REFERENCES

Barron, E. S. G., Barron, G. A. & Klemperer, F. [1936]. *J. biol. Chem.* **116**, 563.
Borsook, H., Davenport, H. W., Jeffries, C. E. P. & Warner, R. C. [1937]. *J. biol. Chem.* **117**, 237.
Crook, E. M. [1941]. *Biochem. J.* **35**, 226.
—— & Hopkins, F. G. [1938]. *Biochem. J.* **32**, 1356.
Deeney, J., Murdock, E. T. & Rogan, J. J. [1942]. *Biochem. J.* **36**, 271.
Elvehjem, C. A. [1942]. *J. Amer. med. Ass.* **120**, 1388.
Hopkins, F. G. & Morgan, E. J. [1936]. *Biochem. J.* **30**, 1446.
Johnson, S. W. & Zilva, S. S. [1934]. *Biochem. J.* **28**, 1393.
Lemberg, R., Legge, J. W. & Lockwood, W. H. [1941]. *Biochem. J.* **35**, 339.
Mawson, C. A. [1935]. *Biochem. J.* **29**, 569.
Platt, R. [1931]. *Brit. J. exp. Path.* **12**, 139.
Schultze, M. O., Harrer, C. J. & King, C. G. [1939]. *J. biol. Chem.* **131**, 5.
Woodward, G. E. & Fry, E. G. [1932]. *J. biol. Chem.* **97**, 465.

Lysine Deficiency in Young Rats

By H. A. HARRIS, A. NEUBERGER and F. SANGER (Benn W. Levy Student),
Departments of Anatomy and Biochemistry, University of Cambridge

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'Essential' amino-acids share with vitamins the property that they cannot be synthesized in the mammalian body and have therefore to be supplied in the diet. They differ from vitamins, however, in several other respects. Whilst vitamins are present only in small quantities in the tissues, amino-acids combined in proteins form a large part of the total mass of the body. The quantitative requirements for essential amino-acids are therefore considerably greater than those for vitamins. On the other hand, the body has a great store of amino-acids in its proteins, and the deleterious effects of a dietary deficiency of a particular essential amino-acid will therefore generally be less sudden and less marked, at least in the adult animal, than those of most vitamin deficiencies.

The clinical picture of a particular amino-acid deficiency will greatly depend on the biological function of the amino-acid concerned. If an amino-acid is needed only as building material for the synthesis of protein, a lack of that amino-acid in the diet will result only in an inhibition of protein synthesis and the effect will be more marked in the young animal than in the adult. The severity of the symptoms will depend on the quantitative requirements of the body relative to its stores of that amino-acid, and also on the extent of irreversible oxidation which occurs during the constant breaking-down and building-up of proteins in the mammalian body [Schoenheimer & Rittenberg, 1940]. This loss, which corresponds to the wear and tear quota of Rubner, may vary considerably for different amino-acids, and the effects of deficiencies on weight and nitrogen equilibrium may vary accordingly.

Some amino-acids, however, are apparently needed not only for the synthesis of proteins, but also for some specific metabolic purpose. For example, methionine acts as a donor of methyl groups and its absence from the diet produces specific symptoms peculiar to this amino-acid. Again, valine deficiency produces characteristic nervous symptoms [Rose & Eppstein, 1939] which suggest a special metabolic role for that amino-acid in the central nervous system. The deficiency or lack of such amino-acids will produce a clinical picture peculiar to the amino-acid concerned and will be superimposed on the general picture of deficiency common to all essential amino-acids which may be called a 'structural' deficiency.

This paper is concerned with lysine deficiency. Lysine was shown to be indispensable for the growth of young animals by Osborne & Mendel [1914; 1916], who also showed that animals could be kept alive for long periods and at fairly constant weight on diets containing very small amounts of lysine. Lack of lysine in young animals produces no specific symptoms apart from cessation of growth, and it has been claimed that it is not essential for maintenance in adults [Burroughs, Burroughs & Mitchell, 1940].

The animal kept at a constant weight by the lysine-deficient diet is, however, not static as regards the development of individual tissues and organs. Its body and tail lengths increase and so do the leg bones [Winters, Smith & Mendel, 1927], and many organs such as the kidneys and the eyes increase in weight, whilst others, like muscle, decrease [Lafon, 1939]. Male genital organs are not affected by lysine deficiency, but in females the

oestrous cycle is suspended [Pearson, 1937], and lactation is more affected than gestation [Hart, Nelson & Pitz, 1918; Fowler, Morris & Wright, 1934; Lafon, 1939]. It can be inferred from the absence of specific symptoms that lysine is required purely as passive material for the synthesis of proteins. This paper contains a description of the changes in the formed elements of the blood and in the blood proteins, and also of the changes in the processes of ossification, caused by a deficiency of lysine in the diet.

EXPERIMENTAL

Diets. The experimental animals, piebald rats of the laboratory stock (mostly males), were put on the diets soon after weaning and littermates were used as controls in each group. The main basal diet (diet A), which was given *ad lib.*, was as follows: gliadin 18%, arachis oil 10%, lard 5%, cod-liver oil 1%, salt mixture (McCollum) 5%, corn starch 61%. Basal diet B was identical with diet A except that gliadin was replaced by the same amount of zein, and a supplement of 10 mg. of *l*-tryptophan was always given daily. The water-soluble vitamins were given as a separate supplement in the following daily doses: aneurin 30μg., pyridoxin 30μg., riboflavin 50μg., calcium-*d*-pantothenate 100μg., nicotinic acid 1 mg., inositol 1 mg. and choline chloride 3 mg. The animals also received a daily dose of 0·5 ml. of a purified aqueous acetone extract of whole liver, of which 1 ml. corresponds to 4 g. fresh liver. This liver extract, which contained 11 mg. N/ml., gave a positive ninhydrin reaction and must be presumed to contain amino-acids.

The amino-acid supplements were fed with the vitamins. Adding the amino-acids to the basal diets did not affect the results. Lysine was given in the control experiments in the form of *l*-lysine hydrochloride, 40 mg./day.

RESULTS

Increase of total body weight

Table 1 shows that if young rats are reared on a diet in which practically all the N is derived from gliadin or zein, they stop growing and their body weight remains constant for long periods, confirming the old observations of Osborne & Mendel [1912, 1914]. Such a diet is by no means devoid of lysine. Osborne, van Slyke, Leavensworth & Vinograd [1915] isolated lysine from a gliadin hydrolysate and, after correcting for solubilities, arrived at a value of 0·64% for the lysine in gliadin. The indirect van Slyke distribution method indicates an even higher value and the lysine content can be tentatively put at about 0·9%. The liver concentrate included in our diet may also contain traces of lysine and the total daily intake of lysine on basal diet A may be as much as 10 mg.

Zein is believed to be completely free from lysine [Vickery, 1938] on the other hand, and basal diet B will contain only traces of lysine, from the liver extract and possibly from nitrogenous impurities in the starch. Since the results obtained with the gliadin and zein diets were almost identical, it can be concluded that the small amount of lysine in gliadin does not greatly affect the deficiency symptoms, at least in young rats.

If the basal diets are supplemented with sufficient lysine, growth occurs at a suboptimal rate (Table 1). We have found, in agreement with other workers, that an addition of 40 mg. lysine hydrochloride daily is sufficient to produce a maximal growth response with a gliadin diet. The increase in body weight, which varied from about 7–14 g./week (Table 1), is only about 40% of that produced by feeding a good protein, such as casein, at the same level. It appeared likely that gliadin might also be deficient in some other indispensable amino-acid. Rose [1938] found gliadin to be a poor source of threonine, and the value of 2·92% found by Winnick [1942] for the threonine content of gliadin is smaller than the values reported for other proteins such as casein or fibrin. An addition of 50 mg.

Table 1. *Total body weights of young rats receiving basal diets with or without added lysine*

Litter no.	Rat	Duration of exp. (weeks)	Basal diet	Lysine hydrochloride added daily (mg.)	Wt. (g.)		
					Initial	Final	Increase/week
5	13	6	A	0	33	32	−0·17
	14	6	A	0	30	29	−0·17
	15	6	A	0	31	29	−0·33
	17	6	A	40	32	83	+8·5
	18	6	A	40	31	90	+9·8
6	1	7	A	0	32	33	+0·14
	2	7	A	0	30	33	+0·43
	3	7	A	0	30	29	−0·14
	4	7	A	40	28	71	+6·2
	5	7	A	40	30	73	+6·2
8	30	5	B	0	31	30	−0·20
	31	5	B	0	31	30	−0·20
	32	5	B	0	33	33	0·00
	33	5	B	40	32	67	+7·00
	34	5	B	40	32	69	+7·40

dl-threonine daily to a gliadin diet supplemented with lysine produced, however, no improved growth response. Likewise, addition of *l*-histidine, in which gliadin is also poor, did not affect the growth of the rats, nor was any improvement effected by addition of isoleucine or cystine to the diet. The suboptimal growth produced by supplemented gliadin diets is not readily explained by the amino-acid composition of this protein and must remain an open question at the moment. A similar lack of success in supplementing zein by different amino-acids has been recently reported by Borchers, Totter & Berg [1942].

If animals on the basal diet are compared with animals reared on a diet to which an optimal amount of lysine has been added, we observe in fact the differences produced by a diet low in lysine and a diet which contains sufficient lysine but is still, for an unexplained reason, incapable of producing optimal growth. Nevertheless, it appears reasonable to ascribe the differences observed between the animals reared on the basal diet and the controls to the insufficient quantity of lysine in the basal diet.

Red cells and haemoglobin

New-born rats have about 2·5–3·5 million red cells/cu.mm. and about 10 g. haemoglobin/100 ml. blood. At the age of 3–4 weeks, i.e. immediately after weaning, we find in our stock average values of 4,700,000 and 10·3 g. Hb respectively. On a casein diet containing all the vitamins these values rise during the following 2 months to about 8,000,000 red cells and 14 g. Hb. The changes which occur on a gliadin diet and a diet supplemented with lysine are shown in Table 2. All the figures are average values obtained from 10 to 12 rats in each group; the animals were about 3 weeks old at the beginning of the experiment. In the earlier experiments haemoglobin was estimated by the method of Herbert [1941], but in the later experiments we used the more accurate method of Rimington [1942].

Table 2. *Increase in number of red cells and haemoglobin in normal and lysine-deficient animals*

No. of weeks on diet	Red cells (millions/ml.)		Hb (g./100 ml.)	
	Normal	Lysine-deficient	Normal	Lysine-deficient
0	4·80 ±0·4	4·80 ±0·4	10·2 ±0·5	10·2 ±0·5
2	5·90 ±0·4	5·35 ±0·5	11·0 ±0·5	10·35 ±0·6
3	7·1 ±0·6	6·02 ±0·4	12·5 ±0·5	10·2 ±0·4
5	7·2 ±0·5	6·24 ±0·5	12·5 ±0·4	10·8 ±0·6
7	7·4 ±0·4	6·21 ±0·5	12·55 ±0·4	10·9 ±0·6

Table 2 shows that with a gliadin diet supplemented with lysine the rise of the cell count and of the haemoglobin content is almost normal. The values may be slightly lower than on a casein diet, but our data were not sufficiently numerous to establish this point with certainty. In the lysine-deficient animals both haemoglobin and red cells rise more slowly and the final values are lower by about 15–20 % than in the controls. This increase indicates that, although the total weight of the animals remains constant, the haematopoietic system continues to develop. The differences found between the lysine-deficient animals and the controls can hardly be interpreted in the sense that lysine deficiency produces an anaemia, but it may be inferred that the rate of development of the haematopoietic system is somewhat reduced by the lysine deficiency.

The effect of lysine deficiency on red cells is of particular interest in connexion with the anaemia produced by deaminized casein [Hogan & Ritchie, 1934], which can be cured by feeding relatively large quantities of lysine [Hogan, Powell & Guerrant, 1941]. Deamination destroys the terminal-NH_2 group of lysine and the resulting hydroxy or unsaturated compound is almost certainly unable to replace lysine in the diet. It appears, however, from the results reported here that the destruction of lysine in itself cannot account for the anaemia observed by Hogan. It must be assumed, in agreement with the conclusions reached by Hogan and his co-workers, that the treatment with nitrous acid produces a toxic substance either derived from lysine or from another amino-acid and that the curative effect of lysine is due to a specific detoxicating action of that amino-acid on the unknown toxic substances.

The conclusion that lysine deficiency does not cause anaemia is also supported by the results of Pearson, Elvehjem & Hart [1937], who found that recovery from nutritional anaemia produced by a milk diet is not greatly influenced by the quality of the protein in the diet during the recovery period. We have repeated their experiments with our gliadin and zein diets on three litters and have obtained results very similar to those of the American workers. It appears, therefore, that the relatively large amounts of lysine required for the formation of haemoglobin are taken from other tissues, if no dietary lysine is available.

Plasma proteins

At the end of the experimental periods the animals were killed and the blood was collected in vessels containing Na oxalate. There were no gross pathological changes in the experimental animals apart from a considerable wasting of muscular tissue in the lysine-deficient rats.

Plasma protein was estimated by multiplying the total N of plasma, as determined by the Kjeldahl method, by 6·25. In some cases the non-protein

nitrogen was estimated separately and deducted from the total N. This correction amounted to about 5–7 % of the total N; since the amount of plasma obtained was small, the determination of non-protein N was omitted in most experiments and a value of 35 mg. N/100 ml. plasma was deducted from the total N to give protein N. Table 3

Table 3. *Plasma protein concentration in normal and lysine-deficient animals*

Litter		No. of animals	Plasma protein (g./100 ml.)
1	Normal	2	5·5, 6·35
	Deficient	3	4·0, 4·3, 4·2
2	Normal	3	5·6, 4·95, 5·2
	Deficient	3	4·2, 4·2, 4·1
3	Normal	3	5·8, 5·6, 5·7
	Deficient	3	4·0, 4·2, 4·2
4	Normal	3	5·5, 5·9, 6·1
	Deficient	3	4·0, 3·9, 4·3

gives the results obtained with lysine-deficient and normal animals. Plasma protein values are considerably lower in the deficient animals; the first three litters had been reared on gliadin diets and the average plasma-protein value in the deficient animals is 4 % compared with one of 5·5 % in the controls. In litter 4, which had been on a zein diet, the plasma-protein values seem to be even lower. In two litters the albumin/globulin ratio was determined by the method of Howe [1921]. There was no significant difference between the lysine-deficient rats and the controls; in both groups the albumin/globulin ratio varied between 1·7 and 2·1.

Growth and ossification

The methods available for the assessment and comparison of rates of growth in animals, apart from mere weighing and measuring of bodily dimensions, depend on radiographic and histological studies. The former have the advantage that radiographs can be taken at any desired intervals during the course of the experiment; the latter have the disadvantage that they involve the sacrifice of the animal. The radiographic method gives a good idea not only of the rate of growth in the spine, tail, skull and long bones, but, with adequate 'soft tissue' technique, yields information about the bulk of the subcutaneous fat and muscular tissue. The histological method consists in recording the number of mitotic figures in the growing tissue or organ. In the case of the long bones, the actual number of cells in the columns of the zone of proliferating cartilage and the zone of calcifying cartilage in the epiphysial region may be counted. This method gives information not only of the rate of proliferation in a given type of cell, but also of the rate of differentiation such as occurs in osteogenesis and in the formation of bone marrow.

A comparison of the radiographs of the control and lysine-deficient rats at the age of 6 weeks, after 3 weeks on the diets, shows the great difference in bodily dimensions (Fig. 1, *a*, *b*). The normal animal is well covered with subcutaneous fat and muscle as compared with the starveling. The width of the epiphysial cartilage at the upper end of the tibia in the normal rat is approximately 1 mm., but that in the lysine-deficient rat is barely visible as a thin line, indicating that union of the epiphysis with the shaft is about to take place. The patella in the normal animal is a mass of bone nearly 3 by 1 mm., in the other it is a mere dot measuring less than 1 sq. mm. The caudal vertebrae in the lysine-deficient rat are smaller in all dimensions and the epiphysial plates are separated from the body of the vertebra by a narrower zone of epiphysial growth cartilage. The degree of calcification in all the bones is generally reduced as compared with the normal.

Two rats, one normal, the other lysine-deficient, aged 9 weeks, were killed after 6 weeks of feeding. Certain organs, including the wrist joint, knee joint and testes, were fixed in Allen's modification of Bouin's fluid at 38°, with a view to studying the minute anatomy of the epiphysial growth cartilage and the mitotic count of the testes. Sections were cut in paraffin and stained with haematoxylin and eosin.

The microscopic sections of the distal end of the radius (Fig. 2 *a*, *b*) show that in the normal rat the first zone of proliferating cartilage averages about 15 chondroblasts to the column as compared with about 7 in the lysine-deficient rat. The second zone of calcified cartilage averages from 3 to 5 cells in the column, but the trabeculae of calcified matrix are more clearly and heavily laid down in the lysine-deficient rat. The third zone of actual osteogenesis and bone marrow formation shows heavier transverse trabeculae of bone in the lysine-deficient rat, indicating arrest of growth. The bone marrow in these animals is composed largely of fat.

The microscopic sections at the upper end of the tibia similarly show in the first zone of columns of proliferating cartilage about 7 cells in the normal and 3 cells in the lysine-deficient rat, so that the evidence gained is definitely in favour of a marked arrest of growth in the lysine-deficient animal. In the case of the testes, the volume in the lysine-deficient animal was approximately 75 % of the normal and the number of mitotic figures in the tubules about 66 %.

It is clear from the comparison of the radiographs of the lysine-deficient rat at 3 weeks and 9 weeks of age, after 6 weeks of deficient feeding, that the internal economy of the animal has altered profoundly. Although the weight has remained constant, it is no longer the same animal. The subcu-

taneous fat has decreased, the muscles have wasted, the long bones have grown by about 20 % in length, but diminished in girth. The caudal vertebrae have increased by about 60 % in length but their diameter has slightly diminished.

DISCUSSION

The most striking effect of the deficient diet which contains only small amounts of lysine is complete cessation of growth without any loss of weight. It is possible that complete absence of lysine might result in a decrease of body weight.

Following the experimental technique of W. C. Rose and others, food was given *ad libitum*; the food intake of the deficient animals steadily diminished during the experiment and averaged during the last weeks only about 60–70 % of that of the controls. It may be argued, therefore, that the results observed are mainly due to the reduced calorie intake. The case for and against paired feeding, which eliminates differences caused by variations of food intake, has been reviewed at some length by Brody [1935]. It is certain, however, that the diminished appetite is the result of the deficiency of the diet and generally follows the failure of growth, as first observed by Hopkins [1912]. The diminished food intake is therefore indicative of a deficiency in the diet and it is immaterial for our purpose, i.e. the description of the symptoms of lysine deficiency, whether the effect is wholly direct or is largely caused by reduced intake of food, which in itself is due to the deficiency in lysine.

The constancy of weight in the deficient animals represents the sum total of changes in continuous operation. Certain organs such as the eye and kidney continue to grow at the expense of others [Lafon, 1939]. The long bones grow in length and the number of red cells/unit volume of blood increase. Whether the total number of red cells in the body and the total amount of haemoglobin change is impossible to say without measuring the blood volume. But growth, even where it occurs, is considerably retarded. This is shown very clearly by the histological analysis of the epiphysial bone and the reduced number of mitotic figures in the testes. Some tissues, such as muscle, lose weight, and it appears likely that the total protein of the body decreases slowly. A disturbance of protein metabolism is also indicated by the low serum protein values, which are reduced to oedema level. Protein appears to be transferred from some organs to others according to a definite system of priorities. The constancy of body weight shows that this transfer is done very economically, at least in lysine deficiency, with only a small loss of lysine through irreversible oxidation. The whole picture, which can best be interpreted as an inhibition of protein formation, is not unlike that produced by ordinary starvation, i.e. by reducing total intake of food [Jackson, 1908; 1932]. It is noteworthy, but not unexpected, that lack of one essential building material in the diet produces essentially the same symptoms as general underfeeding.

SUMMARY

1. A diet containing only small amounts of lysine produces, in young rats, cessation of growth and hypoproteinaemia. The number of red cells and the amount of haemoglobin/unit volume of blood are slightly lower than in the control animals which receive a diet supplemented with lysine and are growing. These low values are interpreted as indicating not so much an anaemia proper as a retarded development of the haematopoietic system.

2. Radiological examination shows a considerable decrease of subcutaneous fat, waste of muscle and reduction of calcification in the bones. The epiphysial cartilage in the long bones is barely visible and histological examination reveals a considerable reduction in the number of chondroblasts in the first zone of proliferating cartilage. In the zone of calcified cartilage the trabeculae of calcified matrix are heavier in the deficient animals than in the controls. Mitotic figures in the testes are reduced as compared with normal animals.

3. The changes observed are assumed to be due to a general inhibition of protein formation. This leads to a reduced growth of some organs which develop at the expense of others and protein is transferred according to a fixed system of growth priorities. The sum total of these changes is constant body weight. The similarity of this picture to that produced by starvation is discussed.

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REFERENCES

Borchers, R., Totter, J. R. & Berg, C. P. [1942]. *J. biol. Chem.* **142**, 697.

Brody, S. [1935]. *Ann. Rev. Biochem.* **4**, 383.

Burroughs, E. W., Burroughs, H. S. & Mitchell, H. H. [1940]. *J. Nutrit.* **19**, 363.

Fowler, A. B., Morris, S. & Wright, N. C. [1934]. *Scot. J. Agric.* **17**, 261.

Hart, E. B., Nelson, V. E. & Pitz, W. [1918]. *J. biol. Chem.* **36**, 291.

Herbert, D. [1941]. *Biochem. J.* **35**, 1166.

Hogan, A. G., Powell, E. L. & Guerrant, R. E. [1941]. *J. biol. Chem.* **137**, 41.

—— & Ritchie, W. S. [1934]. *J. biol. Chem.* **107**, 179.

Hopkins, F. G. [1912]. *J. Physiol.* **44**, 425.

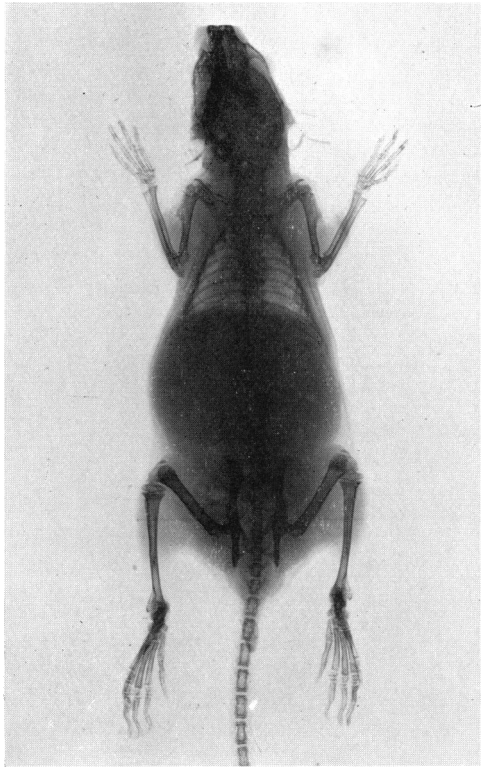

Fig. 1 *a*.

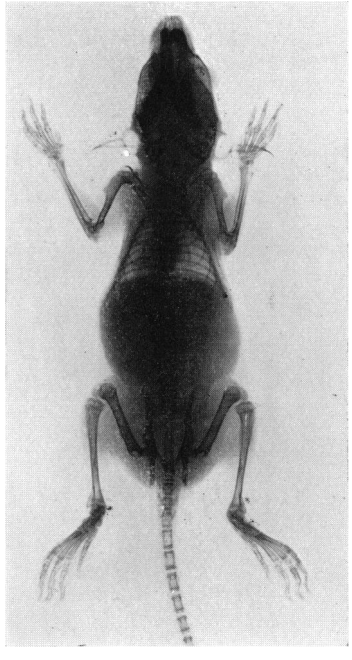

Fig. 1 *b*.

Fig. 1. Radiograph of lysine-deficient animal (1 *b*) and control (1 *a*). The animals were littermates, 10 weeks old and had been 6 weeks on their respective diets.

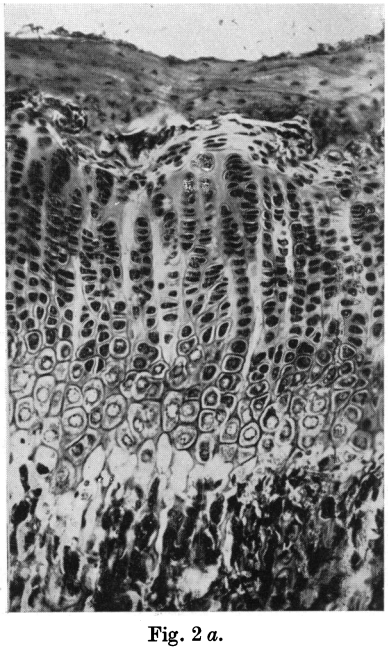

Fig. 2 *a*.

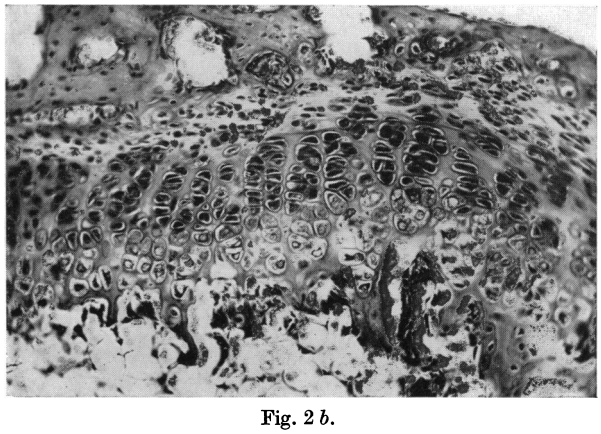

Fig. 2 *b*.

Fig. 2. Microscopic section of the distal end of the radius (*a*) of the normal, (*b*) of the deficient animal.

Howe, P. E. [1921]. *J. biol. Chem.* **49**, 109.
Jackson, C. M. [1908]. *J. exp. Zool.* **19**, 99.
—— [1932]. *Amer. J. Anat.* **51**, 347.
Lafon, M. [1939]. *Ann. Physiol. Physicochim. biol.* **15**, 1.
Osborne, T. B. & Mendel, L. B. [1912]. *J. biol. Chem.* **12**, 473.
—— —— [1914]. *J. biol. Chem.* **17**, 325.
—— —— [1916]. *J. biol. Chem.* **25**, 1.
—— van Slyke, D. D., Leavensworth, S. & Vinograd, M. [1915]. *J. biol. Chem.* **22**, 259.
Pearson, P. B. [1937]. *Amer. J. Physiol.* **118**, 788.
Pearson, P. B., Elvehjem, C. A. & Hart, E. B. [1937]. *J. biol. Chem.* **119**, 749.
Rimington, C. [1942]. *Brit. med. J.* **1**, 177.
Rose, W. C. [1938]. *Physiol. Rev.* **18**, 109.
—— & Eppstein, S. H. [1939]. *J. biol. Chem.* **127**, 677.
Schoenheimer, R. & Rittenberg, D. [1940]. *Physiol. Rev.* **20**, 218.
Vickery, H. B. [1938]. *C.R. Lab. Carlsberg (sér. chim.)*, **22**, 519.
Winnick, T. [1942]. *J. biol. Chem.* **142**, 461.
Winters, J. C., Smith, A. H. & Mendel, L. B. [1927]. *Amer. J. Physiol.* **80**, 577.

The Mechanism of Coprosterol Formation *in vivo*

1. CHOLESTENONE AS AN INTERMEDIATE

BY O. ROSENHEIM AND T. A. WEBSTER, *National Institute for Medical Research, Hampstead, London, N.W. 3*

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In view of the inherent improbability that the organism is able to convert cholesterol by direct hydrogenation into its *cis*-decalin derivative coprosterol, it has been suggested that the reaction proceeds in two stages [Rosenheim & Starling, 1933]. According to this view cholestenone, the intermediate oxidation product of cholesterol, is subsequently reduced in the intestine to coprosterol and partly to *epi*-coprosterol.

Although feeding experiments with cholestenone have since provided considerable experimental evidence for the correctness of this view [Rosenheim & Webster, 1935; Schoenheimer, Rittenberg & Graff, 1935; Rosenheim & Starling, 1937; Anchel & Schoenheimer, 1938], the final proof of the occurrence of cholestenone itself in the animal organism or in its excreta was still lacking. The difficulties of isolating such small amounts of cholestenone as escape complete reduction from the faeces of animals kept on an ordinary mixed diet, which is poor in cholesterol, are obvious. In our search for cholestenone we have, therefore, made use of the fact that brain contains a substance, the administration of which enables the organism to convert large amounts of cholesterol into coprosterol [Rosenheim & Webster, 1941]. By employing Girard and Sandulesco's ketone reagent, we have, under such conditions, been able to isolate cholestenone from the faeces of a dog and of rats fed on brain, and to characterize it both chemically and physically.

The isolation of cholestenone from faeces after feeding on brain necessitated a search for its possible occurrence in brain itself. The ultra-violet absorption spectra of cholesterol fractions obtained from various organs had been examined by Page & Menschick [1930 *a*, *b*; 1931], who were unable to find the characteristic absorption band of cholestenone at 2400 A, in cholesterol prepared from normal brain. Their results do not, however, exclude the presence of traces of cholestenone which would have been removed by the solvents used in the preparation of the various cholesterol fractions. We therefore subjected brain to the treatment which led to the isolation of cholestenone from faeces. Although we obtained evidence that traces of a substance absorbing at 2380 A occur in brain, we were unable to characterize it as cholestenone. In view of its small amount, the possibility that this unidentified substance may be the source of the relatively large amount of cholestenone isolated from faeces after the administration of brain must be considered as remote.

Whilst this work was in progress, Marker, Wittbecker, Wagner & Turner [1942] succeeded in isolating *epi*-coprosterol from the faeces of a dog on a normal diet, thus bringing further support to the view that cholestenone is an intermediate in the formation of coprosterol in the organism.

EXPERIMENTAL

Cholestenone from rats' faeces. Rats of our Institute strain of Wistar rats and of the average weight of 200 g. were used. Six rats were kept on a diet of steamed sheep's brain and bone meal for 4 days, each rat consuming *ca.* 30 g./day of the diet. The housing of the animals, the preparation of the diet and the collection of the faeces have been described previously [Rosenheim & Webster, 1941].

The faeces of the last 3 days (140 g. moist) were collected, dried *in vacuo* over conc. H_2SO_4 and exhaustively extracted with ether in a Soxhlet. The lipids (23·5 g.) yielded on saponification 15·2 g. of unsaponifiable matter, which was dried in a high vacuum. 14 g. of the unsaponifiable fraction in acetic acid-ethanol solution were treated with 5 g. of the