found that their liver function tests were all practically normal. The earlier Finnish reports concerned post-menopausal women; impaired liver function is less likely in younger women. It may also be relevant that hepatotoxic damage due to oral contraceptives seems to be much commoner in certain countries (e.g. Finland and South America).

Liver biopsy and liver function tests in affected patients indicate that this is a cholestatic type of jaundice and is very much akin to the recurrent jaundice of pregnancy which has been described above. In a recent paper from Chile, Orellana-Alcalde and Dominguez (1966) described fifty such patients. Forty-two had previously been pregnant and of these seventeen gave a history of either late pruritus, or pruritis and jaundice of pregnancy. In twenty-six out of the fifty patients symptoms started during the first cycle of treatment with the oral contraceptive. On withdrawing the drug these symptoms disappeared. The biochemical abnormalities noted by the latter authors included moderate elevation of the serum bilirubin in all patients and some increase in serum alkaline phosphatase in most cases. The flocculation tests were all negative. The S.G.P.T. level was mildly elevated in nineteen women but there was a marked increase in this enzyme in fourteen cases; in the latter group, the S.G.P.T. level was highest in those patients with a history of previous jaundice of pregnancy. A decrease in serum cholinesterase, together with a fall in serum albumin and an increase in  $\alpha_1$  globulin has also been demonstrated in patients who have taken oral contraceptives for a month or more (Robertson, 1967). In addition, it has been shown that there is an alteration of tryptophane metabolism

in oestrogen-treated patients (Rose, 1966). This author showed that after a tryptophane load there was a large urinary excretion of 3-hydroxykynurenine (H.K.), xanthurenic acid (X.A.) and 3-hydroxyanthranilic acid (H.A.). It was suggested that this was due to a hormone-induced increase in levels of hepatic enzymes responsible for the conversion of tryptophane to nicotinic acid.

## OTHER COMPLICATIONS OF PREGNANCY

### Abruptio placentae

A number of authors (Little, 1959; Plauche and Mosey, 1962; Boutselis *et al.*, 1963) have shown that serum lactate dehydrogenase (L.D.H.) levels are raised in a number of severe cases of accidental ante-partum haemorrhage. This may be due to leakage of enzyme from infarcted placenta and disintegrating red cells.

Little (1959) noted that ten out of fifty-seven patients had elevations of serum L.D.H. during labour. Of interest was the increased incidence of placental infarction in eight of these ten cases; furthermore, foetal distress was present in two of these cases, one was stillborn with anencephaly and a fourth neonate died from sepsis.

The important observation made by Little (1959) and confirmed by Boutselis *et al.* (1961), was that the serum L.D.H. values were elevated in abruptio placentae. Six out of seven of Little's cases and twenty-six out of thirty-three in the latter series had raised L.D.H. levels, while the other bleeding disorders of pregnancy, including placenta praevia and threatened abortion, were usually associated with normal levels of this enzyme. In addition, Little found a gross correlation between the size of the retroplacental clot and L.D.H. activity. High L.D.H. values were noted when retroplacental blood loss was associated with hypofibrinogenaemia, and also the serum L.D.H. level became even higher in one patient who had a large concealed accidental haemorrhage and developed acute

post-partum renal failure. Marked diuresis on the tenth post-partum day was accompanied by a fall in L.D.H. levels towards normal. Boutselis *et al.* (1961) found a high serum L.D.H. value in one patient with macrocytic anaemia of pregnancy (see below). This enzyme was also elevated in another woman who developed Sheehan's syndrome following a severe post-partum haemorrhage and defibrination.

Hawkins and Whyley (1966) showed that one or more of the iso-enzymes of L.D.H. could be elevated in proven abruptio placentae or in patients with small accidental haemorrhages, even though the total L.D.H. was normal. Elevations of iso-enzyme L.D.H.<sub>4</sub> and <sub>5</sub> were suggestive of placental damage. They described one case of concealed accidental haemorrhage which was suspected because of a very high L.D.H. value; this finding prompted an emergency Caesarean section. They confirmed that the total serum L.D.H. values were usually normal in both placenta praevia and the other bleeding disorders of pregnancy. However, the L.D.H.<sub>4</sub> and <sub>5</sub> iso-enzymes were somewhat elevated in six out of fifteen cases of placenta praevia and in two of these the baby was asphyxiated at birth.

### Anaemia

Very high serum L.D.H. activity was first reported in pernicious anaemia by Hess and Gelm (1955), and Heller *et al.* (1960a) noted markedly increased levels of plasma lactic, malic and 6-phosphogluconic dehydrogenase in megaloblastic anaemia due to other causes. Appropriate treatment of megaloblastic anaemia with either B<sub>12</sub>, folic acid, or both (Heller *et al.*, 1960a; Elliott and Wilkinson, 1963; McCarthy *et al.*, 1966) resulted in a fall of L.D.H. activity towards normal.

In a paper from Nigeria, Fleming and Elliott (1964) estimated serum L.D.H. and S.H.B.D. activities in anaemic and non-anaemic pregnant African women. In Ibadan, folate deficiency in pregnancy is common, and the above authors found that most women with transitional and all those with frankly megaloblastic bone marrow changes had elevated levels of these two enzymes. Enzyme elevation was proportional

to the degree of megaloblastic change and not to the degree of anaemia, and specific therapy with folic acid resulted in a sharp fall of L.D.H. and S.H.B.D. levels before the bone marrow reverted to normal. In their later paper, Elliott and Fleming (1965) demonstrated high L.D.H. and S.H.B.D. activity in marrow plasma obtained from pregnant patients with frank megaloblastosis. The marrow plasma activity of these two enzymes was even higher than the activity in the peripheral blood, and they concluded that the raised marrow plasma level of S.H.B.D. and L.D.H. was due to the accelerated destruction of red cell precursors which are rich in these enzymes. A similar mechanism to explain the high enzyme levels in megaloblastic anaemia had previously been put forward by Heller *et al.* (1960b).

### Ruptured ectopic pregnancy

In 1957 Kelley described a negress, aged 22 who presented with an acute abdomen. The serum amylase was acutely elevated at 1600 Somogyi units, and the diagnoses considered included acute pancreatitis, acute haemolytic crisis complicating sickle cell anaemia, and ruptured ectopic pregnancy. An ectopic pregnancy was found at operation, and a right-sided salpingectomy was successfully performed. It was considered that peritoneal blood could have caused pancreatic inflammation. It was later shown (McGeachin *et al.*, 1958) that there was appreciable amylase activity in the Fallopian tubes, and this could be relevant to the above case.

### Trophoblastic disease

Hydatidiform mole usually presents in the first half of pregnancy with bleeding and undue uterine enlargement. The signs of pregnancy are all evident but the foetal heart sounds cannot be detected. An elevated level of urinary chorionic gonadotrophin occurs but is not always diagnostic in distinguishing normal pregnancy from hydatidiform mole. Uterine evacuation is essential treatment of a mole and therefore any further diagnostic test would be most useful. In this connection,

it was noted by Bressler and Forsyth (1959) that the serum leucine aminopeptidase (L.A.P.) level rose in normal pregnancy, but remained normal in three cases of hydatidiform mole and in one patient with a chorioncarcinoma. Subsequently, Robinson *et al.* (1966b) confirmed that pregnancy associated C.A.P.<sub>1</sub>, C.A.P.<sub>2</sub>, and alkaline phosphatase was absent in seven patients with active trophoblastic disease. Smith and Rutenburg (1966), using L-methionine to inhibit the hydrolysis of L-leucyl-B-naphthylamide by normal serum L.A.P., were able to demonstrate electrophoretically very small amounts of C.A.P.<sub>1</sub> at 9 weeks gestation. This 'non-hormonal' demonstration of pregnancy provided an additional method for distinguishing normal pregnancy from chorioncarcinoma.

### SUMMARY

The object of this chapter was to review the literature on enzyme changes in pregnancy. Special attention was paid to those serum enzymes e.g. oxytocinase (C.A.P.) and alkaline phosphatase (A.P.) which increase during pregnancy. Those enzyme changes that could be of clinical value were stressed. For instance, in early pregnancy, a failure of increase of blood histaminase could indicate abnormal placentation. Likewise, at a later stage, low levels of serum oxytocinase occurred in patients with placental insufficiency, and falling levels of this enzyme in toxæmia suggested impending foetal death. This information could be helpful in deciding when to carry out a caesarean section. It was also found that severe toxæmia was accompanied by a marked increase in the urinary excretion of leucine aminopeptidase (L.A.P.). Mention was also made of those enzyme changes accompanying certain complications of pregnancy, e.g. toxæmia, jaundice, abruptio placentæ and megaloblastic anaemia. The abnormalities of liver function that can occur in some patients on the 'pill' were also noted.

As in other fields of research, a great deal is known about the results of disease, but the causes often remain obscure. In particular, toxæmia remains a 'disease of theories', and we

seem to be no nearer to finding out the cause of this common complication of pregnancy.

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## CHAPTER VII

## The value of enzymology in the detection of cervical carcinoma

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### CARCINOMA OF THE CERVIX, CARCINOMA *in situ* AND CYTOLOGY

It is a widely accepted fact that of 100 fatalities in women, at least one will be from carcinoma of the cervix (Macgregor *et al.*, 1965). It is also very probable that it will be the death of a younger woman, married and usually with a family. Carcinoma of the cervix is an occult disease, and normally by the time symptoms have occurred, the disease has spread too far for a cure. Way *et al.* (1963) said that extension to the lymph nodes reduced the chances of the 5-year survival by 4 to 1, and that extension to the lymph glands may occur without any clinical signs. Way also went on to stress that there were no early symptoms of carcinoma of the cervix. Obviously, in a condition with such a poor prognosis once it is established, and affecting a relatively young section of the community, every effort at earlier diagnosis must be made. Only in the last 20 years, since Papanicolaou (1943) really established the use of vaginal and cervical cytology as a diagnostic test for cervical carcinoma, has it been possible to diagnose the pre-invasive stage.

Papanicolaou techniques have been very widely applied, and many series of results have been published. A few of the more notable are mentioned below. In 1950 Nieburgs and Pund published an account of a cytological survey of 10,000 women in which 3.3 per cent (332) were found to have a smear

suggestive of malignant disease. Of these 332, only 234 were prepared to have any further investigations. 185 cases had carcinoma of the cervix, while forty-nine had biopsies showing benign cervical conditions. Of the 185 cases with carcinoma of the cervix, sixty-eight were pre-invasive and seven were border-line cases; thus 41 per cent of the cases detected were in a 'curable' state. Women with pre-invasive carcinoma of the cervix were all in a younger age group than those with invasive carcinoma of the cervix. It was concluded that if cytology was confined to the cases with symptoms, a large number of treatable cases would be missed because, in their series, 42 per cent of the cases detected had no symptoms. Way *et al.* (1963) estimated that if cytology were confined to the gynaecological out-patients, about 89 per cent of the cases of carcinoma of the cervix would not be detected in a curable state. They found that of 1000 carcinoma of the cervix cases admitted to their unit, only 110 patients had attended gynaecological departments in the 5 years before, when their smears might have been positive.

In 1949, a cervical cytological service was set up for the general public in British Columbia, and between 1949 and 1960 146,833 women were screened, which represented about a third of the population at risk in that province (Boyes, Fidler and Lock, 1962). In this service, 828 pure carcinoma *in situ* were detected, and eighty-seven pre-clinical invasive carcinoma were detected. Of the invasive group, there were forty cases with micro-invasion, and forty-seven cases with frank invasion. Since 1955, the incidence of clinically or symptomatic invasive carcinoma of the cervix has fallen from 28.4 per cent per 100,000 to 19.7 per cent in 1960. In the United Kingdom there have been a number of surveys in which similar results have been reported: Macgregor and Baird (1963), Grant (1963), Way *et al.* (1963), Jones and Metcalf Brown (1965).

Assuming that the progression of carcinoma *in situ* to invasive carcinoma is established, these surveys have emphasized the need for a widespread cytological survey. At the moment the frequency of this progression is not established. Younge, Hertig

and Armstrong (1949) report twelve cases in whom carcinoma *in situ* had been overlooked, of these, five progressed to invasive carcinoma. Carson and Gall (1954) found thirteen cases of carcinoma *in situ* in old cervical biopsy material, they traced nine cases, of which eight had developed invasive carcinoma. Peterson (1956) stated that 30 per cent of untreated carcinoma *in situ* developed carcinoma. Jordan *et al.* (1964) surveyed 379 cases of cervical epithelial abnormality. Of ninety-five major dysplasias, eight developed carcinoma *in situ*, while five of forty-three carcinoma *in situ* became invasive carcinoma.

Boyes, Fidler and Lock (1962), Bryans, Boyes and Fidler (1964), Christopherson, Parker and Drye (1962) all reported a reduction in the incidence of invasive carcinoma in the screened population as compared to the unscreened. The increasing average ages for carcinoma *in situ*, *in situ* with micro-invasion, and clinical invasion carcinoma, support the progression of *in situ* carcinoma to invasive carcinoma. The widely reported racial and social factors are of similar significance in both diseases. In spite of this evidence, other facts have been interpreted to infer that carcinoma *in situ* is of no significance.

Kirkland (1963) followed up forty-three carcinoma *in situ* only treated by cone biopsy and none of these cases subsequently developed invasive carcinoma. Kreiger *et al.* (1963), Green (1964 and 1966) and Lewis (1966) all stress the rarity of invasive carcinoma developing in the cervical stumps after conization. Lewis and Green have both found that in about 40 per cent of cases the conization is incomplete. All three authors feel that if carcinoma *in situ* is of significance, more patients should develop invasive carcinoma. Lewis drew attention to the intense inflammatory and fibrotic reaction which occurs in the cervical tissue following conization; he believed that this might kill the remaining pre-malignant cells.

A more convincing argument against the significance of carcinoma *in situ* has been put forward by Douglas (1962, 1963) and Green (1964, 1966), using the mortality rates from British Columbia. In his most recent article, Green argued that the

British Columbia screening programme should have eliminated at least a third of the invasive cervical carcinoma; the British Columbia mortality figures suggested that this has not happened. McKinnon (1963) doubts that carcinoma *in situ* invariably progresses, and his paper makes a convincing argument against too much reliance on mortality figures. While it is possible to question the significance of carcinoma *in situ* it is difficult to ignore a lesion which may be a forerunner of carcinoma, since this situation exists it is desirable to screen the population for carcinoma *in situ* and invasive carcinoma.

### ENZYMOLOGY OF MALIGNANT CERVICAL TISSUE

The enzymology of carcinoma of the cervix has not been extensively studied owing to the small amount of tissue available. Odell and Burt (1949) and Fishman *et al.* (1963) have demonstrated both histochemically and chemically, increased  $\beta$ -glucuronidase in carcinoma of the cervix. More recently, Latner (1964) drew attention to the elevation of 6-phosphogluconate dehydrogenase and lactate dehydrogenase in carcinoma of the cervix. Latner, Turner and Way (1966) showed that in *in situ* carcinoma of the cervix, while 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase were in the normal range, the iso-enzymes of lactate dehydrogenase showed a shift towards a pattern associated with malignancy. The total lactate dehydrogenase activity was very occasionally elevated. Thiery and Willihagen (1962, 1963, 1966a, 1966b) have related changes in enzyme histochemistry to the morphological characteristics of the tumour. Among the thirteen enzymes studied were 5'-nucleotidase, glucose 6-phosphate dehydrogenase, and lactate dehydrogenase. The changes with these three were most marked.

Goldberg and Pitts 1966 reported that alkaline and acid ribonuclease, deoxyribonuclease types I and II and adenosine deaminase were all increased in invasive cervical carcinoma as compared to cervices with benign gynaecological lesions.

These changes were most marked with the ribonucleases. Ayre and Goldberg (1966) also demonstrated an increase in both isocitric dehydrogenase, 6-phosphogluconate dehydrogenase, but not an increase in supernatant lactate dehydrogenase, though the microsomal activity was increased.

### **$\beta$ -GLUCURONIDASE AND CERVICAL CANCER**

$\beta$ -glucuronidase is a lysosomal enzyme of unknown significance, but appears to be related to cellular destruction. It has been shown to be elevated in both primary and secondary carcinomas from a variety of organs (Fishman and Anylan, 1947). Foetal and rapidly growing tissues also have elevated levels (Levvy *et al.*, 1958).

Odell and Burt (1949 and 1950) demonstrated that the  $\beta$ -glucuronidase activity was increased in the vaginal fluid in cases of carcinoma of the cervix and corpus uteri. An increase in  $\beta$ -glucuronidase activity was associated with pregnancy, *Trichomonas vaginalis* infection, menstruation and non-specific vaginitis. Levels greater than 300 units per ml. of vaginal fluid were suggestive of malignant uterine disease. In patients with benign conditions there was an 18 per cent level of false positive results, but no cases of cervical carcinoma were unassociated with elevated activities. Fishman and his colleagues published a series of papers on  $\beta$ -glucuronidase activity (Fishman *et al.*, 1950; Kasdon *et al.*, 1950; Kasdon *et al.*, 1951a and b; Fishman *et al.*, 1951; Kasdon *et al.*, 1953; Fishman *et al.*, 1954). In women with benign gynaecological lesions the enzyme activity was higher than in normal women. They suggested that the levels at which carcinoma should be suspected should allow for the age of the patient, since the activity rose after the menopause, and suggested the following levels: between 20 and 40 years a level of 250 units per gramme wet weight; between 40 and 60 years, 400 units; and between 60 and 90 years, 800 units. 80 per cent of non-cancerous women came below these levels. In pre-menopausal women, all carcinomas

of the cervix were associated with a raised level, but in post-menopausal women, not all cases of uterine carcinoma had raised levels. Between levels of 0-300 units per gramme weight, the leucocyte count influenced  $\beta$ -glucuronidase activity, but over this level there was no correlation. In post-menopausal and oopherectomized women, administration of stilboestrol caused a fall in the  $\beta$ -glucuronidase activity. Later, they defined normal activity as less than 300 units, suspicious activity between 300 and 400 units, diagnostic activity as over 400 units. In their hands, 81 per cent of cervical carcinomata were found in group 3.

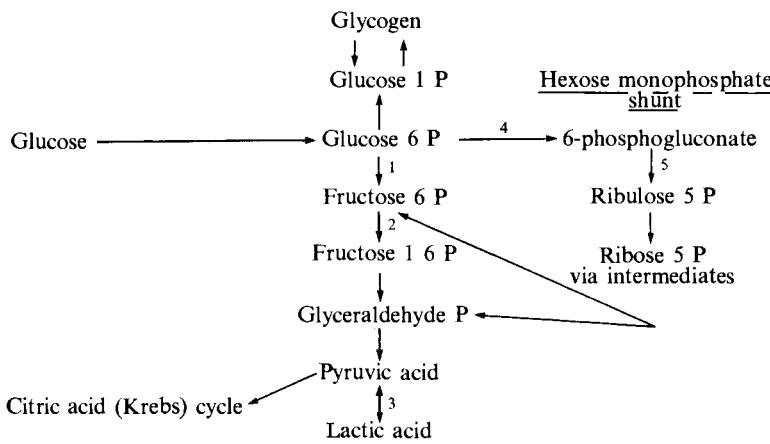
In 1959 Rauramo published a series of fifty cases of carcinoma of the cervix, and over 100 normal controls. He divided his results into the three groups suggested by Fishman's group, and found that 38 per cent of his carcinoma cases were in group I, 14 per cent in group II, while 48 per cent were in group III. In the control group the figures were 68 per cent in group I, 20 per cent in group II and 12 per cent in group III.  $\beta$ -glucuronidase did not correlate with clinical stages of carcinoma of the cervix, nor had it any prognostic value. Lawson (1959), suggested that in pre-menopausal patients treated with radiotherapy and Wertheim's hysterectomy, a rise in  $\beta$ -glucuronidase suggested a hopeful prognosis, while a fall carried a poor prognosis. Lawson (1960) studied  $\alpha$ -mannosidase, a related enzyme, but it was of no additional value. Hatzmichael (1962), using 100 units as the upper limit of normal, found that the  $\beta$ -glucuronidase of the cervical mucus was increased in seventeen of eighteen cases of carcinoma of the cervix. In carcinoma of the endometrium, in one of four cases it was not raised. In *Trichomonas vaginitis*, pregnancy and in post-menopausal women,  $\beta$ -glucuronidase of the cervical mucus was not affected. In conclusion it appears that in benign conditions  $\beta$ -glucuronidase in the vaginal fluid is often raised, while in 10 to 38 per cent of cases with carcinoma of the cervix it is not raised. In benign conditions the cervical mucus enzyme activity reduces the number of false positive results and in malignancy there are fewer false negative samples.

## 6-PHOSPHOGLUCONATE DEHYDROGENASE (6-P.G.D.)

In 1962, Bonham and Gibbs assayed 6-phosphogluconate dehydrogenase in 139 samples of vaginal fluid, ninety-three of which showed no evidence of malignancy and forty-six were from proven carcinoma. Using 100 units per gramme dry weight, only three samples from non-malignant lesions had elevated levels, whilst all forty-six samples from carcinoma had elevated levels.

6-phosphogluconate dehydrogenase is one of the enzymes of the hexose monophosphate shunt. In Fig. 23 the metabolism

### Embden Meyerhoff Pathway



<sup>1</sup> Phosphohexose isomerase.

<sup>2</sup> Aldolase.

<sup>3</sup> Lactate dehydrogenase.

<sup>4</sup> Glucose 6-phosphate dehydrogenase.

<sup>5</sup> 6-phosphogluconate dehydrogenase.

FIG. 23. Glucose 6-phosphate metabolism and related enzymes.

of glucose 6-phosphate in mammalian tissue with some relevant enzymes is diagrammatically represented. The hexose monophosphate shunt provides an alternative pathway to the glycolytic sequence for glucose 6-phosphate metabolism. A number of workers have demonstrated increased activity of the hexose-monophosphate shunt in spontaneous and induced

malignancy, foetal and regenerating tissues (Agranoff *et al.*, 1954; Abraham, *et al.*, 1955; Emmelot *et al.*, 1955; Kit, 1956; Kit *et al.*, 1957). Increased activity of the enzymes of this pathway has been demonstrated in both malignant and in pre-malignant lesions (Weber and Cantero, 1957; Morris, 1963; McNair-Scott *et al.*, 1962). Beaconsfield and Carpi (1964) and Beaconsfield and Liuzzi (1963) showed that the activity of the hexose monophosphate shunt was increased in inflammatory tissue. Ginsberg and Jeacock (1964) and Beaconsfield, Ginsberg and Jeacock (1964) showed that the activity of the hexose monophosphate shunt and its enzymes correlated with placental growth and protein and nucleic acid synthesis.

### Clinical applications

There have been a number of studies into the value of 6-phosphogluconate dehydrogenase as a diagnostic test for carcinoma of the cervix. At the St. Bartholomew's Well Women Clinic, 22 per cent of well women gave elevated enzyme levels using 100 units per gramme dry weight (Table 11). Bonham

TABLE II. *Over-all results obtained in samples from women attending the Well Women's Clinic, using 6-phosphogluconate dehydrogenase as a test for carcinoma in situ*

	<i>Enzyme level less than 100 units per gramme</i>	<i>Enzyme level more than 100 units per gramme</i>	
Malignant cells absent	254	78%	72
Malignant cells present	2		1
Total	256		73

and Gibbs in their original article found a false positive rate of 3.2 per cent. Bonham in 1964 gave the rate as 6.4 per cent. In Table 12 the figures from other workers are given. Cameron and Husain's (1965) figures were grouped according to the patient's age, thus two figures are given. The rates given are all much higher than those found by Bonham and Gibbs.

In Table 12 Muir's results in in-patients and out-patients are

compared, the false positive rates for the two groups are not very different. Lawson and Watkins had a much higher false positive rate than any other series; but in this work, all specimens had been homogenized prior to freeze-drying. Evidence has been obtained suggesting 6-phosphogluconate dehydrogenase is not completely liberated by freeze-drying and the more complete liberation they achieved may explain their results.

TABLE 12.

Authors	<i>False positive rate %</i>
Nerdrum, 1964	28.3
Cameron and Husain, 1965	32-38
Lawson and Watkins, 1965	47
Hoffman and Merritt, 1965	30
Bell and Egerton, 1965	38
<i>Own series</i> —out-patients	30
ward-patients	27

### Findings in normal women (vaginal fluid 6-P.G.D.)

In completely normal pre-menopausal women the enzyme is rarely present, as 81 per cent had no detectable enzyme present, and 4 per cent only had elevated enzyme levels. It has been reported that there are variations in 6-phosphogluconate dehydrogenase activity with the menstrual cycle, when results are grouped according to the day of the menstrual cycle it appears the incidence of increased 6-phosphogluconate dehydrogenase activities is lowest at the ovulatory stage.

#### (i) *Effect of oestrogens on 6-P.G.D. activity*

The number of cornified squamous cells in the vaginal smear is related to the secretion of oestrogens. This is estimated as the number of cornified cells per 100 cells in the smear and called the cornification index. Hoffman and Merritt (1965), using this index, found that a low oestrogen secretion was associated with an elevated 6-phosphogluconate dehydrogenase activity

in 56 per cent of cases. Muir and Canti (1966) obtained elevated levels in 66 per cent of cases.

(ii) *Findings in pregnancy*

12 per cent of 121 samples from pregnant women had elevated enzyme levels, as compared with 4 per cent in normal non-pregnant women. The number of samples with slight enzyme activity increased from 18 to 40 per cent. Cameron and Husain (1965) found a similar increase in activity. This may well be related to the parabasal cell hyperplasia which occurs in pregnancy (Wachtel 1964).

(iii) *Post-menopausal women*

25.4 per cent of normal post-menopausal women had elevated 6-phosphogluconate levels. Cameron and Husain showed 6-phosphogluconate dehydrogenase increased with age in women with benign gynaecological lesions, rising from 38 per cent to 80 per cent at 65 years. Muir and Canti (1966) found that in samples with a parabasal hyperplasia in the smear, the evidence of elevated enzyme levels reached over 90 per cent. In women with an atrophic pattern the increase is slight. The changes in the vaginal epithelium appear to be the cause of the increased enzyme activity.

### **Findings in women with gynaecological lesions**

As mentioned earlier, gynaecological lesions are associated with an increase in the detectable 6-P.G.D. activity, but there is considerable variation with different lesions (Table 13).

In Tables 14, 15 and 16, the effects of the presence of pus cells and red blood cells on the enzyme activity are shown. The presence of red blood cells in smears causes an increase in the amount of enzymes found in the sample. The amount of enzyme would appear to be related to the amount of blood present. Wolfson and Williams-Ashman (1957) and Glock and Mclean (1954) have shown that erythrocytes have 6-phosphogluconate dehydrogenase activity. Muir and Canti (1966) compared the 6-phosphogluconate dehydrogenase

TABLE 13. *6-phosphogluconate dehydrogenase activity in 791 samples grouped according to gynaecological lesions found*

<i>Lesion</i>	<i>Total no.</i>	<i>Nil</i>	<i>Slight enzyme activity</i>	<i>Percentage of total</i>	<i>Elevated enzyme activity</i>	<i>Percentage of total</i>
Hormonal	45	24	9	20	12	26.7
Cervical polyps	71	38	12	16.9	21	29.5
Cervical lesions	396	200	69	17.4	127	32.0
Ovarian cysts and tumours	17	12	3	17.6	2	11.7
Post-menopausal bleeding	81	46	9	11.1	26	32.0
Fibroids	46	28	10	21.7	8	17.3
Prolapse	72	56	6	7.8	13	18.0
Inflammatory	63	24	9	14.2	30	47.6

TABLE 14. *6-phosphogluconate dehydrogenase activity in 100 samples which had an excessive leucocyte count in the smear*

<i>6-phosphogluconate dehydrogenase activity in units per gramme dry weight</i>								
	0	1-19	20-39	40-59	60-79	80-99	100-499	500+
Number of samples	37	8	2	2	1	4	28	18
Percentage of cases	37	8	2	2	1	4	28	18

TABLE 15. *6-phosphogluconate dehydrogenase activity in samples from 63 cases in which the smear showed the presence of excessive erythrocytes*

<i>6-phosphogluconate dehydrogenase activity in units per gramme dry weight</i>								
	<i>Nil</i>	<i>1-19</i>	<i>20-39</i>	<i>40-59</i>	<i>60-79</i>	<i>80-99</i>	<i>100-499</i>	<i>500+</i>
Number of samples	10	4	4	6	2	3	27	7
Percentage of samples	15.9	6.3	6.3	9.5	3.2	4.8	43	11

(6-P.G.D.) activity in vaginal fluid in benign and malignant gynaecological lesions. In samples with an abnormal benign smear but no gross gynaecological lesion, a considerable number of samples had elevated enzyme activities (Table 17). 6-phosphogluconate dehydrogenase and  $\beta$ -glucuronidase activities may also be influenced by *Trichomonas vaginalis* infestation. 6-P.G.D. activity was elevated in 35 per cent of samples from

TABLE 16. 6-phosphogluconate dehydrogenase activity in samples containing increasing amounts of blood

	No. of cases	No enzyme detected	Slight enzyme activity	Percentage of total samples	Elevated activity	Percentage of total samples
Red blood cells present	11	4	4	36.0	3	27.0
Blood present	44	5	12	27.0	27	60.0
Profuse blood present	8	Nil	2	25.0	6	75.0

TABLE 17. 6-phosphogluconate dehydrogenase activity in samples from 52 cases in which the only abnormality was cytological

6-phosphogluconate dehydrogenase activity in units per grammme dry weight								
	Nil	1-19	20-39	40-59	60-79	80-99	100-499	500+
Number of samples	24	—	4	3	1	1	19	—
Percentage of samples	46.6	—	7.7	5.7	1.7	1.7	36.6	—

cases with *Trichomonas vaginalis* infection. Kasdon *et al.* (1951a) found  $\beta$ -glucuronidase was elevated only in those cases with infestation causing symptoms. Monilial vaginitis is not often associated with an elevated 6-phosphogluconate dehydrogenase (Hoffman and Merritt, 1965; Bell and Egerton, 1965; Muir and Canti, 1966). It is of interest that monilial infection is less often associated with a vaginal inflammatory reaction (Wachtel, 1964). This would suggest that the vaginal inflammation causes an elevation of 6-phosphogluconate dehydrogenase.

The results obtained in normal women and in those with gynaecological disorders, suggests that 6-phosphogluconate dehydrogenase activity measures an inflammatory reaction of the vaginal epithelium and is related to pathological changes in the vagina. In about 4.6 per cent of normal women, unexplained 6-phosphogluconate dehydrogenase activity was found. In some cases poor smears may have been taken and the reaction missed, but the significance of these enzyme elevations cannot be assessed.

Brooks and Muir (1967), following Hatzmichael's work on the  $\beta$ -glucuronidase content of the cervical mucus, made a similar study of 6-phosphogluconate dehydrogenase. In the majority of normal women it was found that 6-phosphogluconate dehydrogenase was not present in cervical mucus or the vaginal fluid. In samples from cases with a variety of gynaecological lesions, the cervical mucus 6-phosphogluconate dehydrogenase was more commonly elevated than the vaginal fluid content. This is not surprising since the majority of benign gynaecological lesions consist of chronic cervical disease.

When samples are taken on consecutive days there can be considerable variation in activity. Assuming that 6-phosphogluconate dehydrogenase is related to an inflammatory reaction, it is to be expected that the inflammatory response to an irritant will vary from day to day.

#### **Carcinoma *in situ***

In our series there were eighteen of carcinoma *in situ*; in these cases, seven showed elevated 6-P.G.D. activity. In these seven cases there was a considerable evidence of inflammatory response. In the remaining cases with slight enzyme activity there was evidence of inflammation. In four of seven cases of carcinoma *in situ* the 6-phosphogluconate dehydrogenase content of the cervical mucus was elevated; in equivalent vaginal samples it was elevated in three.

In the literature there is some disagreement as to the number of cases of carcinoma *in situ* associated with elevated levels. Table 18 shows some of the reported results. If all the reported

cases are combined, then sixty-two of 113 recorded cases (54 per cent) were associated with raised enzyme activity.

In benign gynaecological lesions, the presence of an inflammatory response had a considerable effect on the amount of enzyme present in the vaginal fluid. It may well be that in the early stages of the development of carcinoma *in situ* the presence of an adjacent cellular reaction may be the cause of the lesion being associated with an elevated 6-phosphogluconate dehydrogenase. Cameron and Husain (1965) quoting Bitensky and Chayen, suggest that the development of invasive characteristics is associated with elevation of 6-phosphogluconate

TABLE 18. *Carcinoma in situ and 6-P.G.D. activity*

<i>Authors</i>	<i>Level used</i>	<i>Number positive</i>	<i>Total</i>
Cameron and Husain, 1965	80	6	11
Bonham, 1964	80	10	10
Moukhtar and Higgins, 1966	100	12	14
Longnecker and White, 1965	100	14	20
Hoffman and Merritt, 1965	100	12	33
Bell and Egerton, 1965	100	1	7
Present work	80	7	18
Total rate		62	113

dehydrogenase. The histological evidence of micro-invasion would not seem to influence the 6-phosphogluconate dehydrogenase activity in the vaginal fluid (Table 19). It could be suggested that a carcinoma *in situ* with an elevated 6-phosphogluconate dehydrogenase activity is a pre-malignant lesion, while others are not. The inference that the carcinoma *in situ* with an elevated enzyme level is a pre-malignant lesion would be difficult to prove. Theoretically it seems unsound to assume that the vaginal content of 6-phosphogluconate dehydrogenase should be increased at the onset of invasion. While a metabolic pathway may be increased, as judged by substrate utilization, the enzymes of that pathway will not necessarily be increased. During lactation the hexose monophosphate pathway increases at least twenty-fold in breast tissue while the increase in enzyme

activity is nowhere near that level (Mclean, 1958). Thiery (1965) showed that in well-differentiated cervical carcinomas there was little increase in 6-phosphogluconate dehydrogenase activity, but when the tumour became more de-differentiated the enzyme began to increase. This suggested that increase in 6-phosphogluconate dehydrogenase activity corresponded with de-differentiation rather than invasiveness. In conclusion it is not possible to assess the significance of 6-phosphogluconate

TABLE 19. 6-phosphogluconate dehydrogenase activity in samples from cases of carcinoma *in situ* of the cervix

Samples	Micro-invasion	Cytological findings		Enzyme activity	
		Erythrocytes	Leucocytes	1st sample	2nd sample
I	Absent	Absent	Profuse	48	—
II	Present	Absent	Present	nil	nil
III	Absent	Present	Present	nil	—
IV	Present	Present	Present	6	—
V	Present	Present	Moderate	33	—
VI	Absent	Present	Present	91	—
VII	Absent	Present	Present	1410	315
VIII	Present	Present	Present	nil	—
IX	Absent	Absent	Profuse	1390	—
X	Absent	Absent	Profuse	96	259
XI	Absent	Present	Present	455	—
XII	Absent	Moderate	Profuse	531	—
XIII	Absent	Absent	Absent	nil	—
XIV	Absent	Absent	Present	313	—
XV	Present	Absent	Moderate	nil	—
XVI	Absent	Absent	Absent	nil	—
XVII	Absent	Absent	Absent	nil	310
XVIII	Absent	Absent	Absent	nil	—

dehydrogenase elevation in carcinoma *in situ*. It could be merely coincidence or else be related to malignancy; in the latter case either from the carcinoma cell or else from an associated reaction of the tissues.

### Results in established malignancy

#### (a) Carcinoma of the cervix

In sixty-nine samples taken from cases of carcinoma of the cervix five, or 7 per cent, of these samples were not associated

with elevated 6-phosphogluconate dehydrogenase activity. In five cases the enzyme activity of the vaginal fluid rose during radiotherapy treatment, and in one case the enzyme level rose following a non-curative biopsy. All these agents would cause an inflammatory reaction in the cervix and the surrounding tissue.

*Reasons for failure of 6-phosphogluconate dehydrogenase to detect invasive cervical carcinoma and carcinoma in situ*

The failure of fornical cytology to detect both carcinoma *in situ* and invasive carcinoma is established. Way (1963) has shown the lower cell population of a fornical smear compared with a cervical smear. Younge (1958) claimed that a solitary fornical smear was accurate only in 68 per cent of cases. Wied *et al.* (1962) obtained similar results to those of Way in a comparison of fornical and cervical smears. In vaginal fluid enzymology there exists initial disadvantage of using the equivalent of a fornical smear and consequent dilution effects. The lower cell population of the vaginal fluid in carcinoma *in situ* as compared to invasive carcinoma (Wied *et al.*, 1962) could be the cause of carcinoma *in situ* being less frequently associated with elevated 6-phosphogluconate dehydrogenase activity. Brooks and Muir (1967) demonstrated the increased correlation of the 6-phosphogluconate dehydrogenase of cervical mucus with carcinoma of the cervix as compared with the vaginal 6-phosphogluconate dehydrogenase content.

Cameron and Husain (1965) and Bonham and Gibbs (1962) both claim never to have missed a case of invasive carcinoma. If the presence of slight enzyme activity is regarded as a positive result, the false positive rate would rise in normal women to 22 per cent and in cases of benign gynaecological lesions to 47.6 per cent. Our experience would agree with that of Nerdrum (1964) and Lawson and Watkins (1965) who failed to demonstrate elevated enzyme levels in four of thirty-three cases and five of forty-nine cases respectively, of cervical carcinoma (see Table 20). The latter authors failed to demonstrate

any significant difference between 6-phosphogluconate dehydrogenase and  $\beta$ -glucuronidase as a diagnostic test. They felt that, in both cases, the source of the elevated enzyme was not in malignant cells but rather in the interaction between normal and neoplastic tissue, a similar view to that of Muir and Canti (1966).

TABLE 20. *Various authors' results with 6-phosphogluconate dehydrogenase in carcinoma of the cervix*

<i>Authors</i>	<i>Enzyme positive</i>	<i>Total</i>	<i>Per cent</i>
Bonham and Gibbs, 1962	26	26	100
Nerdrum, 1964	33	37	89
Bonham, 1964	52	52	100
Cameron and Husain, 1965	40	40	100
Bell and Egerton, 1965	5	6	83
Lawson and Watkins, 1965	44	49	90
Hoffmann and Merritt, 1965	9	11	82
Longnecker and White, 1965	32	36	89
Moukhtar and Higgins, 1965	31	31	100

TABLE 21. *Various authors' results with 6-phosphogluconate dehydrogenase in carcinoma of the corpus uteri*

<i>Authors</i>	<i>Enzyme positive</i>	<i>Total</i>	<i>Per cent</i>
Hoffmann and Merritt, 1965	7	10	70
Bonham and Gibbs, 1962	12	12	100
Nerdrum, 1964	11	13	88
Longnecker and White, 1965	13	15	87

### (b) Carcinoma of corpus uteri

There were 21 samples taken from patients with carcinoma of the uterus of which only 5 samples had low 6-P.G.D. levels. When a sample was taken of the cervical mucus it was found that the vaginal sample was more often positive (Brooks and Muir, 1967). The elevated level found in carcinoma of the body is more likely associated with post-menopausal reactions of vaginal epithelium than with malignant cells. Table 21 gives the collected published results.

### (c) Other genital carcinomata

6-phosphogluconate dehydrogenase activity was elevated in five of eight cases of carcinoma of the clitoris and the vulva, this could either be due to exfoliative malignant cells in the vagina or generalized inflammation. In cases of carcinoma of the ovary, seven of fifteen cases were associated with elevated enzyme levels. Hoffman and Merritt (1965) found that two of four cases, Longnecker and White (1965) that two of three cases, and Moukhtar and Higgins (1965) that two of two cases were positive.

## THE USE OF OTHER ENZYMES IN THE DETECTION OF CERVICAL CARCINOMA

Muir (1966a) studied the value of glutamic oxaloacetic transaminase, lactate dehydrogenase and aldolase. All these enzymes had the disadvantage that they were present in the vaginal fluid of normal women, and by themselves were no better than 6-phosphogluconate dehydrogenase. Using multiple enzyme screens did not offer any advantage. Estimation of 6-phosphogluconate dehydrogenase and lactate dehydrogenase activity reduced the false positive rate, but did not alter the number of carcinomata detected (Muir, unpublished).

### Phosphohexose isomerase (P.H.I.)

The estimation of phosphohexose isomerase in vaginal fluid could be useful in screening for cervical carcinoma (Muir 1966b). Unlike 6-phosphogluconate dehydrogenase, phosphohexose isomerase is normally present in the vaginal fluid, but in 80 per cent of completely normal women the activity is not more than 3 micromoles per minute per gramme dry weight. When this activity of 3 micromoles or more was used to distinguish between benign and malignant lesions, all carcinoma *in situ* and all but one of nineteen invasive carcinoma *in situ* were detected. Due to a high false positive rate phosphohexose isomerase has no place in the gynaecological department, but

it may be a means of screening for the population at risk. 30–36 per cent of women attending a Well Women Clinic have a gynaecological lesion (Measday, 1963), and this would increase the number with increased vaginal fluid P.H.I. activity. It is possible that phosphohexose isomerase may prove not to be the final answer, but it is unlikely that an absolutely specific enzyme test for carcinoma of the cervix will be found. It would appear to be more rational to look for an enzymological screening technique rather than a replacement for cytology.

### SUMMARY

Carcinoma of the cervix is an important cause of death in young women and successful treatment depends on an early diagnosis before symptoms have occurred. A widespread cytological survey is fully justified so that early carcinoma *in situ* can be diagnosed before the stage of malignant invasion. Various enzymes, e.g.  $\beta$ -glucuronidase, 6-phosphogluconate dehydrogenase and phosphohexose isomerase have been measured in the vaginal fluid with the object of finding a further useful screening test for cervical carcinoma. The literature on this subject has been reviewed. In addition, results are recorded of 6-phosphogluconate dehydrogenase activity in vaginal fluid in 'well women', patients with benign gynaecological lesions, and others with carcinoma *in situ* or frankly invasive carcinoma. The general conclusion was that enzyme activities in the vaginal fluid do not as yet provide a means of diagnosing carcinoma of the cervix. It is hoped that by testing further enzymes and improvements in technique a reliable means of screening for cervical cancer may be found. It is unlikely to replace cytology, but it may well reduce the need for massive cytological screening programmes.

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## CHAPTER VIII

## Inherited metabolic and enzyme abnormalities

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This chapter will be divided into a number of sections dealing with (i) abnormalities of amino-acid metabolism, especially phenylketonuria, (ii) disturbances of carbohydrate metabolism including fructose and lactose intolerance, (iii) enzyme abnormalities in neonatal jaundice, haemolytic anaemias and certain endocrine disorders. The final section (iv) is a review of useful enzyme measurements in children together with normal values for different ages.

In the other chapters of this book various serum enzyme changes resulting from disease were considered. Of greater interest are those enzyme deficiencies which actually cause or occur in genetically determined disorders. The 'inborn errors of metabolism' described by Garrod in 1908 was an important starting point in the study and understanding of normal and abnormal metabolic pathways. The concept of a metabolic block was put forward, and later on the principle of 'one gene one enzyme' was suggested by Beadle (1945).

Garrod described alkaptonuria, cystinuria, albinism and pentosuria which are all life long metabolic errors. In due course, other inborn errors of metabolism such as phenylketonuria (Fölling, 1934) and galactosaemia (Gorter, 1951) were studied. Phenylketonuria and galactosaemia are recessively inherited (i.e. in each condition the parents are heterozygotes), and both diseases are particularly important because untreated they cause mental deficiency. However, in

phenylketonuria, the early introduction of a low phenylalanine diet will result in considerable improvement (Woolf *et al.*, 1955). Likewise, in galactosaemia, a galactose-free diet given to the new-born will result in complete remission of symptoms.

In the case of an inherited enzyme defect it may be possible either to demonstrate a deficiency of the enzyme or measure substances which have accumulated or are excreted. For instance, in galactosaemia, there is a lack of galactose-1-phosphate uridyl transferase in red blood cells (Kalckar *et al.*,

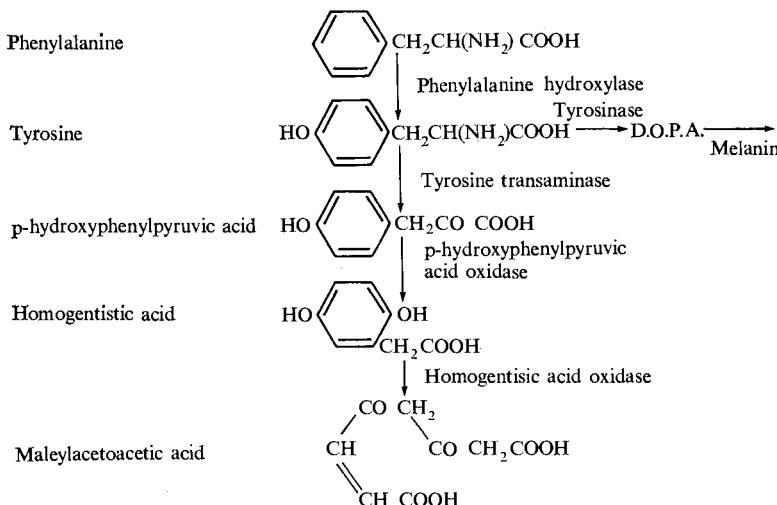


FIG. 24. Indicating metabolic pathway from phenylalanine to homogentistic acid. Homogentistic acid oxidase (homogentisicase) oxidizes homogentistic acid to the aliphatic substance maleylacetoacetic acid.

1956, Isselbacher *et al.*, 1956), and this leads to the accumulation of galactose-1-phosphate (Schwarz *et al.*, 1956). On the other hand in phenylketonuria, the diagnosis is confirmed by finding raised levels of phenylalanine in the plasma. This is due to the absence of hepatic phenylalanine hydroxylase (Mitoma *et al.*, 1957). Another example of an enzyme defect is seen in alkaptonuria, where hepatic homogentisic acid oxidase is absent (La Du *et al.*, 1958) and there is an accumulation of homogentisic acid which is excreted in the urine (see Fig. 24).

Characteristically the urine turns dark on standing. Black pigment is deposited in cartilage (ochronosis) but only later in life is the patient disabled by arthritis.

#### ABNORMALITIES OF AMINO-ACID METABOLISM

##### **Phenylketonuria**

In Great Britain the prevalence of this condition is 2–6 per 100,000 (Munro, 1947). The incidence varies in different countries; it is more common in the Irish and in the west of Scotland. On the other hand, phenylketonuria is almost unknown in the African.

As Fölling pointed out most patients are fair and have blue eyes. This lack of pigmentation is due to impaired melanin formation (this may be compared to albinism in which there is a complete absence of tyrosinase—see Fig. 24). As already mentioned the main feature in phenylketonuria is severe mental retardation, which is usually noted after 6 months. Disturbances of behaviour, epilepsy, E.E.G. changes and eczema are not uncommon, but these complications all improve on a low phenylalanine diet. Dietary restriction of phenylalanine is particularly important during the first 2 or 3 years of life. If no early diagnosis is made then degenerative changes occur in the white matter of the brain, and this structural damage accounts for the poorer response to dietary treatment in the older cases. Leucodystrophy (Schilder's disease) has also been described in four older patients with phenylketonuria (Crome, 1962).

Normally phenylalanine is rapidly hydroxylated to tyrosine. However, in phenylketonuria this cannot occur, the blood level of phenylalanine is increased, and transamination results in markedly increased urinary excretion of phenylpyruvic acid. The urine on standing has a musty smell, and using Fölling's original method the presence of phenylpyruvic acid can be detected by adding 5 per cent ferric chloride solution which gives a blue/green colouration. Phenistix applied to the wet nappy has also been extensively used as an initial screening

test for the early diagnosis of phenylketonuria. Other more reliable methods are now employed and Woolf (1967) recommends the Guthrie (1963) method using blood and chromatography of urine. It will also be possible to detect other abnormal amino-acidurias by paper chromatography e.g. Maple sugar urine disease (leucinosis).

Blood phenylalanine levels may be raised in the premature or normal neonate during the first few days of life, and Woolf (1962) recommended that in 'high risk' infants the blood phenylalanine level should be determined towards the end of the first week and also 3 weeks after birth.

For the study of heterozygotes, the plasma phenylalanine levels can be followed after a phenylalanine load, or the urinary excretion of ortho-hydroxyphenyl acetic acid can be followed by paper chromatography (Armstrong *et al.*, 1955). However, a much more rapid and sensitive method involves the use of thin layer electrophoresis on cellulose (Whatman Chromedia CC41) at 500 volts (approximately 30 volts per cm.), followed by chromatography at right angles to the direction of electrophoresis in the solvent system isopropanol/ammonia/water (200:10:20). This technique (O'Gorman, 1967) provides an excellent method for studying any abnormalities of phenolic acid excretion.

The blood levels of phenylpyruvic acid and o-hydroxyphenyl acetic acid have been determined in patients with phenylketonuria by Jervis and Drejza (1966); however, the estimation of these two compounds does not offer any clinical advantage.

### **Other amino-acid abnormalities**

Most of these abnormalities e.g. hypermethioninaemia, hyperprolinæmia (Schafer *et al.*, 1962), hypoglycinaemia, histidæmia, cystathionuria and homocystinuria can be screened by using amino-acid chromatography of plasma (Scriver *et al.*, 1964). The latter authors claim that plasma is superior apart from the cases where the renal clearance of the substance is high. Presumably in cases of this nature they advocate chromatography of plasma as well as urine.

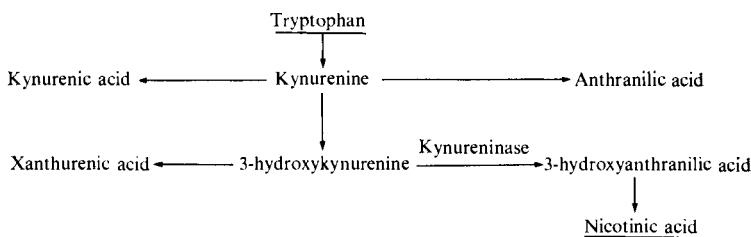
### Tyrosinosis and tyrosyluria

This rare inborn error of metabolism was first described by Grace Medes in 1932. The patient was a male Russian Jew aged 49 who had myasthenia gravis; p-hydroxyphenylpyruvic acid was found in the urine, and it was suggested that there was a deficiency; p-hydroxyphenylpyruvic oxidase. When the patient was given tyrosine, D.O.P.A. was excreted which indicated the opening up of an alternative metabolic pathway (see Fig. 24). Felix *et al.* (1951) found large amounts of tyrosine metabolites in the urine of patients with liver disease, and high excretion of tyrosine metabolites has also been reported in scorbutic patients (Boscott and Cooke (1954)). However, Robinson and Warburton (1966) now state that tyrosyluria does not occur in scurvy, and they claimed that the high excretion of tyrosine metabolites reported by Boscott and Cooke correlated with hepatic dysfunction. Dalgliesh and co-workers (1966) studied aromatic acids in urine by gas-liquid chromatography. They showed that ethyl alcohol alone given to the rat resulted in a large increase in the excretion of a tyrosine metabolite, p-hydroxyphenyl acetic acid.

### Abnormalities of tryptophan metabolism

Amino-acid chromatography was used to investigate two mentally retarded sibs described by Heeley *et al.* (1966). In these two children they found increased excretions of kynurenine, 3-hydroxy-kynurenine and xanthurenic acid. These cases are similar to the case reported previously by Komrower *et al.* (1964) who suggested that the enzyme kynureninase was absent. However, Heeley *et al.* (1966) suggested that kynureninase was present in their cases but that additional pyridoxine (above the daily normal requirement) was required for the conversion of 3-hydroxy-kynurenine to 3-hydroxy-anthranoilic acid. The figure below indicates the metabolic pathway of tryptophan to nicotinic acid. An enzyme deficiency in this pathway could lead to relative lack of nicotinic acid, and symptoms similar to those occurring in pellagra.

On the other hand, a pellagra-like rash together with neurological features also occurs in Hartnup disease (Barron *et al.* 1956). Massive amino-aciduria is accompanied by impaired jejunal absorption of tryptophan and other aminoacids. This leads to diminished synthesis of nicotinamide. It is of interest that the dermatitis and neurological picture in Hartnup disease may respond to large doses of nicotinamide.



#### ABNORMALITIES IN CARBOHYDRATE METABOLISM

This includes a variety of conditions such as benign pentosuria in Jews and symptomless essential fructosuria. On the other hand, in this group of inborn errors of metabolism there are a number of serious conditions such as galactosaemia (Woolf, 1962), which untreated may lead to severe liver damage, cataract formation and mental deficiency, and von Gierke's disease which may present with hypoglycaemic convulsions and early death of the infant. In this glycogen storage disease (type I), glucose-6-phosphatase (Cori and Cori, 1952) is absent and therefore glucose cannot be formed from glucose-6-phosphate. There are a number of other types of glycogen storage diseases which have been reviewed by Hargreaves (1963), but the patient described by McArdle (1951) was particularly interesting. He was a man of 30 who complained of muscle weakness and pain after exercise. Ischaemic exercise of his forearm caused shortening of the flexor muscles with ulnar deviation, and localized swellings of muscle were also produced. Blood lactate levels failed to increase after exercise. Other cases have now been described and histologically muscle glycogen is increased, and there is a deficiency of myophos-

phorylase. Some patients suffer from attacks of myoglobinuria and others develop epilepsy which may be precipitated by post-exertional hypoglycaemia (Salter *et al.*, 1967).

### Fructose intolerance

Chambers and Pratt first described this condition in 1956. The patient was a woman aged 24 who from the age of 10 months had complained of vomiting after eating fruit or cane sugar. Because ingestion of fructose produces vomiting and hypoglycaemic symptoms adults will learn to avoid this sugar. However, in infants repeated ingestion of fructose will lead to failure to thrive, hepatomegaly, jaundice and amino-aciduria. Diagnosis is important because a fructose-free diet will result in remission of all symptoms. The diagnosis of fructose intolerance is confirmed by carrying out a fructose tolerance test. A fructose load will cause prolonged hypoglycaemia which is probably due to the failure of release of glucose from the liver. If a large quantity of fructose is given, a dangerous deterioration of liver function can occur together with increased serum transaminase activity. The primary enzyme deficiency is lack of fructose-1-phosphate splitting liver aldolase (Froesch *et al.*, 1963) and this leads to the accumulation of fructose-1-phosphate. The situation is complicated by secondary inhibition of other enzymes concerned with fructose metabolism. Froesch *et al.* (1963) and also Swales and Smith (1966) who studied an adult case, showed a fructose rise during a fructose tolerance test, with a fall in plasma glucose, symptoms of hypoglycaemia and a fall in plasma phosphate. The rise in the plasma fructose to levels of 20–25 mg. per 100 ml. also occurred in two patients with cirrhosis. There was a fall in the phosphate level in these two cases and in normal subjects given the same dose of fructose per unit body weight.

A patient with fructose intolerance was admitted to the Sheffield Children's Hospital under the care of Dr. J. Black. She was a girl aged 7 whose selection of her own diet was responsible for both the absence of fructose in the urine and excellent state of her teeth. The rapid and significant

hypoglycaemia, after a loading dose of fructose was the main biochemical finding in this girl. The most satisfactory way of determining plasma glucose was found to be via glucose-6-phosphate dehydrogenase and N.A.D.P. The fructose levels were then determined simultaneously by the addition of phosphoglucose isomerase at the end of the glucose reaction (Bergmeyer, 1962). The fructose-1-phosphate aldolase in red cells was normal in this patient as previously shown by Froesch *et al.* (1963). Liver biopsy was not performed in this case because it was considered that the biochemical findings after fructose loading provided adequate confirmation of the diagnosis.

Fructose intolerance has recently been described in five children by Black and Simpson (1967), and two of these infants presented at the age of 7 weeks with vomiting thought to be due to pyloric stenosis. However, the diagnosis of fructose intolerance was confirmed by the oral fructose tolerance test, or satisfactory weight gain on a fructose-free diet. One infant had a liver biopsy, and subsequent examination of a piece of liver, kept in deep freeze showed complete absence of fructose-1-phosphate aldolase activity. As pointed out by these authors, microscopy of the liver in fructose intolerance is misleading and the changes may suggest neonatal hepatitis, early cirrhosis or fatty change.

### **Disaccharidase deficiency**

There may be a primary deficiency of intestinal enzymes which split disaccharides (see Fig. 25). This is seen in combined sucrase and isomaltase deficiency which is inherited as a recessive disorder, and in primary lactase deficiency. In these two primary conditions the administration of sucrose and lactose respectively will cause abdominal distension, diarrhoea and weight loss.

On the other hand, there may be a secondary disaccharidase deficiency due to mucosal damage in certain conditions such as coeliac disease (Plotkin and Isselbacher, 1964). The advent of small-bowel mucosal biopsy (Crosby, 1963) and direct

determination of intestinal disaccharidase activity (Dahlquist, 1964) has been helpful in determining the cause of disaccharide intolerance and in the study of affected families (Kerry and Townley, 1965).

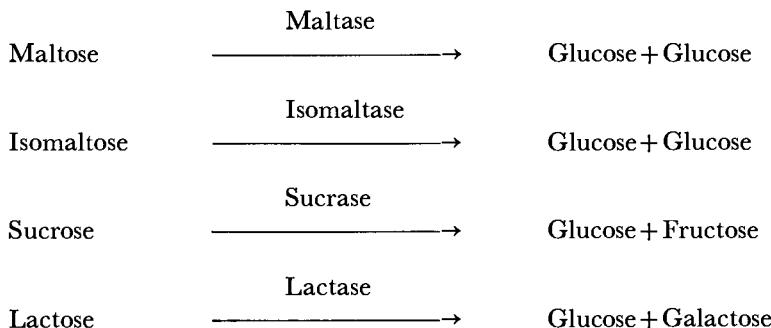


FIG. 25. Indicating the action of the intestinal disaccharidases. Maltose and isomaltose are formed by the action of amylase on starch.

### Lactose intolerance

One child with lactose intolerance and lactosuria was studied in great detail (Smith, 1967) and this led to the development of a method for the estimation of plasma lactose levels (Toseland, 1967). This patient had explosive diarrhoea, and was admitted to the Sheffield Children's Hospital under the care of Dr. J. Black. Lactose was present in the urine, the amounts varying from 0.5 to 1.0 per cent and there was no amino-aciduria. The stool pH was low (5.0–5.4) on several occasions particularly after lactose loading tests, and faecal lactic acid excretion (Podmore and Clarke, 1966) also increased after oral lactose. However, it was noted the glucose curve following a lactose load was never completely flat except on one occasion. Lactose intolerance and lactosuria was first described by Durand (1958). Intact lactose may be absorbed causing toxic symptoms and lactosuria (Holzel *et al.*, 1962). Congenital lactase deficiency is a different entity and is usually not associated with lactosuria.

Another patient investigated had lactase deficiency, very high faecal lactic acid output, but no lactosuria. This patient

had no measurable glucose, lactose or galactose response to a lactose load, and this particular case is more in keeping with the lactose intolerance without lactosuria described by Holzel, Schwartz and Sutcliffe (1959). Intestinal disaccharidase activity was not measured in this patient. However, in the study of three adults with temporary lactase deficiency, the method for determining intestinal disaccharidase activity (Dahlquist, 1964) proved to be very satisfactory. The topic of lactase deficiency has recently been well reviewed by Lifshitz (1966).

#### ENZYME ABNORMALITIES IN OTHER DISORDERS

##### **Neonatal jaundice**

Severe jaundice in the neonatal period may present diagnostic difficulties, and urgent exchange transfusion may be required to prevent brain damage (*kernicterus*). Jaundice may be transient or physiological owing to impaired conjugation of bilirubin. Deficiency of hepatic glucuronyl transferase occurs in the first few days of life, and in the premature infant lack of this enzyme is more likely to cause failure of bilirubin conjugation. In addition, large doses of vitamin K may cause haemolysis and increase the degree of jaundice. Alternatively glucuronyl transferase activity may be inhibited by either a drug like novobiocin, or by an abnormal steroid which is present in the milk of some mothers (see Chapter I). Permanent failure of bilirubin conjugation occurs in the rare Crigler-Najjar Syndrome which is characterized by severe unconjugated hyperbilirubinaemia, *kernicterus* and early death.

In practice the more common cause of neonatal jaundice should first be considered. These include haemolysis due to blood group incompatibility, infections—umbilical sepsis, neonatal viral hepatitis, toxoplasmosis, cytomegalic inclusion body disease and obstructive jaundice as seen in the inspissated bile syndrome and congenital biliary atresia. Enzyme tests may be helpful in differential diagnosis. For instance, an early acute elevation in S.G.P.T. activity will occur in hepatitis.

The estimation of serial serum transaminase activity may be useful in differentiating the inspissated bile syndrome from congenital malformation of the bile ducts (Kove, 1960). In the former condition there is an early rise in transaminase activity in the first week of life, while in the latter condition, increased transaminase activity begins later at about 1 month of age. Biliary atresia may lead to well-established cirrhosis within 3 months (Moore, 1953), and therefore an early diagnosis is important so that plans can be made for surgical correction. Cretinism may also be associated with jaundice and in these cases the serum creatine phosphokinase activity may be elevated but will return to normal after treatment with thyroxine.

Other inherited metabolic and haematological causes have to be considered. For instance, cases of galactosaemia may present with neonatal jaundice and later develop cirrhosis. The early demonstration of absent galactose-1-phosphate-uridyl transferase in the red cells in such a case will be of great diagnostic importance, and the early withdrawal of all milk should be life saving. Fructose intolerance, which is discussed above can also present with jaundice. Glucose-6-phosphate dehydrogenase deficiency may cause neonatal jaundice in patients of Mediterranean extraction (Doxiades *et al.*, 1961) and haemolytic anaemia due to pyruvate kinase deficiency is now a well-defined entity (Valentine and Tanaka, 1966) and some cases have had exchange transfusions in the neonatal period (Tanaka *et al.*, 1961).

### **Endocrine abnormalities**

#### *Cretinism*

A number of enzymes are required within the thyroid to maintain normal function, and an inherited deficiency of one of these enzymes can result in hypothyroidism and goitre. For instance the enzyme which oxidizes iodide may be missing (Stanbury and Hedge, 1950). This type of enzyme deficiency is frequently associated with congenital deafness (Pendred's

TABLE 22. *Normal values of serum enzymes*

<i>Enzyme</i>	<i>Age of subjects</i>	<i>Range of values in normal subjects</i>	<i>Reference</i>
Alkaline phosphatase	0-1 yr. 1-2 yr. 2-6 yr. 6-10 yr. 10-14 yr. 14-18 yr. 26-28 yr. Newborn-2 wk. 2-13 yr. 13-16 yr. 16-18 yr.	10.6-27.5 (King-Armstrong units)*† 8.2-22.7 8.8-20.2 8.0-22.7 2.8-24.1 0.3-21.0 1.6-9.9 2.71-8.86 (King-Armstrong units)† 2.77-6.78 2.01-5.25 1.06-3.14	Clark and Beck
Acid phosphatase	3 mo.-adult	5-120 (spectrophotometric units)	Kove
Glutamic-oxaloacetate transaminase (G.O.T.)	3 mo.-adult	5-40	Kove
Glutamic-pyruvate transaminase (G.P.T.)	Newborn-3 mo.	5-90 (spectrophotometric units)	Kove
Lactic acid dehydrogenase (L.D.H.)	3 mo.-adult	5-40	Lending <i>et al.</i>
	Newborn	44.0-254.0 (spectrophotometric units)	Gautier <i>et al.</i>
	0-30 da.	24.0-102.1 (spectrophotometric units)	
	1 mo.-2 yr.	21.2-6.40	
	3-12 yr.	11.2-4.88	
Pseudocholinesterase	Newborn (term)	0.97-4.46 (I.U./ml.)†	Lehmann <i>et al.</i>
	1-4 wk.	2.41-5.27	
	1-2 mo.	2.23-6.16	
	Adults	2.45-5.58	

<i>Amylase</i>	Birth-1 yr. 1 yr.-12 yr. 12 yr.-adult	0-127 (Somogyi units)† 8-183 19-103	Gautier <i>et al.</i>
<i>Aldolase</i>	0-2 yr. 2-5 yr. 5-15 yr. 15-25 yr. Newborn Neonate-adult‡	2.7-25.5 (Bruns units)† 3.5-18.3 3.8-16.6 3.3-15.5 Male < 1.80 I.U. (mean = 0.4 I.U.) Female < 1.80 I.U. (mean = 0.4 I.U.)	Clayton <i>et al.</i>
<i>Creatine kinase</i>	Newborn 1-12 mo. 1-2 yr. Adult	May normally range up to 12 I.U. 58-302 (Goldberg-Rutenberg units)† 70-218 85-193 86-194	Vasella <i>et al.</i> Richterich <i>et al.</i>
<i>Leucine aminopeptidase</i>			Kaplan and Ruark

\* Original data were presented in Bessey-Lowry-Brock (B.L.B.) units. These have been converted to King-Armstrong (K.A.) units letting 1 B.L.B. unit = 2.32 K.A. units.

† Mean values  $\pm$  2 standard deviations.

‡ Variable serum levels have been reported in children under 6 years of age. This is possibly related to the inability of this age group to restrict strenuous activity (which elevates serum creatine kinase) prior to the obtaining of serum.

By courtesy of Professor R. R. Howell and the C. V. Mosby Company.

Syndrome). Other types of cretinism may be due to inability to concentrate iodide in the thyroid, failure of coupling of iodotyrosines to form tri-iodothyronine and thyroxine, and a deficiency of deiodinase. Lack of this latter enzyme will result in excessive loss of iodotyrosines from the thyroid and iodine deficiency.

#### *Adrenogenital syndrome*

This condition may present with virilism, virilism and hypertension, or features of adrenocortical insufficiency.

An inherited enzyme deficiency results in impaired cortisol synthesis. Low cortisol levels stimulate a 'feed back' mechanism resulting in increased A.C.T.H. production and hyperplasia of the adrenal cortex. Androgen is therefore produced in large amounts and the female neonate will show evidence of male pseudohermaphroditism. However, in males the genitalia may be normal at birth, and the infant may present with gross dehydration and salt depletion due to urinary loss, vomiting and diarrhoea. Fatal circulatory collapse is likely and early treatment with hydrocortisone is essential. In this type of adrenocortical insufficiency there is complete failure of C<sub>21</sub> hydroxylation and grossly impaired synthesis of cortisol and aldosterone. A different enzyme deficiency occurs in cases with hypertension. Here, the failure is in C<sub>11</sub> hydroxylation, and this results in the accumulation of 11-deoxycorticosterone which causes salt retention and hypertension. The biochemical aspects of the adrenogenital syndrome have been fully discussed elsewhere (Stempfel and Tomkins, 1966).

#### SERUM ENZYME MEASUREMENTS IN CHILDREN

The review by Howell (1966) focuses attention on the problem of serum enzyme estimations in children. Howell demonstrated the dangers of failing to measure enzyme activity in a large number of 'normal' children in exactly the same age group, particularly in respect of lactic acid dehydrogenase (see Table 22).

A study has recently been carried out at the Sheffield Children's Hospital on the levels of serum  $\alpha$ -hydroxybutyric acid dehydrogenase (S.H.B.D.) in children of all ages (Hirst, 1967, Fig. 26). These results were obtained on an autoanalyser coupled to a flow cell in a Beckman DB spectrophotometer. It was only in this way that sufficient results could be obtained

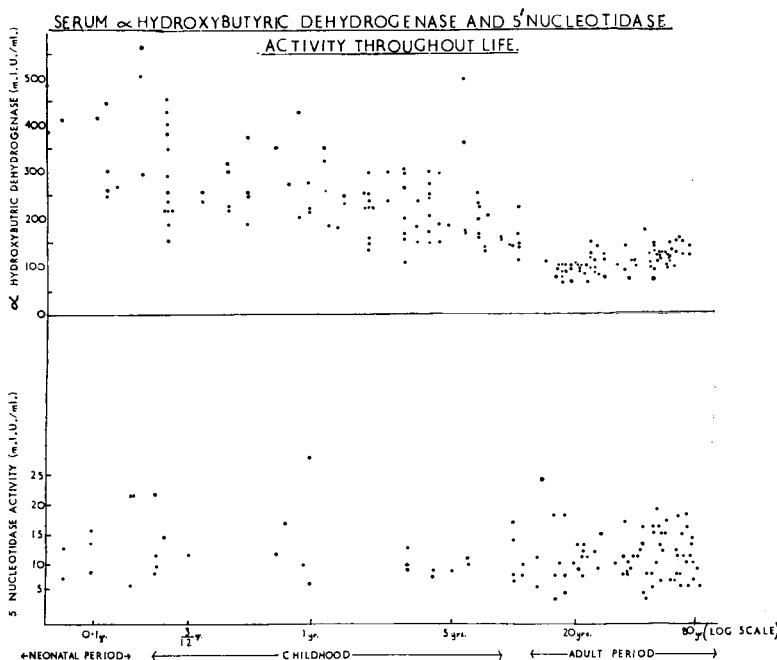


FIG. 26. By courtesy of D. Hirst and A. Belfield. Indicating the levels of serum hydroxybutyric dehydrogenase and 5'-nucleotidase activity throughout life.

in order to make comments about a normal range. It will be noted from Fig. 26 that the wide normal range for S.H.B.D. in young children does not flatten out to the lower 'adult' level until the age of 14. These results are interesting and are paralleled by the results of their serum alkaline phosphatase activity. The correlation between the rate of bone growth and the plasma alkaline phosphatase activity can be seen in the classical figure by Clarke and Beck (1950). As expected the serum

5'-nucleotidase activity was not increased in growing children (Belfield, 1967 Fig. 26). The main value of the determination of 5'-nucleotidase activity is to establish the source of a raised serum alkaline phosphatase level (Wachstein and Sigismondi,

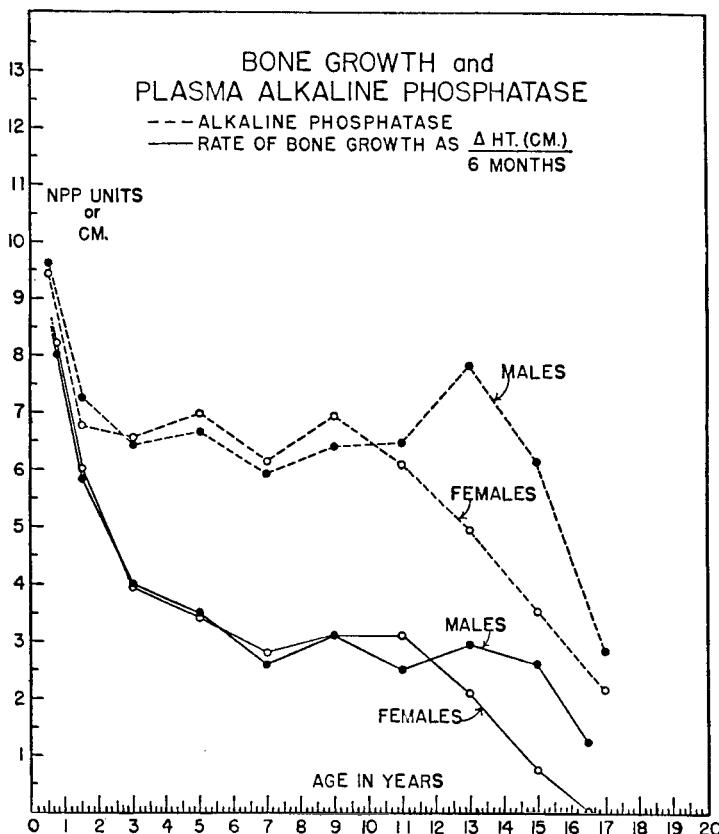


FIG. 27. Comparison of the rate of bone growth and plasma alkaline phosphatase levels in infancy and childhood. (Clark and Beck, 1950). By courtesy of the C. V. Mosby Company.

1958; Young, 1958). Although fewer estimations of serum 5'-nucleotidase were obtained (owing to a lack of automated procedure), normal levels of this enzyme confirmed the bony origin of the raised serum alkaline phosphatase levels in growing children.

The 5'-nucleotidase determinations (Belfield, 1967) were carried out using activation with magnesium rather than the nickel inhibition of Campbell's method (1962). Problems caused by turbidity prompted the investigation of this modified procedure, and it is interesting to note that Hill and Sammons (1966) also reported difficulties due to turbidity with the Campbell method in automation. In his review, however, Howell (1966) expresses the view that 'measurement of this enzyme in serum has no diagnostic advantage over several other enzymes'. During the above study of 5'-nucleotidase determinations in the United Sheffield Hospitals, only once was a specific request made for liver/bone differentiation of a raised serum alkaline phosphatase level.

### **Serum aspartate and alanine aminotransferase (S.G.O.T. and S.G.P.T.)**

There is widespread use of the adult normal range being applied to children in spite of the findings of Kove (1960) who showed that in the first 3 months of life the S.G.O.T. levels can be elevated up to three times the adult upper level of normal, and the S.G.P.T. up to twice the normal adult upper level.

### **Serum creatine phosphokinase: (C.P.K.)**

The estimation of this enzyme is important in paediatrics. For instance Vassella *et al.* (1965) made a careful study of 216 patients. All fifty-five cases with the Duchenne type of progressive muscle dystrophy had increased C.P.K. activity, but patients with neurogenic muscle atrophy, as in Werdnig-Hoffmann's disease had normal levels.

Various methods exist for the determination of serum C.P.K. activity e.g. Hughes (1962), Vasella *et al.* (1965), Richterich *et al.* (1963), Tanzer and Gilvarg (1959), Finley and Anderson (1966). There was an interesting discussion on this subject at the VIth International Congress of Clinical Chemists, where most speakers had little faith in what they called the 'colorimetric methods'. However, Whitehead and Shuttleworth (1965) reported favourably on spectrophotometric and

fluorometric methods. All reported favourably on the addition of SH groups to sera either prior to storage at  $-20^{\circ}\text{C}$ . or immediately prior to enzyme assay. SH groups were usually supplied in the form of cysteine or the more expensive dithio-threitol. Using these refined techniques carriers of muscular dystrophy could readily be detected.

From a practical point of view the kit supplied by 'Calbiochem Ltd.' has been found to be particularly useful especially for small numbers of determinations. It should be emphasized that the only way of establishing a normal range for children of different ages is by the use of automated procedures. However, concentrations of enzymes, substrates and co-enzymes (e.g., N.A.D.) can make automation very expensive; particularly as these solutions stand at room temperature for several hours. Two ways of overcoming these problems include (i) the use of fluorimetry, where in order to obtain satisfactory readings very low concentrations can be used and (ii) employing a flow cell through an ultra-violet spectrophotometer that has facilities for scale expansion.

### SUMMARY

A number of 'inborn errors of metabolism' have been considered. Particular attention was paid to phenylketonuria, fructose and lactose intolerance, neonatal jaundice and certain endocrine abnormalities. The demonstration of an absent enzyme, e.g. in galactosaemia, may be of great diagnostic importance. Alternatively, in certain enzyme deficiencies, it may be more convenient to measure metabolites which have accumulated. Treatment is often very satisfactory; for instance, mental deficiency in phenylketonuria will be prevented by introducing a low phenylalanine diet. Screening for early cases is therefore essential. The possibility that an enzyme deficiency in an otherwise fatal metabolic disease could be treated by organ transplantation is worth considering.

The final section of this chapter includes some useful enzyme measurements in children together with normal values for

different ages. The best way of establishing a normal range is by the use of an automated procedure.

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## CHAPTER IX

## Enzyme changes in diabetes mellitus

The enzyme changes in diabetes will be considered under the following sections mentioned below.

- (i) Adaptive enzyme changes, e.g. due to altered carbohydrate metabolism in the liver.
- (ii) Enzyme changes related to arterial disease and the 'diabetic angiopathy'.
- (iii) Acute elevation of serum enzymes associated with keto-acidosis.
- (iv) Abnormal liver function tests in diabetes mellitus.
- (v) Enzyme changes resulting from one of the common complications of diabetes.
- (vi) Results of studies in which serum and urinary leucine aminopeptidase (L.A.P.) were measured in well-controlled diabetes mellitus and in a number of other patients with some of the serious complications of this disease will be recorded (Mullan, 1966).

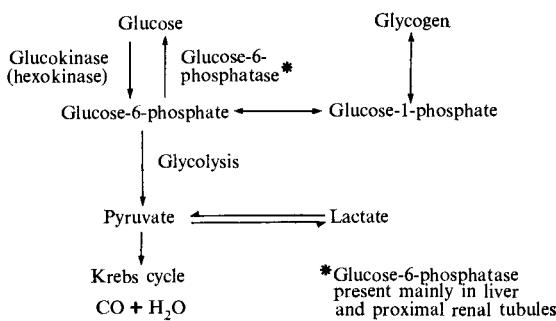
Since the discovery of insulin by Banting and Best in 1922, it has become clear that a simple deficiency of circulating insulin is not the only cause of diabetes. There are other important factors which 'diminish the effectiveness of insulin' (Joslin, 1959). These factors include undue conjugation of insulin with protein in the liver (Antoniades *et al.*, 1962), and the presence of various circulating insulin antagonists (Vallance-Owen, 1962).

### **(i) Adaptive enzyme changes**

Insulin carried to the liver in portal venous blood may be

destroyed by insulinase (Mirsky, 1957). This author made the interesting observation that insulinase activity varied according to the diet. For instance, hepatic insulinase activity remained low when a high protein diet was given to rats that were previously fasting. In contrast, a high carbohydrate diet resulted in the return of insulinase activity to normal in 24 to 48 hours. Mention was also made of a child with hypoglycaemia who had an extremely low level of insulinase activity in the liver.

In diabetes mellitus a number of important adaptive enzyme changes occur in the liver and other tissues. Hepatic glucokinase (hexokinase) is markedly decreased and the concentration



\* Glucose-6-phosphatase present mainly in liver and proximal renal tubules.

of glucose-6-phosphatase is doubled (Cahill *et al.*, 1959). Both these enzymes catalyse essentially 'unidirectional' reactions and, in untreated diabetes, the liver is unable to take up glucose in spite of hyperglycaemia. Treatment with insulin results in a fall of blood sugar and after a lag period glucose phosphorylation returns to normal with a fall of glucose-6-phosphatase activity. Cahill *et al.* (1959) considered that the primary biochemical lesion in the liver of the diabetic animal was diminished glucokinase activity.

On the other hand, muscle hexokinase catalyses the irreversible conversion of glucose to glucose-6-phosphate, and insulin accelerates the membrane transport of glucose into the muscle cell.

### (ii) $\beta$ -glucuronidase and diabetic microangiopathy

Of particular interest has been the recent report of increased serum  $\beta$ -glucuronidase in diabetes mellitus (Miller *et al.*, 1966). The method of Fishman (1965) was employed, and this depends on the enzymic hydrolysis of the substrate phenolphthalein-glucuronide, to form glucuronic acid and phenolphthalein. The latter compound is measured colorimetrically, and  $\beta$ -glucuronidase activity is expressed in microgrammes of chromogen formed per 100 ml. of serum per hour. Enzyme activity was measured in 100 diabetics and sixty-three controls and the mean  $\beta$ -glucuronidase value was 2160 and 1290 respectively. In both groups the mean activity of this enzyme was highest in the 40 to 49 age group. In addition diabetics on insulin or with atherosclerosis tended to have higher serum enzyme levels compared to mild cases on dietary restriction or oral hypoglycaemic agents only. Miller *et al.* (1966) also found increased serum  $\beta$ -glucuronidase activity in forty non-diabetics with coronary artery disease. In this connection, Herman and Gorlin (1965) have confirmed that pre-clinical diabetes (demonstrated by a raised fasting blood sugar or an abnormal intravenous glucose tolerance test) occurred in a high proportion of patients with premature coronary artery disease. The diagnosis in their patients was confirmed by coronary angiography.

Winegrad and De Prati (1965) suggested that in diabetes mellitus the non-insulin-sensitive glucuronic acid cycle was used to greater degree in the metabolism of glucose in diabetic patients. This resulted in the increased production of uridine diphosphate glucuronic acid, which was necessary for the formation of mucopolysaccharides and glycoproteins. These substances were laid down in capillary basement membrane in diabetic patients (Spiro, 1963), and these changes represented the main biochemical lesion of the so-called diabetic microangiopathy (Bloodworth, 1963). Miller and his co-workers have put forward the idea that increased serum  $\beta$ -glucuronidase activity in diabetics may help to prevent the

deposition of undue amounts of polysaccharides in their arteries and capillaries.

**(iii) Enzyme changes in diabetic acidosis, during treatment of ketosis and in coma**

There is not much information on the question of serum enzyme levels in diabetic acidosis. However, Seige (1961) noted increased serum transaminase activity in a proportion of newly diagnosed uncontrolled diabetics, and in some patients the serum L.D.H. may be raised (Wróblewski and La Due, 1955). More recently Vélez-Garcia *et al.* (1966), using a cysteine stimulated technique, measured serum C.P.K. activity in a variety of conditions. During the course of these studies, they unexpectedly found that this enzyme was elevated during treatment of diabetic ketosis. Prior to treatment serum C.P.K. levels were normal and peak activity was reached 24 to 48 hours after starting treatment. A similar increase in serum C.P.K. activity was noted during treatment with insulin and intravenous fluids of two patients with non-ketotic hyperosmotic coma. This increase in serum C.P.K. activity could be related to the acute shift of fluid and electrolytes between the various body compartments, and presumably the increased serum C.P.K. activity was derived from muscle.

Previous authors (Somogyi, 1940; Gray *et al.*, 1941) found low levels of serum amylase in diabetic coma, and Tully and Lowenthal (1958) stated that a raised serum amylase in cases of diabetic coma indicated acute pancreatitis. Of interest was the finding by Finn and Cope (1963) of gross elevation of plasma amylase in eight out of eleven patients in diabetic coma. This increased plasma enzyme activity was not due to acute pancreatitis, and post-mortem examination was normal in five patients. One patient who died had no elevation of the plasma amylase, but in five other cases who died the plasma amylase was 800 units or over. It was suggested that in these patients the elevation of this enzyme could be due to release of liver amylase in patients with grossly disordered carbohydrate utilization (Janowitz and Dreiling, 1959).

TABLE 23. Well-controlled Diabetes mellitus and normal L.A.P. levels

Case	Age	Sex	Duration years	Therapy	Control	FH	Thin Av. Obese	Complications	Serum L.A.P.	24 hr. urinary L.A.P. (10 N.V.) (24 hr. vol.)	Proteinuria	B.P. Bl. urea
1	36	M	2/12	Chlorpropamide	Good	-ve	Av.	None	171 (7/3/67)	152 (1.56 l.)	0	130/80
2	49	M	5	Chlorpropamide	Fair	-ve	Obese	Depression	105 (23/7/65)	125 (1.225 l.)	0	130/90
3	41	M	3	Chlorpropamide and phenformin	Good	+ve	Thin	Retinopathy	166 (23/6/65)	63 (2.35 l.)	0	34 mg. %
4	61	M	10	Chlorpropamide → insulin	Good	-ve	Thin	None	153 (21/3/67)	93 (1.55 l.)	0	160/80
5	49	F	11	Chlorpropamide → insulin	Good	-ve	Obese	Retinopathy and hard exudates	117 (3/3/67)	36 (0.8 l.)	0	140/90
6	32	M	2/12	Small dose insulin	Fair	+ve	Av.	None	100 (24/3/65)	32 (1.02 l.)	0	130/75
7	53	M	2	Small dose insulin	Fair	+ve	Av.	None	100 (24/3/65)	95 (0.95 l.)	0	30 mg. %
8	62	F	6/52	Small dose insulin	Fair	-ve	Thin	Chronic bronchitis and asthma	117 (28/7/65)	65 (0.87 l.)	0	130/80
9	17	M	6/12	Insulin	Fair	-ve	Thin	None	153 (28/2/67)	52 (1.16 l.)	0	44 mg. %
10	30	F	14	Insulin	Good	-ve	Thin	None	87 (23/7/65)	65 (1.0 l.)	0	190/110
11	50	M	3	Insulin	Fair	+ve	Av.	Depression	141 (24/3/65)	41 (1.29 l.)	0	17 mg. %
12	15	M	6/52	Insulin	Fair	-ve	Thin	coal gas poisoning	189 (29/9/65)	153 (1.57 l.)	0	120/80
									MALE AV.	96		
	9	M							FEMALE AV.	142		
	3	F								107		
TOTAL	12		AV. 4 years							55		

TABLE 24. *Diabetes mellitus with increased L.A.P. levels*

Case	Age	Sex	Duration years	Therapy	Control	FH	Thin Av. Obese	Complications	Serum L.A.P.	L.A.P. (to N.Y.) (24 hr. vol.)	Proteinuria	B.P.	Bl. urea
19	46	F	2½	Insulin	Poor	-ve	Thin	P.H. of pneumonia and diabetic ketosis	175 (30/6/65)	315 (1.02 l.)	±	140/90 36 mg. %	
20	23	F	10	Insulin	Poor	+ve	Thin	Previous diabetic ketosis	157 (23/6/65)	125 (3.12 l.)	±	150/100 40 mg. %	
21	27	M	11	Insulin	Poor	-ve	Thin	Admitted x 17 since 1954. Low I.Q.	231 (9/6/65)	163 (2.24 l.)	±	130/80 40 mg. %	
									211 (10/8/66)	185 (1.36 l.)	±		
22	20	M	11	Insulin	Poor	-ve	Av.	No complications	216 (9/9/65)	107 (0.62 l.)	±	140/90	
23	40	M	15	Insulin	Poor	-ve	Thin	Low I.Q. identical twin not diabetic	207 (15/9/65)	92 (2.04 l.)	±	140/90	
24	56	M	23	Insulin	Fair	-ve	Av.	Retinopathy	216 (11/9/65)	163 (1.81 l.)	+	140/90	
25	29	M	8½	Insulin	Poor	-ve	Thin	Cataracts (demolition worker)	225 (1/4/67) (10/4/67)	147 (3.27 l.) 167 (3.71 l.)	0	115/80 48 mg. %	
26	15	F	5	Insulin	Fair	-ve	Av.	No complications	201 (9/7/65)	115 (1.32 l.)	±	130/70 51 mg. %	
27	76	F	18	Insulin	Fair	-ve	Thin	Retinopathy	198 (7/3/67)	81 (1.08 l.)	±		
28	34	F	8	Insulin	Poor	-ve	Av.	Increasing insulin requirements	153 (26/2/67)	195 (1.24 l.)	0	130/75 32 mg. %	

29	51	F	4	Phenformin	Poor	-ve	Obese	Rash with chlorpropanamide	140 (28/7/65)	108 (1.2 l.)	0	150/90 31 mg. %
30	49	M	6/12	Insulin	Poor	-ve	Av.	P.H. recurrent acute pancreatitis.	180 (25/4/66)	186 (2.36 l.)	0	130/80 36 mg. %
31	37	M	7	Insulin	Fair	-ve	Av.	Subtotal pan-createctomy 1962 Recent haematuria	212 (22/11/65)	135 (2.0 l.)	+	50 mg. %
7	M							<i>B. coli</i> infection. Satisfactory response to furadantin			0	20 mg. %
TOTAL	13	F		Av. 9 years								
					MALE AV.				212	142		
					FEMALE AV.				170	156.5		

**(iv) Abnormal liver function tests in diabetes**

In young patients with diabetic ketosis the liver is often tender and palpable but with stabilization the liver diminishes in size. Hepatomegaly in these insulin-sensitive cases is due to the deposition of excessive amounts of glycogen. On the other hand, middle-aged obese diabetics often have marked fatty infiltration of the liver. In spite of these pathological changes the crude liver function tests are usually normal. However, in a small proportion of diabetics, there may be some elevation of the serum alkaline phosphatase with an abnormal thymol turbidity and impairment of bromsulphthalein excretion (Camerini-Davalos *et al.*, 1962). Slight elevation of the serum alkaline phosphatase would correlate with the finding of some increase in serum L.A.P. levels in poorly controlled diabetic men (see Table 24). Goldbarg *et al.* (1963) also found some increase in serum  $\gamma$ -glutamyl transpeptidase in thirty out of eighty-five diabetics. The highest values for this enzyme occurred when one or more liver function tests were abnormal.

**(v) Enzyme changes secondary to complications of diabetes**

It has been confirmed that the levels of serum L.D.H., S.G.O.T., S.G.P.T. and alkaline phosphatase were normal in well-controlled diabetes mellitus (Nakamura *et al.*, 1964). The latter workers studied 161 patients but excluded four cases with associated liver disease. Thus, acute elevation of S.G.O.T. in a diabetic would be good confirmatory evidence of myocardial infarction. Furthermore, the diagnosis of myocardial infarction may be easily missed in a relatively young patient in diabetic coma; in such a case, pulmonary oedema may be precipitated by over-enthusiastic intravenous therapy.

The other important complication, i.e. diabetic nephropathy, will give rise to increased urinary loss of enzymes, e.g. L.D.H. and L.A.P. The changes in serum and urinary L.A.P. will be discussed in the next section of this chapter.

**(vi) Leucine aminopeptidase (L.A.P.) activity in diabetes mellitus**

Serum and urinary L.A.P. activity was measured by the method of Goldbarg, Pineda and Rutenburg (1959). The figure indicates the upper limits of normal for serum L.A.P. (mean + S.D.  $\times 2$ ) and urinary L.A.P. (mean + S.D.  $\times 3$ ). As can be seen, the L.A.P. levels given by these authors tend to be lower in normal women.

<i>NORMAL L.A.P. VALUES:</i> (Goldbarg, Pineda and Rutenburg, 1959)	<i>Serum</i>	<i>24 hr. urinary</i>
	<i>L.A.P.</i>	<i>L.A.P. (10 N.V.)</i>
Female	< 184	< 79
Male	< 200	< 169

Table 23 includes data on twelve well-controlled diabetics. Control was considered to be good when the fasting blood sugar was under 200 mg. per cent on most occasions, and when there was no history of frequent ketosis or hypoglycaemic attacks. Two patients had diabetic retinopathy, one woman suffered from chronic bronchitis and asthma, and another two patients were admitted to hospital suffering from depression. In all cases the serum and urinary L.A.P. levels were normal. It is interesting to note that, except for one case, the 24-hour urinary volumes were all under 1.6 litres.

Patients with increased serum or urinary L.A.P. levels are shown on Table 24. The serum was taken from fasting patients after stabilization in hospital, and a 24-hour collection of urine was made at the same time. Nine out of thirteen of these patients had been poorly controlled. The usual complications were encountered, and in two patients (cases 21, 23) low intelligence made good control impossible. One patient (case 21) had a total of seventeen admissions either with severe hypoglycaemia, which had caused transient hemiplegia, or severe diabetic ketosis with abdominal pain, tender liver enlargement, neck stiffness and a normal cerebrospinal fluid.

It was interesting to note that in six out of seven poorly controlled men the serum L.A.P. level was above the upper limit of normal, while in all four poorly controlled diabetic

women the urinary L.A.P. excretion was increased but the serum level of this enzyme was normal.

At first it was thought that normal serum and urinary L.A.P. levels might be an index of good diabetic control. However, further studies on six men (see Table 25) showed that patients previously poorly controlled could have normal serum and urinary L.A.P. levels. Particular note should be made of case 18. Slight proteinuria due to ketosis in this patient was not associated with an increased urinary excretion of L.A.P. After restabilization, the serum and urinary L.A.P. levels were normal, but in spite of this the patient died 1 year later with severe hypertension, renal failure and blindness. This case emphasizes the sinister prognosis in patients with childhood onset diabetes who develop severe hypertension.

Table 26 gives clinical details of four diabetic patients, all of whom had heavy proteinuria. The first three patients (cases 32, 33, 34) have all, up to the present time, remained fairly well. However, the last patient in this group (case 35) had severe hypertension and retinopathy. His condition deteriorated and he died in renal failure 1½ years later. Histology of his kidneys (see Figs. 28 and 29) revealed extensive glomerular damage with nodular diabetic glomerulosclerosis, and Fig. 29 also shows fibrinoid necrosis of a blood vessel.

As can be seen (Table 26) there was a marked increase in urinary L.A.P. excretion in all patients with heavy proteinuria and in three cases the serum L.A.P. level was above the upper limit of normal. In case 33 the serum L.A.P. was normal but the high urinary L.A.P. value indicated diabetic kidney disease. This latter patient was a heavy smoker and the presence of a hilar shadow on his chest X-ray at first suggested the possibility of a bronchial carcinoma. After a course of tetracycline the pulmonary shadow cleared completely and his oedema also disappeared.

The next group of cases (Table 27) consisted of seven elderly patients with severe peripheral arterial disease. However, in case 40, the main disability was due to acute infection of a large neurotrophic ulcer. This patient was treated with antibiotics

TABLE 25. *Poorly controlled Diabetes mellitus and normal L.A.P. levels*

Case	Age	Sex	Duration years	Therapy	Control	FH	Thin Av. Obese	Complications	Serum L.A.P.	L.A.P. (10 N.V.) (24 hr. vol.)	24 hr. urinary Proteinuria	B.P. Bl. urea
13	33	M	5	Insulin	Poor	-ve	Thin	None	96 (16/765)	131 (1.64 l.)	0	120/90
14	50	M	17	Insulin	Poor	-ve	Av.	Bilateral cataracts and retinopathy	185 (28/765)	162 (1.66 l.)	±	170/95
15	44	M	10	Insulin	Poor	+ve	Thin	Retinopathy and retinal detachment peripheral neuropathy	144 (9/9/65)	69 (1.02 l.)	0	53 mg. % 140/80
16	19	M	1½	Insulin	Poor	-ve	Thin	None	162 (9/9/65)	83 (2.022 l.)	0	40 mg. % 140/85
17	17	M	14	Insulin	Poor	+ve	Av.	Intermittent proteinuria since 1963	171 (25/4/66)	107 (1.3 l.)	±	48 mg. % 120/80
18	21	M	16	Insulin	Poor	+ve	Av.	Ketotic	133 (24/3/65)	106 (0.46 l.)	++	36 mg. % 140/80
								Restabilized	122 (29/3/65)	161 (2.04 l.)	0	—
								Died 1 year later with fulminating Kimmelmel Wilson syndrome			++	200/140
TOTAL	6	M	10½ years					MALE AV.	145	122		

TABLE 26. *Diabetes mellitus with heavy proteinuria*

<i>Case</i>	<i>Age</i>	<i>Sex</i>	<i>Duration years</i>	<i>Therapy</i>	<i>Control</i>	<i>FH</i>	<i>Thin Av.</i>	<i>Complications</i>	<i>Serum L.A.P.</i>	<i>24 hr. urinary I.A.P. (to N.V.)</i> (24 hr. vol.)	<i>Proteinuria</i>	<i>B.P.</i>	<i>Bl. urea</i>
32	31	M	16	Insulin	Fair	-ve	Thin	Trauma toe infection. Retinopathy. D.U.	243 (19/5/65) 24 (5/6/65)	474 (2.3 l.) 408 (2.66 l.)	++	150/90	
33	41	M	16	Insulin	Fair	-ve	Thin	Ankle oedema. Chest infection	277 (19/6/65) 31 (23/7/65)	368 (2.22 l.) 475 (1.22 l.)	+++	66 mg. %	
34	33	M	31	Insulin	Poor	-ve	Av.	Intermittent claudication. Angiography: L. femoral artery block	207 (3/4/67)	331 (1.84 l.)	++	120/70	
35	41	M	20	Insulin	Poor	-ve	Thin	Severe retinopathy. Oedema. Peripheral neuropathy Died 1½ years later with fulminating Kimmelstiel Wilson syndrome*	203 (12/8/65)	289 (2.49 l.)	++	46 mg. %	
											Serum Alb.	3.0	
											Glob.	2.0 g./100 ml.	
											Bl. cholesterol	365 mg./100 ml.	
<b>TOTAL</b>	<b>4</b>	<b>M</b>	<b>Av. 21 years</b>								<b>MALE AV.</b>	<b>196</b>	<b>392</b>

\* See Fig. 28 showing extensive renal damage.

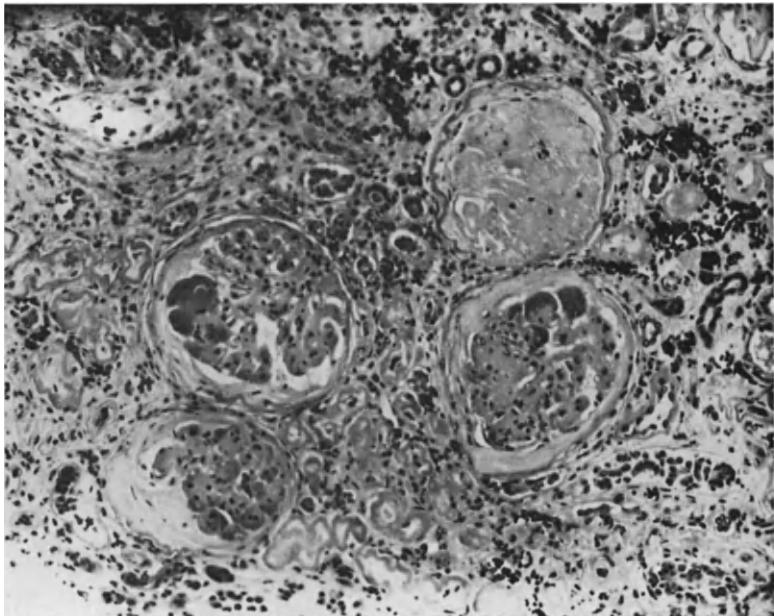


FIG. 28. Photomicrograph showing extensive glomerular damage with nodular diabetic glomerulosclerosis. By courtesy of Dr. L. Henry.

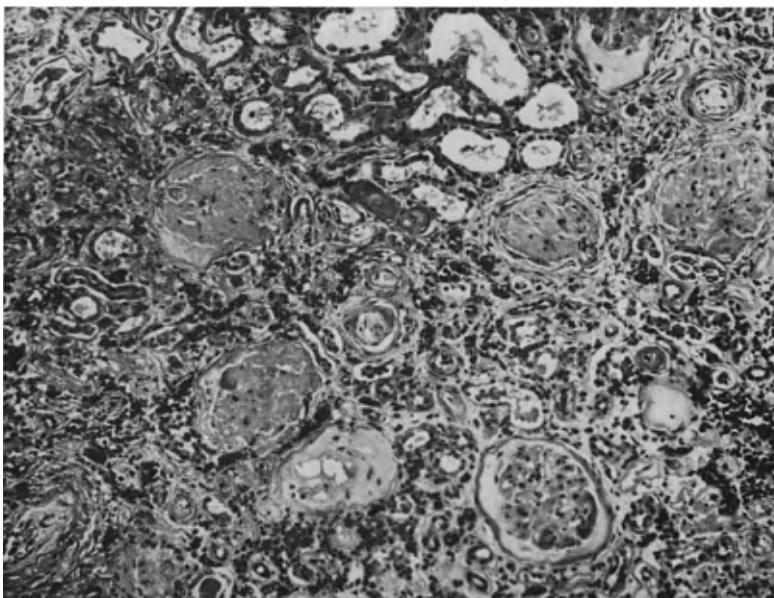


FIG. 29. Photomicrograph showing fibrinoid necrosis of a vessel. By courtesy of Dr. L. Henry.

TABLE 27. *Diabetes mellitus and infected gangrene*

Case	Age	Sex	Duration years	Therapy	Control	FH	Thin Av.	Obese	Other complications	Serum L.A.P. (24 hr. vol.)	<sup>24 hr. urinary</sup> <sup>L.A.P. (to N.V.)</sup>	Proteinuria	B.P. Bl. urea
36	75	M	2	Insulin	Poor	-ve	Av.		Cataracts. Gangrene R foot. Previous amputation L leg	122 (19/7/65)	264 (1.45 l.)	+	150/70 52 mg. %
37	56	F	1	Chlorpropamide	Fair	-ve	Obese		Severe <i>B. coli</i> infection R foot	211 (9/6/65)	542 (1.16 l.)	+	160/90 68 mg. %
38	83	F	10	Chlorpropamide	Fair	+ve	Av.		Gangrene L big toe. Previous amputation R leg	159 (9/6/65)	147 (0.76 l.)	-	170/90 34 mg. %
39	57	M	NK.	Chlorpropamide	Poor	-ve	Obese		Gangrene R foot	441 (5/6/65)	848 (1.55 l.)	-	130/85 36 mg. %
40	64	M	36	Insulin	Poor	-ve	Thin		Infected perforating ulcer R foot. Previous amputation L leg. Peripheral neuropathy E.P. attacks	257 (5/6/65)	434 (4.52 l.)	+	160/90 44 mg. %
41	77	M	8	Chlorpropamide →Insulin	Poor	-ve	Thin		Gangrene L foot. Previous amputation R leg	116 (24/3/65)	235 (0.76 l.)	+	120/80 42 mg. %
42	72	M	NK.	Chlorpropamide	Fair	-ve	Av.		Ischaemic ulcer R small toe. Intermittent claudication	135 (4/8/65)	191 (1.96 l.)	-	160/100 38 mg. %
									FEMALE AV.	185	344.5		
									MALE AV.	214	395		
TOTAL	7									AV. 69 years			

and rest, and the blood supply of the foot was just sufficient to allow for the eventual healing of the ulcer. As can be seen, the urinary L.A.P. excretion was raised in all these patients with infected gangrene. Both the serum and urinary L.A.P. values were particularly high in case 39. In this patient there was no proteinuria or evidence of renal disease, and it is likely that the high serum L.A.P. level resulted in a 'spill over' of this enzyme into the urine. Raised L.A.P. activity may well be due to release of enzyme from necrotic tissue or bacteria. These observations make it clear that a raised urinary L.A.P. excretion in patients with gangrene need not necessarily indicate underlying diabetic nephropathy.

Various miscellaneous conditions complicating diabetes are listed in Table 28. Two patients with frequent hypoglycaemic attacks (cases 43, 44) and one youth with multiple insulin secreting islet cell adenomata (case 45) had normal L.A.P. levels.

As explained in Chapter VII high serum L.A.P. values are found particularly in the last trimester of pregnancy. In case 46 the patient had mild toxæmia of pregnancy and the urinary L.A.P. level was raised. Between the two pregnancies the L.A.P. levels returned to normal. In her second pregnancy the serum urinary L.A.P. values were lower and the patient had no toxæmia.

In case 47, severe hypertension was associated with increased urinary L.A.P. excretion. Likewise in case 48 with proven chronic pyelonephritis and hypertension, the urinary L.A.P. excretion was increased. There was also some elevation of serum L.A.P. in cases 47 and 48. In case 49 the serum L.A.P. was somewhat elevated and this was due to liver congestion secondary to congestive cardiac failure.

To conclude, it should be stressed that there may be a number of reasons for an increased urinary excretion of L.A.P. in diabetic patients. For instance, renal damage due to pyelonephritis or hypertension will result in an increased urinary loss of this enzyme. Likewise, heavy proteinuria, complicating diabetic nephropathy, is associated with increased

TABLE 28. *Miscellaneous conditions complicating diabetes*

Case	Age	Sex	Duration	Therapy	Control	FH	Thin Av. Obese	Other complications	Serum L.A.P.	<sup>24 hr.</sup> <i>L.A.P. (to N.Y.)</i> (24 hr. vol.)	Proteinuria	B.P.	Bl. urea
<i>Frequent hypoglycaemic attacks</i>													
43	33	M	19	Insulin	Poor	-ve	Thin	Retinopathy	164 (19/6/65)	36 (0.7 l.)	0	120/80	
44	60	M	17	Insulin	Poor	-ve	Thin	Retinopathy	122 (9/9/65)	60 (1.15 l.)	0	40 mg. %	
45	16	M	2	Multiple islet cell adenoma	Admitted in coma. (Blood sugar 13 mg. %)				167 (9/8/65)	52 (0.81 l.)	0	175/95	
46	23	F	6	Insulin	Fair	-ve		G1 32/52 Pregnant Non-pregnant level GII 34/52 (No toxæmia)	885 (9/6/65) 180 720	214 (2.0 l.) 50 165	±	30 mg. %	
47	40	M	1½	Diet only	Good	-ve	Av.	Brain stem thrombosis	225 (5/6/65)	194 (0.86 l.)	+	150/90	
											+	26 mg. %	
												E.C.G. L.V.+	
<i>Pregnancy, mild toxæmia and diabetes</i>													
48	61	F	18	Diabetes	Good	-ve	Obese	Amputation L. leg cataracts. Repeated urinary infections. Chronic pyelonephritis confirmed by PM.	234 (7/4/65)	287 (2.01 l.)	++	180/115	
49	57	M	NK.	Diabetes	Good	-ve	Av.	Ischaemic heart disease. Congested liver. Mild jaundice	240 (5/6/65)	171 (0.89 l.)	+	140/85	
50	66	F	NK.	Nil	-ve	Obese		Abd. pain for 5 days. Laparotomy. Mesenteric thrombosis. Died	126 (31/3/65)	—	o	70 mg. %	
												E.C.G. L.B.B.B.	

urinary excretion of L.A.P. The other important mechanism is a 'spill over' of this enzyme from the circulation into the urine. This is clearly seen in diabetic patients with infected gangrene who may have high serum and urinary L.A.P. levels. The reasons for slight elevation of serum L.A.P. in some poorly controlled diabetics needs further investigation, but may well be related to liver dysfunction in patients with intermittent ketosis.

### SUMMARY

A number of enzyme abnormalities in diabetes mellitus and its complications have been reviewed. Some interesting adaptive enzyme changes which are associated with diabetes mellitus have also been noted.

In the final section of this chapter the results of enzyme studies in a series of diabetics have been recorded. Serum and urinary leucine aminopeptidase (L.A.P.) activity was measured in well and poorly controlled diabetics, and others with gangrene or diabetic nephropathy. Heavy proteinuria was accompanied by a marked increase in L.A.P. excretion. However, patients with gangrene had high serum L.A.P. levels which resulted in excessive 'spill over' of this enzyme into the urine. It was also noted that the serum L.A.P. tended to be elevated in some poorly controlled diabetic men. This could be related to impaired liver function in patients with intermittent ketosis.

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## CHAPTER X

## Enzyme changes in urinary tract disease and hypertension

Much of the literature on enzyme changes in urinary tract disease has only appeared within the last decade, and the subject has been made difficult by controversy on certain issues; for instance, recent papers do not support the original claim that a raised urinary L.D.H. is a good screening test for renal or bladder carcinoma.

In this chapter the subject will be reviewed in sections which will include (1) Enzyme studies in the nephron, (2) raised serum enzymes in renal disease and uraemia, (3) increased urinary excretion of enzymes in various conditions including parenchymal renal disease, infections and malignant tumours of the urinary tract. Finally, (4) enzyme changes in a small group of patients will be described.

There may be a number of reasons for the excessive loss of an enzyme in the urine. The causes include:

- (a) 'Spill-over' of enzyme into the urine, e.g. increased loss of amylase in pancreatitis. However, in macroamylasemia, which is a newly recognized cause of elevated serum amylase activity, the large size of the macromolecular complex of amylase prevents its urinary loss (Berk *et al.*, 1967).
- (b) Increased urinary leak due to glomerular damage, e.g. the nephrotic syndrome. The amount of enzyme in the urine will depend on the molecular weight of the enzyme and the degree of proteinuria.
- (c) Acute tubular or parenchymal damage will result in

- release of renal enzymes into the urine in, for example, acute tubular necrosis, pyelonephritis and glomerulonephritis.
- (d) Severe reno-vascular disease, e.g. renal infarction, or malignant hypertension.
- (e) Any acute inflammatory reaction of the urinary tract will result in increased enzyme activity in the urine.

It should also be pointed out that an increased serum level of an enzyme does not necessarily result in increased urinary loss of this enzyme. Thus Rosalki and Wilkinson (1959) made the interesting observation that, in spite of raised serum levels of L.D.H. and G.O.T. in three cases of myocardial infarction, there was no increase in urinary loss of these two enzymes. Likewise, it has been shown that in spite of increasing serum levels of L.A.P. in single pregnancy, the urinary loss of this enzyme remained at a low level. However, if glomerular damage developed (e.g. eclampsia) then there was a marked increase in urinary loss of L.A.P. (see Chapter VI).

### I. ENZYME ACTIVITY IN THE NEPHRON

Enzyme activity has been measured in various parts of the nephron. For instance, Dubach (1965) using histochemical methods studied five enzymes (alkaline phosphatase, glucose-6-phosphate, lactic, malic and isocitric dehydrogenase) and showed that apart from glucose-6-phosphate-dehydrogenase, enzyme activity was always less in normal glomeruli compared to the tubules. Pollack and Mattenheimer (1962) have also carried out meticulous microdissection of the nephron. Minute samples of tissue were weighed and ultramicrochemical assay methods were employed to determine enzyme activity in various parts of the nephron. The activity of alkaline phosphatase, G.O.T., G.P.T., L.D.H. and carbonic anhydrase was less obvious in the normal human glomerulus as compared to the convoluted tubules and medulla. The exception to this was acid phosphatase; the activity of this enzyme was higher in the glomerulus compared to the rest of the nephron. Alkaline

phosphatase activity was highest in the proximal convoluted tubule, while carbonic anhydrase activity was greatest in the distal convoluted tubule. These authors have also confirmed that there was a considerable species variation in the distribution of various enzymes in the nephron.

Similar studies have been carried out in various metabolic disorders. For instance, only slight glucose-6-phosphatase activity could be demonstrated in the kidney in glycogen storage disease (Cori, 1954), and in hypophosphatasia, alkaline phosphatase activity was almost absent in all parts of the nephron (Pollack *et al.*, 1960).

## 2. RAISED SERUM ENZYMES IN RENAL DISEASE AND URAEMIA

In 1958 West and Zimmerman reported elevation of serum L.D.H. activity in forty-three out of a total seventy-one patients with a variety of kidney disorders. Increased enzyme activity was found not to be proportional to the degree of uraemia, but higher serum L.D.H. values tended to occur when the serum albumin was reduced. Of particular interest was the finding of increased serum L.D.H. activity in all four patients with the nephrotic syndrome. In this connection, raised levels of plasma cholinesterase have also been recorded in the nephrotic syndrome by Kunkel and Ward (1947). They felt that this was due to increased hepatic synthesis of this enzyme. West and Zimmerman (1958) also showed that the S.G.O.T. levels were normal in uraemia. Only six out of sixty-three cases had raised S.G.O.T. levels and in five of these cases there was evidence of liver damage.

It has been known for many years that blood diastase activity is raised in chronic renal failure (Myers and Killian, 1917). Marked elevation of plasma amylase has been recorded in acute renal failure (Meroney *et al.*, 1956) and in the cases studied there was no evidence of acute pancreatitis. Enzyme levels remained high in spite of repeated dialysis and it was considered that diminished urinary excretion was an important

factor in maintaining raised plasma levels of this enzyme. Histological evidence of pancreatitis may be present at autopsy in a high proportion of patients with uraemia (Baggenstoss, 1947, 1948) and it should also be noted that acute pancreatitis is a well-recognized complication of hypercalcaemic renal failure (e.g. case 54, Table 29) hyperparathyroidism, accidental hypothermia and in patients on steroid therapy.

Elevation of plasma ribonuclease has also been recorded in uraemia (Rabinovitch *et al.*, 1959) and the highest levels of this enzyme were found in two anuric patients treated by haemodialysis. More recently, raised serum C.P.K. levels have been noted in five out of eleven patients with renal insufficiency (Eschar and Zimmerman, 1967) and two of these cases were receiving treatment by peritoneal dialysis.

### 3. INCREASED URINARY EXCRETION OF ENZYMES

In Britain Rosalki and Wilkinson (1959) first noted an increase in total urinary L.D.H. excretion in nine patients with acute renal disease and in five of these the urinary G.O.T. was also raised. They felt that this increased enzyme activity in the urine was due to renal cellular injury, and was not related to the degree of proteinuria.

#### Parenchymal renal diseases

##### (a) *Nephrotic syndrome*

Crockson (1961) showed that heavy proteinuria in the nephrotic syndrome was associated with an increased urinary loss of L.D.H., and that the kidney handled this enzyme in the same way as other proteins of medium molecular weight. The mean serum L.D.H. was similar in patients with minimal, membranous and proliferative histological change but the mean L.D.H. clearance (ml. per minute) was greatest in the membranous group and least in patients with minimal change. Other workers (Szász *et al.*, 1965) studied a number of enzymes (G.O.T., G.P.T., L.A.P.,  $\beta$ -glucuronidase and alkaline

phosphatase) in thirty children with the nephrotic syndrome. Significant enzymuria was found only when proteinuria exceeded 1 g. per day.

(b) *Other nephropathies*

Increased urinary L.D.H. has been reported in various other renal diseases including acute and chronic glomerulonephritis, diabetic nephropathy and disseminated lupus erythematosus (Wacker *et al.*, 1964). 80 per cent of cases of chronic pyelonephritis were found to have raised urinary L.D.H. and in most of these there was no bacteriuria. On the other hand, patients with biopsy proven healed glomerulonephritis and others with essential hypertension had normal urinary L.D.H. levels. It was considered that patients with hypertension and a raised urinary L.D.H. could well have underlying pyelonephritis.

Increased urinary L.A.P. excretion has been found in the nephrotic syndrome, diabetic nephropathy and malignant hypertension (Mullan, 1966). Increased urinary loss of L.A.P. has also been reported in acute tubular damage, in patients treated with nephrotoxic antibiotics and after investigation by intravenous pyelography (Bergmann and Scheler, 1964).

(c) *Renal damage and urinary lysozyme*

Other urinary enzymes have been studied in an attempt to find a more specific index of renal damage. For instance, in 1964, Prockop and Davidson reported some interesting studies on the urinary and serum lysozyme in patients with renal disease. Work in this field stemmed from the discovery in 1922 by Sir Alexander Fleming of a 'remarkable bacteriolytic element found in tissues and secretions'. This lyosomal enzyme lysed bacteria by hydrolysing cell wall mucopolysaccharides. In practice, lysosomal activity is determined using a substrate solution containing cells of *micrococcus lysodeikticus* (Litwack, 1955). The mucoid envelopes of these organisms are broken down and the process is measured photometrically.

Prockop and Davidson found that in normal individuals or patients without evidence of renal disease, the urinary lysozyme

activity was less than 5 microgrammes per ml. In some cases, marked elevation of blood urea nitrogen levels was associated with only slight lysozymuria. Thirty-one of thirty-eight cases with proteinuria ( $>0.1$  g. per litre) had increased urinary lysozyme activity, but only slight lysozymuria was present in some cases with heavy proteinuria. Urinary lysozyme activity was not correlated with the degree of haematuria, pyuria or bacteriuria. Leucocytes contain lysosome (Flannagan and Lionetti, 1955) and it was surprising that enzyme activity was not increased when there was a high urinary white cell count. Prockop and Davidson also found that the serum lysozyme level was raised in eighteen out of twenty-one patients with increased urinary activity of this enzyme. Five patients with pre-renal uraemia had raised serum activity but there was no increase in urinary lysozyme excretion. These workers also demonstrated marked lysozymuria in rats injected with mercuric chloride and this suggested that renal tubular damage could cause increased excretion of this enzyme in the urine. The measurement of this enzyme in acute tubular necrosis in man could well prove to be of interest.

### **Urinary infections and pyelonephritis**

A great deal of time and energy is being spent in the management of patients with 'end stage' renal disease. Clearly it is also equally important to study the natural history of kidney disease. In particular, the diagnosis and effective treatment of asymptomatic urinary infection could well prevent the development of silent pyelonephritis. Chronic pyelonephritis is certainly a common finding at necropsy and the early detection of bacteriuria (Kass, 1957) or the use of chemical or enzyme tests in high risk patients (e.g. pregnant mothers and diabetics) could well be helpful. As pointed out in the *Lancet* (1964) full bacteriological investigation of the urine in large numbers of patients attending the out-patient department may be too time consuming. Counting urinary bacteria is the best way of confirming actual urinary infection (a count of over 100,000 organisms per ml. is significant), but other rapid screening

methods have been devised. For instance, Kincaid-Smith *et al.* (1964) showed that 6 per cent of 3000 pregnant women had significant bacteriuria and of these 57 per cent had a positive catalase test but a high proportion (86 per cent) had a positive T.T.C. test. In the latter test, bacteria reduce the colourless salt triphenyl tetrazolium chloride (T.T.C.) into a coloured dye. The former test depends on the ability of bacteria to liberate oxygen from hydrogen peroxide, and catalase activity is usually determined by the disc flotation method (Braude and Berkowitz, 1961). The latter workers found that the catalase test was a reliable way of detecting significant bacteriuria, and that this test could also be positive in patients with known renal disease but normal urines. Moutsos *et al.* (1962) went on to show that hypertension by itself did not give rise to a positive test, but that this test was likely to be useful in the detection of underlying pyelonephritis.

Brenner and Gilbert (1963) made a careful study of L.D.H., G.O.T. and catalase in infected urine. In normal controls there was no G.O.T. or catalase activity and only slight L.D.H. activity. These enzymes were raised in urine containing pus or bacteria but in thirty-eight patients with no increase in enzyme levels, there was only one with a positive culture for *Escherichia coli*. These workers made the interesting observation that the three urinary enzymes were nearly always increased in infections due to *Proteus* and *Klebsiella-Aerobacter*, but were much less frequently elevated in *E. coli* infections.

The fact that increasing degrees of pyuria was associated with increasing activity of each urinary enzyme clearly indicated that acute inflammation was the important cause of increased enzyme activity in the urine. The amount of enzyme derived from bacteria was probably slight; lysed bacteria of the same species showed variability or absence of L.D.H. and G.O.T. activity.

The whole question of the selective release of enzymes from bacteria has recently been reviewed by Heppel (1967). One experimental method, employing 'osmotic shock' is of particular interest; in this procedure the cells are exposed to E.D.T.A. in

0.5 molar sucrose followed by sudden osmotic transition to cold dilute MgCl<sub>2</sub>. If treated in this way *E. coli* will release various hydrolytic enzymes and certain other proteins. Fortunately, the shocked cells remain viable and the biochemical changes during the recovery phase can then be investigated.

Brenner and Gilbert (1963) also produced experimental pyelonephritis in rats by the intracardiac injection of suspensions of *Proteus mirabilis* and *E. coli*. There was marked increase in urinary L.D.H. and G.O.T. which were measured one week later, and both organisms produced extensive suppurative lesions in both kidneys. G.O.T. and L.D.H. are found in high concentration in kidney tissue, and it was considered that experimental pyelonephritis produced increased urinary excretion of enzymes because of renal damage associated with an acute inflammatory reaction.

$\beta$ -glucuronidase has also been measured in the urine of patients with renal disease. It is a lysosomal enzyme (i.e. it is contained within a cytoplasmic granule or lysosome) and Bank and Bailine (1965) have found that urinary glucuronidase activity was increased in acute and chronic pyelonephritis. In lower urinary tract infections, in spite of an excess of pus cells, there was no excess of this enzyme in the urine. Of interest was one patient with unilateral renal disease. An I.V.P. showed contraction of the right kidney, and the urinary  $\beta$ -glucuronidase excretion was clearly greater on the affected side. In another case, with severe congestive cardiac failure and heavy proteinuria, there was no increased urinary loss of  $\beta$ -glucuronidase which suggested that the mechanism was not an increased glomerular leak of this enzyme. Furthermore, these workers showed experimentally that increasing the blood levels of  $\beta$ -glucuronidase in rats did not result in increased urinary loss of this enzyme. The conclusion was that renal tubular damage could result in the release of this enzyme into the urine.

### Malignant diseases of the urinary tract

*The L.D.H. controversy.* As in other fields of clinical enzymology, the introduction of a new enzyme test may at first be

considered to be diagnostic of a certain condition. For instance, Wacker and Dorfman (1962) considered that elevation of urinary L.D.H. was diagnostic of renal or bladder carcinoma. Eighteen out of nineteen patients with malignant disease of the urinary tract had elevation of urinary L.D.H. activity, and these authors felt that the estimation of urinary L.D.H. would be a useful screening procedure for the early detection of silent bladder and kidney carcinoma. Other workers (Macalalag and Prout, 1964) also considered that the tumour-bearing kidney was the source of elevated urinary L.D.H. They found increased total L.D.H. activity in kidney tumour tissue, and an increased proportion of L.D.H.<sub>4</sub> and <sub>5</sub> in this tissue. In addition they noted that in three patients the total urinary L.D.H. from the renal pelvis of the tumour-bearing kidney was raised compared to the other side.

However, many other papers have appeared indicating that increased urinary L.D.H. is not a useful screening test for urinary tract carcinoma. Thus, Riggins and Kiser (1963) found that the urinary L.D.H. activity failed to differentiate benign from malignant disease of the urinary tract. Urines containing an excess of pus cells or lysed red cells increased urinary L.D.H. activity and were excluded from their series. 15 per cent of patients with malignant disease had normal levels of urinary L.D.H., while 70 per cent of those with benign lesions had raised levels of this enzyme. The latter group included patients with infections, tuberculosis, calculi, nephrosis, prostatic hypertrophy and renal cysts. Schmidt (1966) confirmed that the total urinary L.D.H. could be derived from a number of sources, including pus cells, red cells, bacteria, seminal and prostatic fluid. Significantly, there was no increase in total urinary L.D.H. in sixty-seven patients all of whom had macroscopically normal urine. Furthermore, this group included three cases of invasive bladder carcinoma and one case of renal adenocarcinoma. Likewise Gelderman (1965) confirmed that the L.D.H. iso-enzyme patterns in those cases with increased total urinary L.D.H. corresponded with L.D.H. iso-enzyme pattern of the cells found in the urinary sediment.

In fact the urinary L.D.H. iso-enzyme pattern in cases of bladder cancer did not differ from the pattern in other patients without evidence of malignant disease of the urinary tract. Moreover, the total urinary L.D.H. was not related to the size or extent of invasion of the bladder tumour, but was increased when there was an excess of pus cells or red cells in the urine.

#### *$\beta$ -glucuronidase in carcinoma of the urinary tract*

Boyland, Wallace and Williams (1955) studied  $\beta$ -glucuronidase activity in the urine and found raised values in patients with carcinoma of the bladder. These workers suggested that increased  $\beta$ -glucuronidase activity in the urine contributed to the development of the disease by the liberation of carcinogenic aminophenols from conjugated compounds, which were normally inactive. In this connection it is well known that there is a very high incidence of bladder carcinoma in men working in close contact with  $\alpha$ - and  $\beta$ -naphthylamine and benzidine. The above workers found that raised enzyme activity was not related to urinary tract infection and that  $\beta$ -glucuronidase activity was greater in bladder cancer tissue compared to normal bladder mucosa. Serum  $\beta$ -glucuronidase activity tended to be above normal in these patients, and it was considered that the urinary enzyme was derived from the circulating blood and probably not from kidney tissue.

Schistosomiasis may also be important in the aetiology of bladder carcinoma, and in this connection it is of interest that Fripp (1960) found high levels of  $\beta$ -glucuronidase in the urine of patients with urinary schistosomiasis (*Schistosoma haematobium*). Treatment with lucanthone hydrochloride resulted in a rapid fall in the urinary excretion of this enzyme. In his preliminary report Fripp (1960) did not measure the serum levels of  $\beta$ -glucuronidase. Urinary schistosomiasis is accompanied by haematuria, and raised serum levels of this enzyme could give rise to increased urinary excretion of  $\beta$ -glucuronidase.

It has also been shown by Watts, MacVicar and Goldberg

(1966) that the urinary  $\beta$ -glucuronidase was increased in some patients with carcinoma of the cervix, and that radiotherapy caused a further increase in urinary excretion of this enzyme. It was suggested that the tumour itself was the source of  $\beta$ -glucuronidase, and that irradiation resulted in release of enzyme from malignant tissue and subsequent excretion by the kidneys. In addition it has been noted that the surgical removal of a bladder tumour will result in a fall in the urinary excretion of  $\beta$ -glucuronidase (Kerr *et al.*, 1963). Likewise, normal urinary values of this enzyme were found in patients who had had successful radiotherapy of bladder cancer (Haife and Van der Werf-Messing, 1962).

*Acid phosphatase and L.D.H. iso-enzymes in carcinoma of the prostate*  
To conclude, mention should be made of the importance of serum acid phosphatase in cases of prostatic carcinoma. In metastasizing carcinoma of the prostate the total acid phosphatase is usually raised and the bulk of the enzyme is inhibited by L-tartrate (Cook *et al.*, 1962). Occasionally, however, patients with bone secondaries may have marked elevation of the serum alkaline phosphatase but a normal serum total acid phosphatase.

The interesting work of Denis *et al.* (1962, 1962, 1963) should also be noted. They studied L.D.H. iso-enzyme patterns in serum and prostatic tissue. In patients with widespread prostatic carcinoma there was an increase in L.D.H.<sub>5</sub> fraction in both prostatic tissue and in the serum. When there was a favourable response to hormone therapy the serum L.D.H. iso-enzyme pattern returned to normal.

#### 4. ENZYME CHANGES IN URINARY TRACT DISEASE AND HYPERTENSION

##### Cases studied

Enzyme studies have been carried out in three patients with the nephrotic syndrome, a woman with the adult Fanconi Syndrome, and two cases of malignant hypertension (Table 29).

In case 51, there was very heavy proteinuria (24 g. protein per 24 hours) and the serum albumin was reduced to 2.5 g./100 ml. Estimation of the 24-hour urinary L.A.P. showed marked elevation on two occasions. Figs. 30 and 31 show the renal histology in this case; severe membranous glomerulonephritis

TABLE 29. *Renal disease and hypertension*

<i>Case</i>	<i>Sex</i>	<i>Age</i>	<i>Diagnosis and complications</i>	<i>Date</i>
51	M	46	<i>Nephrotic syndrome.</i> Oedema R leg, thrombosis R femoral vein and ? small cortical vein thrombosis → R hemiparesis. <i>Renal biopsy.</i> Membranous glomerulonephritis (or D.L.E.)	7/1/66 1/7/66
52	M	33	<i>Nephrotic syndrome.</i> Oedema of ankles 15/12 No improvement with prednisolone. Some improvement with diuretics	25/6/66
53	F	32	<i>Nephrotic syndrome.</i> Oedema of legs and urinary infection. Large doses of prednisolone → Cushing's syndrome	1/4/65
54	F	44	<i>Adult Fanconi syndrome.</i> Pseudo-fractures. Aminoaciduria, intermittent glycosuria. Radiological cure with vit.D but died 2 yrs. later with uraemia and acute pancreatitis. <i>Post-mortem:</i> Kidneys small: nephrocalcinosis	15/3/65
55	M	51	<i>Malignant hypertension.</i> Convulsions → # humerus Grade IV retinopathy Well controlled on Ismelin 40 mg./day	26/4/65 7/5/66 10/8/67
56	F	34	<i>Malignant hypertension and L.V.F.</i> Anuria, repeated dialyses. <i>Renal implant.</i> Died 4/12 after admission <i>Post-mortem.</i> Donor kidney: massive papillary necrosis. Patient's kidneys: contracted, chronic pyelonephritis	On admission 12/8/66

TABLE 29 (*contd.*).

Serum L.A.P. ( <i>10 N.V.</i> )	<i>24-hr. urinary</i> <i>L.A.P.</i> ( <i>10 N.V.</i> )	Proteinuria	B.P.	Blood urea <i>mg/100 ml.</i>	Other investigations
167 570 (1.1L)		+++	150/90	36	Serum alb. 2.5 glob. 1.7 ↑ $\alpha_2$ ↓ $\gamma$ No L.E. cells.
153 455 (1.38L)		24 g./24 hrs.			E.S.R. 64 mm./hr.
95 554 (2.24L)		+++	140/80	33	Serum alb. 2.3 glob. 3.3 ↑ $\alpha_2$ E.S.R. 47 mm./hr.
163 306 (0.62L) ( <i>E. coli</i> ++) Centrifuged specimen 182 (No <i>E. coli</i> seen)		+++	140/95	78	Serum alb. 2.4 glob. 1.5 ↑ $\alpha_2$ and $\beta$ E.S.R. 53 mm./hr.
108 228 (1.27L)		++	130/100	40-72	Alk. phosphatase 54 → 15 K.A. units Serum amylase > 1000 units
172 371 (1.3L) 276 (1.46L)		++	220/140 150/95	54	
252 446 (0.85L)		++	260/140	360	S.G.O.T. 55 King units Alk. phosphatase 11 K.A. units

was present and the pathologist also suggested the possibility of disseminated lupus erythematosus. The absence of L.E. cells, reduced serum  $\gamma$  globulin, and subsequent follow up supported the diagnosis of nephrotic syndrome due to membranous glomerulonephritis. The development of a right

femoral vein thrombosis in this patient may well have been related to diminished plasma volume (Chamberlain *et al.*, 1966) and increased blood coagulation.

Cases 52 and 53 also had leg oedema, hypoalbuminaemia, heavy proteinuria and increased urinary excretion of L.A.P. In all three cases of the nephrotic syndrome the serum L.A.P. levels were normal, while in three out of four patients with

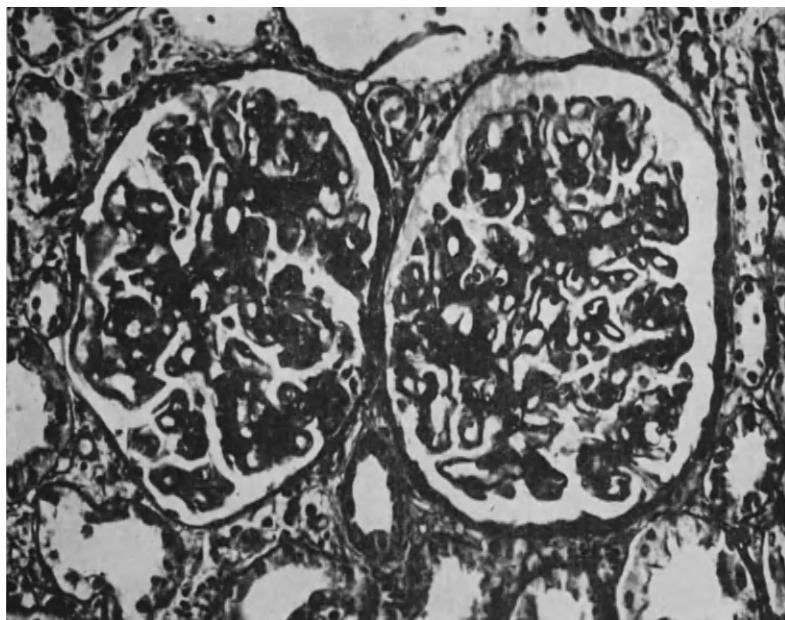


FIG. 30. Photomicrograph showing severe membranous glomerulonephritis. Renal biopsy specimen. By courtesy of Dr. L. Henry.

diabetic nephropathy the serum levels of this enzyme were above the upper limit of normal (see Table 26, Chapter IX).

Case 53 was interesting because at the time of urinary L.A.P. estimation the urine was heavily infected with *E. coli*. 306 mg.  $\beta$ -naphthylamine represented the total 24-hour urinary L.A.P. excretion of this infected urine. The same aliquot of urine was then centrifuged for 5 minutes at 3000

revolutions per minute, and the L.A.P. estimation was repeated on the supernatant giving a lower value of 182 mg.  $\beta$ -naphthylamine per 24 hours. Microscopic examination of this supernatant urine showed that bacteria were absent. The above results suggested that L.A.P. of bacterial origin contributed to the increased total excretion of urinary L.A.P.

Case 54 was a proven case of the so called adult Fanconi Syndrome which has been fully described elsewhere (Mullan,

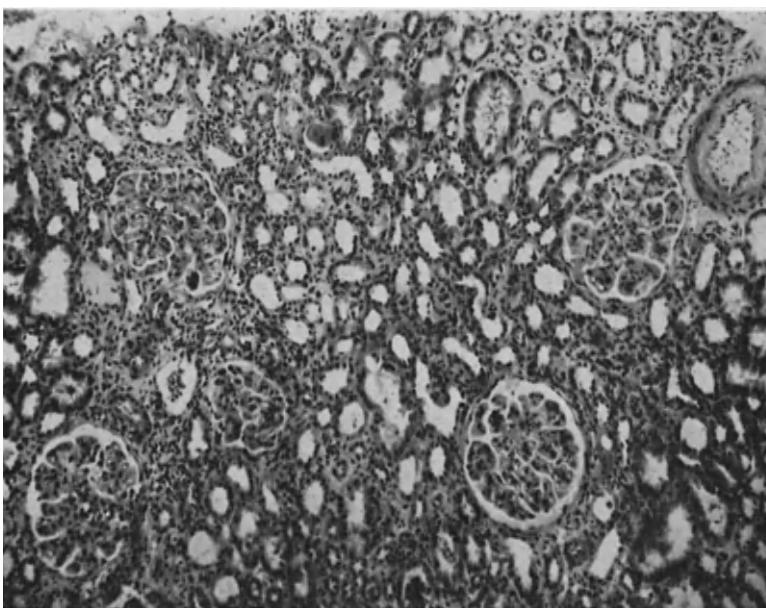


FIG. 31. Photomicrograph by courtesy of Dr. L. Henry. Low power view of same specimen.

1966). The patient had typical features including osteomalacia, pseudo-fractures, amino-aciduria, hypophosphataemia, glycosuria, systemic acidosis and hypokalaemia. Electrophoresis of her urine (kindly performed by Dr. F. V. Flynn) demonstrated 'tubular protein' migrating in the region of the  $\alpha_2$  globulin, and in addition, the urinary excretion of L.A.P. was elevated at 228 mg. of  $\beta$ -naphthylamine per 24 hours. This patient had

other interesting enzyme changes. In particular, because of her osteomalacia, the serum alkaline phosphatase was elevated at 54 King units. Vitamin D therapy resulted in radiological cure, and the alkaline phosphatase value fell to 15 King units. As expected, her serum L.A.P. level was normal; serum L.A.P. values are normal when the elevation of serum alkaline phosphatase activity is due to a bone disorder (Kowlessar *et al.*, 1961). Two years later this patient was admitted with uraemia and an acute abdomen. The serum amylase was over 1000 Somogyi units and the diagnosis of acute pancreatitis was confirmed at necropsy. The kidneys were small and there was evidence of nephrocalcinosis. Hypercalcaemic renal failure may have been brought on by vitamin D therapy, and it has been suggested that this hazard can be avoided by treatment with phosphate and alkalis only (Wilson and Yendt, 1963).

Case 55 was of interest because the patient had been treated elsewhere with anticonvulsants for supposed epilepsy. During one of these attacks he had fractured his humerus. Nobody had recorded the blood pressure which was in fact 220/140 mm. Hg! He also had a grade IV retinopathy. The previous convulsive attacks were probably due to hypertensive encephalopathy. Malignant hypertension in this patient was associated with an increased excretion of L.A.P. in the urine. However, after controlling his blood pressure with Ismelin the urinary excretion of L.A.P. fell appreciably.

The author would like to thank Dr. Margaret Platts for supplying the clinical details of case 56. This patient was of historical interest because she was the first patient to receive a cadaveric renal implant in Sheffield. As indicated she presented with anuria, severe malignant hypertension and left ventricular failure. In spite of uraemia, the levels of S.G.O.T. and alkaline phosphatase were normal. However, the total urinary excretion of L.A.P. was greatly elevated at 446 mg.  $\beta$ -naphthylamine per 24 hours indicating severe damage of the implanted donor kidney, at a time when this kidney was secreting about 800 ml. of urine per day. Four months after admission the patient died and post-mortem examination

revealed massive papillary necrosis of the donor kidney, and chronic pyelonephritis of both her own kidneys.

In future studies, serial estimation of urinary enzymes, e.g. L.A.P.,  $\beta$ -glucuronidase or L.D.H., could well be useful in the early diagnosis of rejection of an implanted kidney.

The final table (30) gives some enzyme levels in seven men

TABLE 30. *Carcinoma of prostate*

<i>Case</i>	<i>Age</i>	<i>Diagnosis and complications</i>	<i>Total acid phosphatase King units</i>	<i>Formaldehyde stable acid phosphatase</i>	<i>Alkaline phosphatase</i>	<i>Serum L.A.P.</i>
57	67	Large prostatic carcinoma X-Rays: Non-functioning L kidney. No bony deposits	5	4	15	196
58	70	Palpable carcinoma prostate	5	2	9	128
59	62	Palpable carcinoma prostate	5	2	8	120
60	76	Palpable carcinoma prostate	13	12	22	100
61	65	Palpable carcinoma prostate	37	24	8	198
62	71	Urinary retention due to carcinoma prostate. X-Rays: 2nd deposit in lumbar spine	40	40	64	180
63	81	Died of C.V.A. (L) atrial clot. Mild diabetes. Haemorrhagic cystitis. Ca. prostate found incidentally at P.M.				104
64	64	Benign prostatic enlargement				96

with carcinoma of the prostate and one case with benign prostatic enlargement only. Mr. J. Williams kindly allowed these patients to be investigated.

The normal range for total serum acid phosphatase is 1 to 3 King units (King and Wootton, 1965) and values of 5 King units and over are suspicious of prostatic disease. Red cell acid phosphatase is almost completely inhibited by formaldehyde, and therefore in cases 58 and 59 the levels of acid phosphatase are not diagnostic of prostatic carcinoma. On the other hand, the high levels of formaldehyde stable acid phosphatase in cases 60, 61 and 62 clearly confirm the diagnosis. The normal

range for serum alkaline phosphatase is 3 to 13 King units, and the patient (case 62) with radiological evidence of secondary deposits in his lumbar spine had the highest serum alkaline phosphatase level, i.e. 64 King units. In spite of elevated levels of serum alkaline phosphatase in cases 57, 60 and 62, the serum L.A.P. values were normal in all cases. Case 63 was added simply to emphasize that unsuspected carcinoma of the prostate is not an uncommon post-mortem finding.

### SUMMARY

The enzyme changes in renal disease have been reviewed and some of the experimental work in this field has been mentioned. Enzymuria occurs in parenchymal kidney disease, infections and malignant tumours of the urinary tract. In some instances, raised serum enzyme levels of e.g. amylase and leucine aminopeptidase (L.A.P.) will give rise to increased 'spill over' of these enzymes into the urine. On the other hand, elevated S.G.O.T. is not accompanied by increased urinary loss of G.O.T. It is also important to stress that certain serum enzymes, e.g. amylase, C.P.K. and L.D.H. may be elevated in uraemia, and therefore these enzyme tests will lose their diagnostic significance.

In the final section of this chapter, the clinical features and enzyme changes occurring in some patients with renal disease, malignant hypertension and prostatic carcinomata have been described. It was suggested that the estimation of certain urinary enzymes would be of value in diagnosis of rejection of kidney transplants.

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## CHAPTER XI

## Cerebrospinal fluid abnormalities

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### INTRODUCTION

The enzymatic activity of the cerebrospinal fluid (C.S.F.) in neurological disease was first studied by Kaplan *et al.* in 1939. However, it was not until the middle 1950s that more general interest in the cerebrospinal fluid enzymes became apparent with several reports of the activities of lactic dehydrogenase and glutamic-oxaloacetic transaminase in the C.S.F. in neurological illnesses. This was a natural progression following reports of the changes in the serum concentration of these enzymes in systemic disease, particularly myocardial infarction and hepatocellular disease, which appeared a little earlier. In general as enzymes are composed of large protein molecules, their size precludes their passage across the blood-cerebrospinal fluid barrier, except where the latter's integrity is broken in cerebral haemorrhage and meningeal inflammation. Their presence in the cerebrospinal fluid is due to their release from the cells of tissue enclosed within the meninges, that is the brain, brain stem, cerebellum and spinal cord. It must also be remembered that tumour cells within the central nervous system, and the cells of granulomatous tissue involving the central nervous system as in sarcoidosis, may contribute enzymes to the cerebrospinal fluid.

This chapter deals first with previous work on C.S.F. enzymes in a number of neurological conditions, and this survey is followed by the results obtained in cases studied by the author in Sheffield.

**Disseminated sclerosis, C.S.F., G.O.T. and L.D.H.**

In 1957 Green *et al.* reported the analysis of four hundred specimens of cerebrospinal fluid for glutamic-oxaloacetic transaminase (G.O.T.) activity in various neurological conditions. In twelve patients with disseminated sclerosis, half had increased transaminase activity, but this was marked in only three patients with severe exacerbation of the illness. Normal values were obtained in patients with stable chronic disease. Cerebrospinal fluid collected during air encephalography or air ventriculography showed an increase in activity after the injection of each increment of air. Again, in 1958, Green studied twenty patients with disseminated sclerosis and found elevated C.S.F. transaminase levels in eleven cases. However, five patients with acute disease had normal values. In addition the C.S.F. lactate dehydrogenase (L.D.H.) activity was elevated in only three out of ten cases. Likewise, Brodell *et al.* (1959) confirmed that cases of disseminated sclerosis with 'recent clinical evidence of activity' showed no significant increase in C.S.F. transaminase activity.

**Brain tumours: C.S.F. activity of G.O.T., L.D.H., P.H.I. and C.P.K.**

In 1957 Green *et al.* found no increase in C.S.F. transaminase activity in fourteen patients with brain tumours, and in the same year Fleischer *et al.* (1957) showed that patients with brain tumours showed a significant rise only in the serum transaminase. However, the following year Green *et al.* (1958) recorded some cases in which C.S.F. transaminase activity was elevated. He also noted that the C.S.F. L.D.H. was more often increased in these patients.

Again in 1959 Green *et al.* studied the glutamic oxaloacetic transaminase and lactic dehydrogenase in the cerebrospinal fluid of brain tumour subjects, in normal human brain and in brain tumour homogenates. The lactic dehydrogenase of the C.S.F. was increased in all sixteen patients with brain tumours, the highest values being present in patients with malignant

tumours. The glutamic oxaloacetic transaminase activity was increased in nine of these patients. Homogenates of brain tumour tissue had greater transaminase and lactic dehydrogenase activity than normal human brain tissue. Apparently the elevated enzyme activities of the cerebrospinal fluid which occurred could not be fully explained by the enzyme concentrations of the tumours. Green and his colleagues were uncertain of the origin of the additional cerebrospinal fluid enzymes but speculated that they might have appeared from necrotic brain tissue adjacent to the tumours or from necrotizing tumour cells.

Other C.S.F. enzymes have been studied. For instance, Thompson *et al.* (1959) showed that phosphoglucomutase (P.H.I.) activity in the C.S.F. was increased in twenty-one out of thirty-three patients with malignant brain tumours. More recently, Herschkowitz and Cumings (1964) estimated the C.S.F. creatine phosphokinase (C.P.K.) in sixty-six patients with neurological disease. Elevated enzyme activity was found in eleven patients with astrocytomas. Herschkowitz and Cumings also found that brain tissue contained more enzyme than tumour tissue, suggesting that increase in enzyme in the cerebrospinal fluid may reflect brain damage and necrosis.

Mellick and Bassett in 1964 showed that the C.S.F. G.O.T. activity was normal in seven patients with meningiomas, three with acoustic neuromas, three with pituitary adenomas and one case with a craniopharyngioma. On the other hand, the C.S.F. G.O.T. was elevated in eleven patients with malignant glioblastomas.

Herschkowitz and Cumings (1964) also confirmed that the C.S.F. C.P.K. activity was normal in five patients with meningioma and two with craniopharyngioma. More recently, Nathan (1967) studied ninety subjects. Of the twenty-two patients with elevated C.S.F. C.P.K. levels, twelve had tumours of the central nervous system (C.N.S.) and four had cerebrovascular disease. Of especial interest was that all four patients with chromophobe adenoma had elevated levels, whilst all three cases of meningioma had normal C.S.F. C.P.K.

values. Other workers (Lisak and Craig, 1967) investigated a wide variety of neurological disease conditions and found that C.S.F. C.P.K. values were normal and of no diagnostic value.

#### **Leukaemia, lymphoma and metastatic brain tumour, C.S.F. L.D.H.**

Wróblewski *et al.* (1957) drew attention to a blood C.S.F. barrier for lactic dehydrogenase. The lactic dehydrogenase activity of normal adult serum showed a range of 200–680 units per ml., whereas its activity in the C.S.F. of normal subjects ranged from 10 to 40 units per ml. They found that the lactic dehydrogenase activity of the serum and C.S.F. varied independently of each other. Of 110 subjects studied, thirty had no evidence of disease of the nervous system, and the lactic dehydrogenase activity of this group was within the normal range. The remaining eighty had various neurological diseases, and the spinal fluid lactic dehydrogenase was elevated in eight patients with leukaemic involvement of the brain, two patients with lymphomatous involvement of the brain and ten patients with metastatic carcinoma of the brain. It was notable that three subjects with primary brain tumours had normal values. They concluded that elevated spinal fluid lactic dehydrogenase was indicative of involvement of the central nervous system by metastatic carcinoma, lymphoma or leukaemia provided it occurred in the absence of clinical findings suggestive of cerebrovascular accident or meningitis.

In 1958 Wróblewski *et al.* reported on the lactic dehydrogenase activity of the spinal fluid of 180 patients. In those with intracerebral or meningeal leukaemia, lymphoma or metastatic carcinoma the spinal fluid lactic dehydrogenase was above the normal range. In one case treated with intrathecal methotrexate the lactic dehydrogenase returned to normal. The C.S.F. lactic dehydrogenase activity of five patients with primary brain tumour was normal. Patients with malignant neoplasia not involving the nervous system had normal cerebrospinal fluid lactic dehydrogenase (Wróblewski, 1959).

### The lipoidoses

Aldolase, glutamic-oxaloacetic transaminase and lactic dehydrogenase were measured simultaneously in blood and C.S.F. by Aronson *et al.* (1958) in patients with central nervous system lipoidoses. These included nine cases of infantile amaurotic family idiocy and two cases of Niemann-Pick disease. Included in this study was the determination of these enzymes in thirty-three control patients without neurologic disease. Parallel values of serum and C.S.F. glutamic-oxaloacetic transaminase and lactic dehydrogenase were invariably elevated in infantile amaurotic family idiocy while only the serum and C.S.F. glutamic oxaloacetic transaminases were increased in Niemann-Pick disease. The cerebrospinal fluid aldolase was usually elevated in all cases of central nervous system lipoidoses whereas serum aldolase values were but slightly increased. Significant elevation of these enzymes in the C.S.F. was also observed in occasional patients with actively progressive cerebral degeneration.

### Cerebrovascular accidents, serum and C.S.F. G.O.T. levels

In 1957 Lieberman *et al.* reported elevated transaminase levels in the cerebrospinal fluid and serum of subjects having had a cerebrovascular accident. Raised levels were detected as frequently in the serum as in the C.S.F., although not invariably elevated in either, and the greatest elevation, whether it occurred in blood or C.S.F., appeared within 3 to 5 days of the stroke. Further increases could be correlated with clinical extension of brain damage. They demonstrated a blood/C.S.F. barrier for glutamic oxaloacetic transaminase, because some cases showed a striking dissociation between levels of enzyme activity in simultaneously drawn specimens of blood and C.S.F.

Likewise, Fleischer *et al.* (1957) confirmed increases in C.S.F. and serum G.O.T. levels in patients with cerebrovascular accidents, and Myerson *et al.* (1957) showed that the

serum G.O.T. activity was increased in twelve out of twenty-four patients with cerebral thrombosis or embolus, whereas the C.S.F. transaminase was only minimally elevated in three out of sixteen cases.

Brodell *et al.* (1959) estimated the C.S.F. transaminase activity in thirty-nine patients with cerebrovascular accidents. They found that large infarcts caused an elevation of cerebrospinal fluid transaminase during the first 10 days after the onset, but that small infarcts were unlikely to give a rise. Infarcts involving less than an estimated 200 grammes of cerebral tissue at autopsy showed a poor correlation with the transaminase values. The C.S.F. transaminase did not correlate with the protein content.

#### **Subarachnoid haemorrhage. C.S.F. L.D.H. and G.O.T.**

Increased C.S.F. enzyme activity has also been reported in subarachnoid haemorrhage. Thus Wróblewski *et al.* (1958) noted increased C.S.F. lactate dehydrogenase activity in two cases whereas sixteen patients with 'cerebrovascular accidents' had normal values. Katzman *et al.* (1957) also noted increased C.S.F. transaminase activity in four out of ten patients with subarachnoid haemorrhage.

#### **Structural brain damage, L.D.H. iso-enzymes**

Lactic dehydrogenase iso-enzymes in normal and pathological spinal fluids were reported by Cunningham *et al.* (1965). The pathological fluids were obtained from patients without structural damage to the nervous system (neurosis, headaches, idiopathic epilepsy), patients with structural damage to the nervous system (head injury, cerebrovascular disease, hydrocephalus, cerebral tumour, presenile dementia), and four acute and four chronic cases of disseminated sclerosis. Because the number of cases in each of the pathological categories was small, the cases with structural damage were grouped together and compared with the group without structural damage. Both L.D.H.<sub>2</sub> and L.D.H.<sub>3</sub> were significantly increased in the

C.S.F. in the group with structural damage as compared with the group with no structural derangement.

### **Cerebral atrophy, C.S.F. transaminase**

In 1961 Jensen and Osterman reported their results of glutamic oxaloacetic transaminase levels of the C.S.F. in patients with cerebral atrophy. They studied twenty-seven patients with cerebral atrophy and twenty-one normal persons. They found a significant elevation of the transaminase levels in all patients with cerebral atrophy and they claimed the rise in transaminase activity to be present before there was any pneumoencephalographic evidence of cerebral atrophy. Twenty of the patients had presenile dementia.

### **Epilepsy, C.S.F. G.O.T. and C.P.K. activity**

In cases of epilepsy, Mellick and Bassett (1964) found significant elevation of C.S.F. G.O.T. activity when the cerebrospinal fluid was removed within 48 hours of the seizure. Herschkowitz and Cumings (1964) also found increased C.S.F. C.P.K. levels in six out of seven patients with symptomatic epilepsy. This C.S.F. enzyme was also increased in six out of seven patients with progressive hydrocephalus, and in all five patients with benign intracranial hypertension.

### **Meningitis: C.S.F. L.D.H. and G.O.T. activity**

Increased C.S.F. enzyme activity in cases of meningitis is not surprising, and Wróblewski *et al.* (1957) confirmed raised C.S.F. lactate dehydrogenase levels in six patients. Likewise, Jakoby and Jakoby (1958) found greatest C.S.F. lactate dehydrogenase activity in four cases of meningitis. These patients had a mean value of 48 units, whereas the normal was 10 units. Patients with cerebrovascular accidents had a mean value of 31 units, and the four patients with secondary tumours of the brain had significantly but slightly elevated values, the mean value being 15 units. The C.S.F. glutamic oxaloacetic transaminase was also estimated by Grendahl and Kloster (1965) in patients with acute infections

of the nervous system. Elevated values were observed in bacterial and viral meningitis, but the values were elevated more in the patients with bacterial meningitis.

### Other C.S.F. enzymes

#### (i) *Desoxyribonuclease and ribonuclease activity in C.S.F.*

Kovacs (1954) analysed cerebrospinal fluid for desoxyribonuclease and showed that it was not as frequently demonstrated in the C.S.F. as ribonuclease. The majority of specimens from cases of meningitis, syphilis, epilepsy, hydrocephalus and concussion showed desoxyribonuclease activity. There was a striking absence of this enzyme from the cerebrospinal fluid of patients with poliomyelitis, which is to be contrasted with Kovacs's (1953) earlier finding of very high activity of ribonuclease in poliomyelitis. Houck (1958) measured the ribonuclease content of the C.S.F. and found that patients with cerebrovascular accidents, brain tumour and acute disseminated sclerosis had elevated levels.

#### (ii) *Cholinesterase activity in C.S.F.*

The cholinesterase activity of cerebrospinal fluid was measured by Jefferson (1954) who reported that when the total protein content of the C.S.F. was abnormally high pseudocholinesterase was increased, whereas there was a less well-defined tendency for true cholinesterase activity to decrease with abnormally high C.S.F. protein values. He found a significant degree of correlation between C.S.F. total protein and the ratio of true- to pseudo-cholinesterase. In fifteen cases of disseminated sclerosis there was no significant change in the C.S.F. cholinesterase. Plum and Fog (1960) in analysing the C.S.F. in ninety-six cases of disseminated sclerosis found strikingly low cholinesterase activity in sixty-nine cases. The remainder of the patients had relatively low levels of activity, but these findings were not specific for disseminated sclerosis. Pseudo-cholinesterase levels in plasma and cerebro-spinal fluid were reported by Webster and Mackenzie (1957). They studied fifteen cases of disseminated

sclerosis and found that there was no significant difference from normal values.

(iii) *Glutathione reductase activity in C.S.F.*

Glutathione reductase of the cerebrospinal fluid was estimated in thirteen subjects with neurological disease by Manso and Wróblewski (1958). Five had ruptured lumbar discs, two cerebral haemorrhage and one each had cerebral concussion, migraine, acute labyrinthitis, hydrocephalus, tuberculous meningitis and meningococcal meningitis. Glutathione reductase activity was increased only in the patient with acute bacterial meningitis.

### DISCUSSION AND AUTHOR'S CASES

From this review of the previous work it is seen that by far the greatest interest has centred on the glutamic oxaloacetic transaminase and lactic dehydrogenase activity of the cerebrospinal fluid. The reports have been varied and sometimes conflicting. Hain and Nutter (1960) found that lactic dehydrogenase and glutamic oxaloacetic transaminase increased linearly with age and thought that the conflicting results reported previously could have been due to the fact that the ages of patients and controls had not been matched. Spolter and Thompson (1962) also found an increase in lactic dehydrogenase and glutamic oxaloacetic transaminase activity with increasing age and increasing protein concentration of the C.S.F.

However, in an analysis of 500 samples of cerebrospinal fluid for lactic dehydrogenase and glutamic oxaloacetic transaminase activity no significant correlation was found between the ages of the patients and the activities of these enzymes (Davies-Jones, 1967). There was also no relationship between the enzyme activities and the protein concentration of the C.S.F.

The conditions studied included patients with disseminated sclerosis, primary brain tumour, secondary carcinoma of the

central nervous system (C.N.S.), and others with carcinomatous neuropathy associated with a distant primary malignant tumour (Davies-Jones, 1967). Miscellaneous conditions in which C.S.F. L.D.H. and G.O.T. were measured included cases of dementia, epilepsy, viral meningitis and acute polymyositis. Patients with acute cerebrovascular accidents were not studied. Although such cases may have alteration in enzyme levels in the C.S.F. or serum, the diagnosis is usually clear from the history, and in most cases there is no diagnostic problem.

## Results

### (i) *Brain tumours*

The C.S.F. transaminase (G.O.T.) activity was normal in nineteen patients with primary tumours of the central nervous system, but in one patient with a very necrotic astrocytoma (grade IV) the C.S.F. lactate dehydrogenase activity was increased. Eight had a C.S.F. protein of over 100 mg. per cent, and all patients had normal enzyme levels in the C.S.F., except the patient with a necrotic astrocytoma. On the other hand, there was a striking association between the presence of secondary carcinoma of the nervous system and elevation of both glutamic oxaloacetic transaminase and lactic dehydrogenase activities of the C.S.F. Fifteen patients were studied and thirteen had elevation of enzyme activities well above the normal range (mean  $\pm 2$  S.D.) Six of this group of thirteen patients had a normal C.S.F. protein concentration.

### (ii) *Carcinomatous neuropathies*

All fourteen patients with the neuropathy of carcinoma (including three with cerebellar degeneration) that were studied had significantly elevated transaminase activity of the C.S.F., and one had a slightly elevated lactic dehydrogenase level. Three of these cases had elevated protein concentrations, 65, 70 and 400 mg. per cent. In several of the patients with C.N.S. disease caused by either local or remote carcinoma and elevated C.S.F. enzymes the serum activities of the enzymes

were consistently normal. Three patients with myasthenic myopathy had normal C.S.F. enzymes. All thirty patients with peripheral neuropathy from other various causes had normal C.S.F. enzyme activities, as did fifteen patients with carcinoma having no neurological complications. It was also noted that four of the eight patients with cerebellar degeneration of undetermined cause had significantly elevated C.S.F. transaminase activity, so that this finding in the C.S.F. of a patient with cerebellar dysfunction is of no aetiological significance. However, the additional finding of elevated C.S.F. lactic dehydrogenase activity in a patient with cerebellar disease would point strongly to the presence of a secondary carcinomatous deposit.

(iii) *Disseminated sclerosis and other miscellaneous conditions*

Of 107 patients with disseminated sclerosis only two had slight elevation in C.S.F. transaminase activity. It was also observed that two out of nine patients with dementia (one presenile, the other senile) had significantly raised transaminase activity but normal lactic dehydrogenase content of the C.S.F. Of sixteen subjects with epilepsy, one had slight but significant elevation in transaminase activity but he gave a history of a recent fit. The single patient admitted with status epilepticus showed marked increase in both lactic dehydrogenase and transaminase levels. One patient of seven with viral meningitis had elevation of both enzymes in the C.S.F. with return to normal levels on resolution of the disease. One man with acute polymyositis plus minimal signs of cerebellar dysfunction had significant transaminase elevation. However, no underlying carcinoma was found after detailed search.

## SUMMARY

In the first part of this chapter the C.S.F. enzyme changes in neurological diseases were reviewed. The C.S.F. transaminase (G.O.T.) and lactate dehydrogenase (L.D.H.) activities were measured in a number of neurological diseases and the results

have been recorded. Elevation of C.S.F. lactic dehydrogenase and glutamic oxaloacetic transaminase activity was highly suggestive of metastatic malignant disease of the central nervous system, with the occasional exception that very necrotic highly malignant gliomas have rarely been found associated with these changes. On the whole, primary brain tumours are not associated with C.S.F. enzyme changes. Disseminated sclerosis has no association with alteration in C.S.F. enzyme activity, whether during activity or remission. The important observation was also made that there was a striking association between the neuropathies of carcinoma and increased C.S.F. transaminase activity. The relatively easy and quick estimation of these enzymes may well be worth while in patients with polyneuropathy or suspected metastatic malignant disease of the central nervous system.

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# Index

- Abruptio placentae: L.D.H., 112  
 L.D.H. iso-enzymes, 113
- Acid phosphatase: Gaucher's disease, 17  
 prostatic carcinoma, 204, 210
- Activators, 8
- Adrenogenital syndrome, 164
- African heart disease, *see* Heart disease
- Africans, *see* East Africans and Heart disease
- Age: relation to C.S.F. enzyme activity, 224
- Alanine aminotransferase, *see* S.G.P.T.
- Alanine aminotransferase (S.G.P.T.) children, 167  
 heart disease: Africans, 58 ff., Fig. 8  
 hepatitis, 160  
 infective hepatitis, 79  
 neonatal jaundice, 160
- Alkaline phosphatase: biliary obstruction: increase, 16  
 children, 166  
 heat-stable fraction, 94  
 immunology, 94  
 bone disease, 17  
 placenta: histochemistry, 94  
 placental origin, 93  
 pregnancy, 92  
 prostatic carcinoma, 210, Table 30  
 rickets, 15
- Alkaline phosphatase iso-enzymes: bone disease, 33  
 liver disease, 33
- Amino-acid metabolism: abnormalities, 153 ff.
- Amoebiasis, 69 ff.
- Amoebic liver abscess, 71-3  
 differential diagnosis, 76  
 disease: liver function tests, 73
- Amylase, *see also* Macroamylasaemia
- Amylase, diabetes, 178  
 pancreatitis, 178  
 uraemia: increase, 196-7
- Anaemia: pregnancy complications, 113
- Anaemia, megaloblastic: pregnancy: L.D.H. and S.H.B.D. increase, 113
- Analmia, pernicious: L.D.H. increase, 113
- Annular subvalvular left ventricular aneurysm: Africans, 58
- Antibody studies: *Entamoeba histolytica*, 76
- A.P., *see* Alkaline phosphatase
- Apnoea, scoline, *see* Scoline apnoea
- Ascites: enzyme activity, 18
- Aspartate aminotransferase, *see* Serum glutamic oxaloacetic transaminase (S.G.O.T.)
- Aspartate aminotransferase (S.G.O.T.): African heart disease 58 ff., Fig. 7

- Aspartate aminotransferase  
children, 167  
hepatic necrosis, 51  
myocardial infarction, 37-8, 44, 48
- Bacterial enzymes, 200, 207-8  
Bacteriuria: catalase test, 200
- Biliary obstruction: alkaline phosphatase increase, 16
- Bladder carcinoma: and schistosomiasis, 203  
and  $\beta$ -glucuronidase, 203
- Bone disease: alkaline phosphatase iso-enzymes, 33  
serum A.P. increase, 17
- Brain damage: L.D.H. iso-enzymes, 221
- Brain tumours: C.S.F. enzymes, 217-19, 225  
metastatic: C.S.F. enzymes, 219
- Bronchial carcinoma: L.D.H. iso-enzymes, 31
- Caeruloplasmin: heart disease:  
Africans, 59 ff. Fig. 11
- Caeruloplasmin: myocardial infarction, 46
- Cancer: muscle wasting, 17
- C.A.P. *see* Cystine aminopeptidase
- Carbohydrate metabolism: abnormalities, 156
- Carcinoma, *see also* Brain tumours
- Carcinoma: bladder, 202-3  
bronchial: L.D.H. iso-enzymes, 31  
cervix, 126 ff., 140 ff.  
 $\beta$ -glucuronidase, 130  
6-P.G.D., 132 ff.  
P.H.I., 143
- clitoris: 6-P.G.D., 143
- corpus uteri, 142
- enzyme changes, 17
- ovary: 6-P.G.D., 143
- prostate: acid phosphatase, 17, 204, 210, Table 30
- L.D.H. iso-enzymes, 204
- urinary tract, 202-3
- vulva: 6-P.G.D., 143
- Carcinoma *in situ*: cervix, 127-9, 138  
6-P.G.D. levels, 138 ff., Table 19
- Carcinoma, invasive: 6-P.G.D., 140-2
- Carcinomatous neuropathies: C.S.F. enzymes, 225
- Cardiomyopathies: enzyme changes, 50
- Catalase test: bacteriuria, 200
- Cell membrane: permeability, 12
- Cerebellar degeneration: C.S.F. enzymes, 225-6
- Cerebral atrophy: C.S.F. enzymes, 222
- Cerebral lipoidoses, 220
- Cerebrospinal fluid: enzyme activity 18, 216 ff.  
brain tumours, 217-19, 225  
carcinomatous neuropathies, 225  
cerebellar degeneration, 225-6  
cerebral atrophy, 222  
cerebrovascular accidents, 220  
disseminated sclerosis, 217, 226  
epilepsy, 222  
meningitis, 222  
relation to age, 224  
C.S.F. protein levels, 224  
subarachnoid haemorrhage, 221
- Cerebrospinal fluid enzymes:  
Cholinesterase, 223  
C.P.K. levels, 217 ff.  
G.O.T. levels, 217 ff.  
glutathione reductase, 224  
L.D.H., 217 ff.  
ribonuclease, 223
- Cerebrovascular accidents: serum and C.S.F. enzymes, 220
- Cervical mucus:  $\beta$ -glucuronidase, 131  
6-P.G.D., 138
- Cervix uteri: carcinoma, 126 ff.  
 $\beta$ -glucuronidase, 129, 130  
6-P.G.D., 140
- Phosphohexose isomerase (P.H.I.) 143

- 'Cheese reaction', 11  
 Children: alkaline phosphatase, 166  
 C.P.K. levels, 167-8  
 enzyme measurements, 162-3  
 (Table 22), 165-8  
 5'-nucleotidase, 166  
 S.G.O.T., 167  
 S.G.P.T., 167  
 S.H.B.D., 165
- Cholinesterase: Africans, 58 ff.  
 C.S.F. levels, 223  
 congestive heart failure, 51  
 heart disease: Africans, 58 ff.,  
 Fig. 10  
 nephrotic syndrome, 18, 196-7
- Cholinesterase variants, 30
- Cirrhosis: liver function tests, 80
- Classification of enzymes, 5-7
- Co-enzymes, 8
- Congenital dystrophy: muscle, 167
- Congestive heart failure: changes in cholinesterase, M.A.O., and S.G.O.T., 51
- Cord blood: enzyme changes, 91
- Coronary insufficiency: enzyme changes, 45
- Corpus uteri: carcinoma, 142
- C.P.K., *see* Creatine phosphokinase
- Creatine phosphokinase (C.P.K.):  
 C.S.F. levels, 217 ff.  
 comparison with L.D.H.,  
 S.G.O.T. and S.H.B.D., 48,  
 Fig. 6  
 in children, 167-8  
 in cretinism: increase, 161  
 in diabetic ketosis, 178  
 in myxoedema: increase, 12
- Creatine phosphokinase iso-enzymes, 27  
 tetanus, 32
- Cretinism: associated with jaundice:  
 C.P.K. increase, 161
- Cystine aminopeptidase (C.A.P.), 96 ff., 99 ff.  
 chemical estimation, 104  
 decrease in severe toxæmia, 109  
 effect of liver disease, 106  
 effect of pH, 107
- estimation: clinical applications, 105  
 in parturition, 103  
 placental origin, 102  
 species variations, 102
- Cytological surveys: cervical carcinoma, 126-9
- D.A.O., *see* Histaminase
- Dermatomyositis, 16
- Diabetes: amylase, 178  
 L.A.P. levels, 183-91, Tables 23-28  
 myocardial infarction, 182
- Diabetes mellitus, 175 ff.  
 glucose-6-phosphatase, 176  
 $\beta$ -glucuronidase, 177  
 hepatic glucokinase, 176
- Diabetic gangrene: L.A.P. increase, 184, 189
- Diabetic ketosis, 178
- Diabetic microangiopathy, 177
- Diabetic nephropathy, 184, 189
- Diamine oxidase (D.A.O.): pregnancy, 92
- Disaccharidase deficiency, 158, Fig. 25
- Disseminated sclerosis, 223  
 C.S.F., 217, 226
- Drug interactions with enzymes, 11
- East Africans: enzyme studies, 78 ff.
- Embden Meyerhoff pathway, 132, Fig. 23
- Endocrine abnormalities, 161
- Endometrium: L.D.H. iso-enzymes, 88, 89
- Endomyocardial fibrosis: Africans, 58
- Enovid, *see* Oral contraceptives
- Entamoeba histolytica*: antibody studies, 76
- Enzyme changes: in disease, 15  
 physiological, 14, 15
- Enzyme determination: spectrophotometry, 8
- Enzyme diuresis, *see* Urinary loss of enzymes
- Enzyme inhibitors, 9, 160

- Enzyme measurements: children, 164-8  
 Enzyme-substrate complex, 2  
 Enzyme units, 2-3  
 Enzymes: classification, 6-7 (Table 2)  
     distribution, 13  
     nomenclature, 6-7  
 Enzymuria, *see* Urinary loss of enzymes  
 Epilepsy: C.S.F. enzymes, 222  
 Experimental studies: myocardial infarction, 14, 44  
     pancreatitis, 14  
 Fanconi syndrome (adult) 204 ff.  
 Fructose intolerance, 157-8  
 Fructose - 1 - phosphate aldolase, 157-8  
 Galactosaemia, 151, 156  
 Galactose-1-phosphate uridyl transferase deficiency: galactosaemia, 152  
 Gaucher's disease: acid phosphatase increase, 17  
 $\alpha_2$  globulin in pregnancy, 88  
 Glucokinase, hepatic: diabetes mellitus, 176  
 Glucose-6-phosphatase: diabetes mellitus, 176  
     deficiency: von Gierke's disease, 156  
 $\beta$ -glucuronidase: carcinoma:  
     bladder, 203  
     cervix, 129, 130  
     diabetes mellitus, 177  
     renal disease, 201  
 Glucuronyl transferase deficiency, 160  
     inhibitors, 10, 11  
 Glutamic oxaloacetic transaminase (G.O.T.), *see* aspartate aminotransferase  
     G.O.T.: C.S.F. levels, 217 ff.  
     urine: raised in renal disease, 197  
 Glutathione reductase: C.S.F., 224  
 Glycogen storage diseases, 156  
 Gynaecological lesions: 6-P.G.D., 135-8  
 Hartnup disease, 156  
 Heart disease, *see also* Congestive heart failure and Rheumatic heart disease  
 Heart disease: Africans (Nigerians), 57 ff.  
 Heart muscle disease: Africans, 58  
 Hepatic congestion: myocardial infarction, 42  
 Hepatic necrosis: S.G.O.T., 51, 52  
 Hepatitis: S.G.P.T., 160  
 Hepatitis, infective: S.G.P.T., 79  
     liver function tests, 79  
 Hepato-biliary disease: 5'-nucleotidase, 166  
 Hepatoma, malignant: liver function tests, 80  
 Hexokinase, *see* Glucokinase  
 Hexose monophosphate shunt, 132, Fig. 23  
 Histaminase, *see* Diamine oxidase  
 Hydroxybutyric acid dehydrogenase  
     *see* Serum  $\alpha$ -hydroxybutyric acid dehydrogenase  
 Hypertension, 198  
     malignant: enzyme studies, 204 ff.  
 Immigrants: tropical diseases: Great Britain, 69 ff.  
 Infarction, *see* Myocardial infarction and Pulmonary infarction  
 Infective hepatitis, *see* Hepatitis, infective  
 Inflammation: 6-P.G.D. increase, 137-9  
 Insulinase, 176  
 International units: conversion table, 3-5  
 Iso-enzymes, 25 ff.  
     diagnostic value, 30  
     genetic abnormalities, 30  
     methods of separation, 28  
     molecular theory, 26  
     physiological significance, 27  
     species variations, 25, 28  
     starch gel electrophoresis, 29

- Jaundice: complicating pregnancy, 110  
neonatal, 160
- Karmen units, 8
- Ketosis, diabetic, 178
- Kidney, *see* Nephrotic syndrome and Renal disease
- Kidney, *see also* Renal transplant
- Kwashiorkor: liver function tests, 82
- Lactase deficiency, 159
- Lactic dehydrogenase (L.D.H.):  
abruptio placentae, 112  
anaemia: pregnancy, 113  
pernicious, 113  
C.S.F. levels, 217 ff.  
comparison with C.P.K., S.G.O.T. and S.H.B.D., 48  
leukaemia, 219  
lymphoma, 219  
metastases: brain, 219  
nephrotic syndrome: increase, 196, 197  
renal diseases: increase, 198  
uraemia: increase, 196  
urinary excretion: renal diseases, 197  
urinary tract carcinoma, 202
- Lactic dehydrogenase (L.D.H.) isoenzymes, 25 ff.  
abruptio placentae, 113  
brain damage, 221  
bronchial carcinoma, 31  
endometrium, 89  
myocardial infarction, 46  
organ transplants, 33  
prostatic carcinoma, 204  
starch gel electrophoresis, 25  
tetanus, 32
- Lactose intolerance, 159
- L.A.P., *see* Leucine aminopeptidase
- L.D.H., *see* Lactic dehydrogenase
- Leucine aminopeptidase (L.A.P.):  
diabetes mellitus, 183-91,  
Tables 23-28  
pregnancy, 95
- prostatic carcinoma, 210, Table 30  
urinary excretion, 198, Table 29
- Leukaemia: L.D.H., 219
- Lipoidoses, cerebral, 220
- Liver abscess, *see* Amoebic liver abscess
- Liver damage: oral contraceptives, 111
- Liver disease: alkaline phosphatase iso-enzymes, 33  
effect on C.A.P. activity, 106
- Liver function tests: amoebic liver disease, 73  
cirrhosis, 80  
diabetes, 182  
effect of oral contraceptives, 111  
infective hepatitis, 79  
kwashiorkor, 82  
malignant hepatoma, 80
- Luteinizing hormone: effect of oral contraceptives, 89
- Lymphoma: L.D.H., 219
- Lysozyme: urine, 198
- McArdle's syndrome, 156
- Macroamylasaemia, 194
- M.A.O., *see* Monoamine oxidase
- Meningitis: C.S.F. enzymes, 222
- Menopausal changes: 6-P.G.D., 135
- Menstruation: endometrial L.D.H., 89
- Metabolism: carbohydrate, 156  
inborn errors, 151 ff.  
tryptophan, 155-6
- Metastases: brain: L.D.H., 219
- Michaelis constant (Km), 2
- Microangiopathy, diabetic, 177
- Monoamine oxidase (M.A.O.), 87  
congestive heart failure, 51  
placenta: toxæmia, 107
- Muscle, *see also* Heart muscle disease
- Muscle: alcoholic myopathy, 12  
congenital dystrophy, 167  
diseases, 16  
prolonged exercise, 12  
tetanus, 12
- Muscle wasting: cancer, 17  
enzyme changes, 17

- Myocardial infarction: diabetes, 182  
 enzyme changes, 37, Fig. 6  
 differential diagnosis, 42  
 serum G.O.T. increase, 44  
 serum metals, 46  
 experimental studies, 14, 44  
 hepatic congestion, 42  
 L.D.H. iso-enzymes, 46  
 S.G.O.T., 37-8, 44, 48  
 S.H.B.D., 48
- Neonatal enzyme changes, 15  
 Neonatal jaundice, 160  
 Nephron: enzyme activity, 195  
 Nephrotic syndrome, 197  
 cholinesterase increase, 196  
 enzyme studies, 204 ff.  
 L.A.P. excretion: increase, 198,  
 Table 29  
 L.D.H. increase, 196-7
- Neuropathies, carcinomatous:  
 C.S.F. enzymes, 225
- 5'-nucleotidase: children, 165-7  
 hepato-biliary disease, 33  
 pregnancy, 93
- Oestrogens: effect on 6-P.G.D., 134  
 Oral contraceptives, 88-9, 110  
 effect on liver function tests, 111  
 effect on luteinizing hormone, 89  
 effect on serum proteins, 88  
 liver damage, 111
- Organ transplantation, 34  
 kidney, 209  
 L.D.H. iso-enzymes, 33  
 metabolic disease, 168
- Ovulation, 88
- Oxytocin: structure, 103, Fig. 20
- Oxytocinase, *see* Cystine aminopeptidase (C.A.P.)
- Pancreatitis: amylase, 178  
 associated with uraemia, 197  
 differential diagnosis, 42  
 experimental studies, 14
- Parenchymal renal diseases, 197
- Pendred's syndrome, 161
- 6-P.G.D., *see* Phosphogluconate dehydrogenase
- Phenylketonuria, 153-4
- P.H.I., *see* Phosphohexose isomerase
- 6-phosphogluconate dehydrogenase (6-P.G.D.), 132 ff.
- carcinoma, 132  
*carcinoma in situ*, 138 ff., 141, Table 19  
 cervical mucus, 138  
 cervical carcinoma, 141  
 diagnostic applications, 133-4  
 effect of oestrogens, 134  
 in gynaecological lesions, 135 ff.  
 in pregnancy, 133, 135  
 increase: in inflammation, 137-9  
 normal levels, 134  
 post-menopausal levels, 135  
*Trichomonas vaginalis*, 137  
 vaginal fluid, 132  
 normal levels, 134-5
- Phosphohexose isomerase (P.H.I.):  
 cervical carcinoma, 143
- Pill, contraceptive, *see* Oral contraceptives
- Pitocinase, *see* Cystine aminopeptidase
- Placenta: alkaline phosphatase, 94  
 monoamine oxidase: in toxæmia, 107
- Pregnancy: alkaline phosphatase, 92  
 complications: abruptio placentæ, 112  
 anaemia: L.D.H., 113  
 jaundice, 110  
 trophoblastic disease, 114  
 diamine oxidase (histaminase), 92  
 enzyme activity, 86 ff.  
 $\alpha_2$ -globulin, 88  
 L.A.P., 95  
 5'-nucleotidase, 93  
 6-P.G.D. increase, 135  
 serum enzymes, 90  
 serum proteins, 87  
 ectopic: rupture, 114  
 multiple: urinary L.A.P. increase, 95
- Prostate: carcinoma: acid phosphatase, 17, 204, 210, Table 30  
 enzyme levels, 210, Table 30

- Prostate (*cont.*)  
 L.A.P., 210  
 L.D.H. iso-enzymes, 204
- Proteins, *see also* Serum proteins
- Protein structure of enzymes, 1
- Pseudo-cholinesterase, *see* Cholinesterase
- Pulmonary infarction: enzyme changes, 43
- Pyelonephritis, 199  
 experimental, 201
- Renal damage, 198
- Renal disease, *see also* Nephrotic syndrome,
- Renal disease:  $\beta$ -glucuronidase, 201  
 L.D.H. increase, 198  
 serum enzymes increase, 196 ff.  
 urinary excretion of L.D.H., 197  
 urinary G.O.T., 197  
 parenchymal, 197
- Renal transplant, *see* Organ transplantation: kidney
- Rheumatic heart disease: Africans, 58
- Ribonuclease: C.S.F., 223
- Rickets: alkaline phosphatase increase, 15
- Schistosomiasis: associated with bladder carcinoma, 203
- Scoline apnoea, 30  
 due to amoebic liver abscess, 73  
 due to bronchial carcinoma, 73  
 due to cholinesterase deficiency, 30, 73, 90  
 due to liver disease, 73
- Serum glutamic oxaloacetic transaminase (S.G.O.T.), *see* Aspartate aminotransferase and Glutamic oxaloacetic transaminase
- Serum metals: myocardial infarction, 46
- Serum proteins: pregnancy, 87
- S.G.O.T.: children, 167  
 comparison with C.P.K., L.D.H. and S.H.B.D., 48
- congestive heart failure, 51  
 heart disease: Africans, 58 ff., Fig. 7  
 hepatic necrosis, 51 ff.
- Serum glutamic oxaloacetic transaminase: children, 167  
 myocardial infarction, 38, 44
- Serum glutamic pyruvic transaminase (S.G.P.T.), *see* Alanine aminotransferase
- S.G.P.T.: heart disease: Africans, 58 ff., Fig. 8  
 hepatitis, 160  
 infective hepatitis, 79
- Serum  $\alpha$ -hydroxybutyric acid dehydrogenase (S.H.B.D.): anaemia: pregnancy, 113  
 children, 165  
 comparison with C.P.K., L.D.H. and S.G.O.T., 48  
 estimation, 31  
 heart disease: Africans, 58 ff.  
 Fig. 9  
 myocardial infarction, 48
- Serum transaminases: children, 167  
 neonatal jaundice, 160
- Spectrophotometric method of enzyme determination, 8
- Starch gel electrophoresis: iso-enzymes, 29  
 L.D.H., 25
- Subarachnoid haemorrhage: C.S.F. enzymes, 221
- Synovial fluid: enzyme activity, 18
- Tetanus, 12, 32  
 C.P.K. and L.D.H. iso-enzymes, 32
- Tissue damage and increased enzyme activity, 15
- Toxaemia of pregnancy, 107 ff.  
 liver function tests, 109  
 M.A.O.: placenta, 107-8  
 urinary L.A.P. increase, 95 ff.  
 108
- Trichomonas vaginalis: 6-P.G.D., 137

- Trophoblastic disease: pregnancy complications, 114
- Tropical diseases: immigrants: Great Britain, 69
- Tryptophan metabolism: abnormalities, 155-6
- Tumours, *see* Brain tumours; Cancer; Carcinoma; Leukaemia; Lymphoma; Metastases
- Tyrosinosis, 155
- Uraemia, 196-7  
    amylase increase, 196-7  
    associated with pancreatitis, 197  
    L.D.H. increase, 196
- Urinary loss of enzymes, 194, 197
- Urinary tract carcinoma: L.D.H., 202
- Urinary tract disease, 194 ff.
- Urinary tract infections, 199
- Urine, *see also* Bacteriuria  
    L.A.P. excretion, 198, Table 29  
    L.A.P. increase: pregnancy, 95  
    L.H.D. increase: nephrotic syndrome, 197  
        lysozyme, 198
- Uterus: cancer *see* Cervix uteri and Corpus uteri
- Vaginal fluid: carcinoma *in situ*, 138  
     $\beta$ -glucuronidase increase, 131  
    6-P.G.D., 132 ff.  
        increased in inflammation, 139  
        normal levels, 134-5  
    phosphohexose isomerase (P.H.I.), 143
- Vaginitis: enzyme changes, 137
- Von Gierke's disease, 156