Table 1. Normal Values of the Activities of Glutamic-oxaloacetic and Glutamic-pyruvic Acid Transaminases in Dogs

	Art. fem. ser.		V. hep. ser.		D. thor. lymph		Hepatic lymph	
	GOT	GPT	GOT	GPT	GOT	GPT	GOT	GPT
Mean	70	53	78	80	67	52	121	70
Extreme values	23–134	11–89	20–124	20–195	7–126	8–119	80–136	38–125
Number of cases	19	18	18	15	20	19	5	4

glutamic-oxaloacetic acid transaminase and of glutamic-pyruvic acid transaminase were determined 15 min. before and 15 min. after the injection.

Table 1 summarizes the values found in normal controls, and Fig. 1 illustrates the values found after hepatic damage.

Compared with the values of the controls, the concentrations found after hepatic damage are significantly greater in all our experiments.

So far as we know, no data on transaminase activity in the hepatic veins or in the lymph of the various lymphatic ducts have been published. According to our results, the activities of both glutamic-oxaloacetic and glutamic-pyruvic acid transaminase are considerably increased in every fraction of the blood and lymph after hepatic damage; the actual rise is to some extent variable. (In the experiment represented in Fig. 1 the greatest change occurred in the serum from the hepatic vein.)

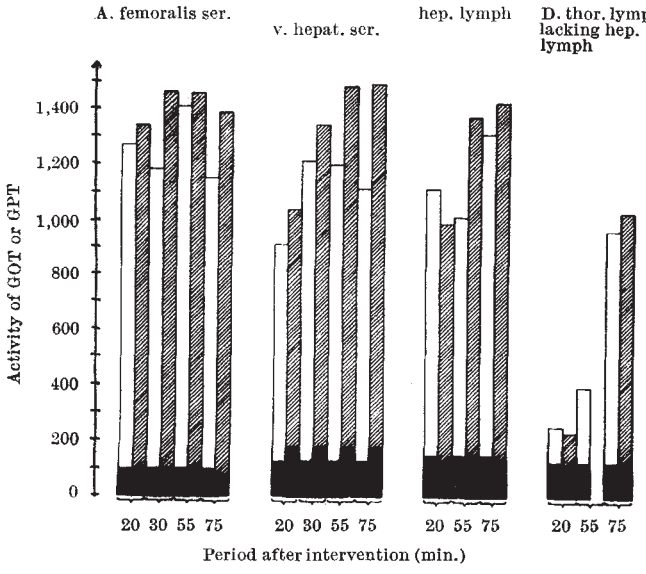

Fig. 1. Activity of glutamic oxaloacetic acid transaminase (white); and of glutamic pyruvic acid transaminase (hatched); mean of control periods (black)

Histological examination of the liver tissue showed intense hyperæmia, œdema and damage to the liver cells (vacuoles, hyalin degeneration).

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Effect of Insulin on Sulphydryl Groups in Muscle

It has been shown that the free —SH content of nerve tissue increases under the influence of stimulation. This suggested the possibility that the number of titratable —SH groups may be directly or indirectly related to the ratio of potassium to sodium ions in the cell and consequently to its surface potential[1,2].

Flückiger and Verzár[3], Zierler[4] and Creese, D'Silva and Northover[5] have observed that addition of insulin *in vitro* to rat-muscle preparations causes gain in potassium ions and loss of sodium ions by the cell and an increase of its resting potential. It seemed, therefore, interesting to investigate whether these changes would be accompanied by a corresponding fall in titratable —SH groups.

Diaphragms were removed from male *CF* Wistar rats (100–145 gm.). Each hemidiaphragm was washed, and suspended in 150 ml. of a glucose–saline medium buffered to *p*H 7·4 and containing 6·15 μmoles potassium ion/ml. To one of the hemidiaphragms was added insulin ('Iletin', Lilly) at a concentration of 0·1 unit/ml.; the other served as a control. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbling through both preparations for 2 hr. at room temperature.

Content of potassium ions was determined in eight pairs of hemidiaphragms after 2 hr. incubation. The muscle was blotted and submitted to the wet-ashing procedure described by Ballantine and Burford[6]; content of potassium ions was measured by means of a flame photometer using an internal standard. The mean potassium ion content of the insulin-treated hemidiaphragm was 130·4 (±33·5 *S.D.*) μmoles/gm. of wet weight; the control muscles contained 82·3 (±32·8) μmoles/gm. The increase in potassium ion content, 48·1 μmoles, is 58·5 per cent of the controls.

Thirty pairs of hemidiaphragms were used for the determination of —SH groups. The tissue was frozen with dry-ice, ground in a Potter–Elvehjem homogenizer and extracted with the suspension medium. After centrifugation at 4,000 r.p.m. for 20 min., —SH groups were determined in the supernatant by means of the amperometric titration method of Benesch, Lardy and Benesch[7]. In 26 experiments the —SH content of the insulin-treated hemidiaphragm decreased by an average of 22·1 per cent. The control muscle contained 5·32 (±1·08 *S.D.*) μmoles/gm. wet weight; under the influence of insulin the —SH groups fell to 4·14 (±0·84) μmoles. The difference is significant at the level of $P = 0{\cdot}05$–$0{\cdot}02$. In four experiments —SH groups increased by an average of 1·22 μmoles/gm.: it could not be ascertained whether these results were due to a technical failure or to some particular condition of the animals.

In some experiments, the extract was dialysed in a shaking dialyser against 25 vol. of the extraction

medium. As in brain tissue[2], the major proportion (80 per cent) of the —SH groups in muscle was bound to non-dialysable molecules, presumably to proteins. The decrease caused by insulin affected about equally the dialysable and non-dialysable sulphydryl fractions.

The fact that insulin causes hyperpolarization of the cell surface, increase of the ratio of potassium to sodium ions and decrease of —SH groups can be interpreted in various ways. In terms of the membrane theory, it could be assumed that insulin, by its action on metabolic processes, increases the efficiency of the ionic 'pumps' or that it acts directly on the membrane and alters its permeability properties. The latter action is assumed to operate in facilitating the entry of glucose into the cell[8]. There is also some evidence that glycogen fixes potassium ions[9] so that increased glycogen synthesis may result in higher intracellular potassium.

In view of certain observations on protein configuration in resting and stimulated cells, insulin can be assumed to act primarily by modifying the structure of certain protein molecules. Stimulation and subsequent depolarization, with lowered ratio of potassium to sodium ions, are accompanied by an 'unmasking' of titratable —SH groups and other ionized side-groups, suggesting a general 'loosening' of the structure of protein molecules[1,2,10]. In the opposite direction, hyperpolarization, with raised intracellular ratio of potassium to sodium ions could be characterized by a decrease in ionized side-groups and tendency to a more rigid protein configuration. Further investigations are being conducted to determine whether modifications of protein structure represent a determining factor in the mechanism of cellular rest and excitation.

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Reduction of Neotetrazolium Chloride by Vitamin K_3

Menadione (vitamin K_3, 2-methyl-1.4-naphthaquinone) has become increasingly associated with pathways of electron transfer. It has been implicated in the nitrate reductase sequence; in the processes of photosynthetic phosphorylation; in a non-phosphorylative oxidative pathway; in menadione reductase and in the general respiratory chain and associated processes of oxidative phosphorylation (cf. ref. 1). Menadione has recently been associated with an enzyme sequence connecting the oxidation of succinate with the reduction of neotetrazolium chloride[1]. The vitamin K_3 involved in such reactions can be followed by spectrophotometric rate measurements[2].

This communication shows that it is also possible to demonstrate that vitamin K_3 is involved in redox reactions by adding neotetrazolium chloride; reduced menadione reacts directly and non-enzymically with neotetrazolium chloride at *p*H 7·4 and 37° C., yielding the intensely coloured insoluble formazan. As stated by Shelton and Rice[3], the absorption maximum of the formazan varies with the method of reduction: enzymic reduction yields a product with a maximum absorption at 510 mμ; chemical reduction (for example, by reduced menadione or dithionite) yields a product with an absorption maximum at 540 mμ.

In the following experiments the reaction mixture used contained 1 ml. of a phosphate – versene mixture (1·0 ml. phosphate buffer, *p*H 7·4, 0·1 molar: 0·05 ml. sodium versenate, *p*H 7·4, 0·1 molar) and 0·15 ml. of 1 per cent neotetrazolium chloride. Other additions mentioned in Table 1 were made in a volume of 0·1 ml. Incubation was at 37° C. and the formazan was extracted with 4 ml. of ethyl acetate; the extinction was obtained using a Hilger 'Uvispek' spectrophotometer. Vitamin K_3 was added as a suspension in phosphate buffer, bovine plasma albumin and ethanol.

Table 1. Extinction read at 540 mμ in a 'Uvispek' Spectrophotometer

Path-length 1 cm., incubation time 30 min. except for reduced menadione (5 min.), and cysteine + vitamin K_3 (15 min.); reagents and volumes described in the text. All values refer to the addition of 1 μmole of reagent

Addition	Extinction	Addition	Extinction
Menadione	0·007	Ascorbic acid + vitamin K_3	0·225
Reduced menadione	0·237	Glutathione + vitamin K_3	0·003
Ascorbic acid	0·066	Cysteine + vitamin K_3	0·960
Glutathione	0·002	Reduced diphosphopyridine nucleotide + vitamin K_3	0·036
Cysteine	0·009	Vitamin K_1 + ascorbic acid	0·123
2,3 Dimercapto-1-propanol	1·490	Vitamin E + ascorbic acid	0·091
Hydroquinone	0·000		
Reduced diphosphopyridine nucleotide	0·000		
Vitamin K_1	0·015		
Vitamin E	0·006		

Reduced menadione was prepared as described by Fieser[4]; a grey powder was obtained which had absorption maxima at 241 and 330 mμ as mentioned by Colpa-Boonstra and Slater[2]. Direct reduction of neotetrazolium chloride by the following reagents was also tried: reduced diphosphopyridine nucleotide (Boerhinger, 90 per cent pure); glutathione (Distillers Co., Ltd.): ascorbic acid (Roche); cysteine; 2,3 dimercapto-1-propanol (BAL, Boots Pure Drug Co., Ltd.); hydroquinone; vitamin E (L. Light and Co.); vitamin K_1 (Roche).

Other than reduced menadione, only 2,3 dimercapto-1-propanol reacted directly with neotetrazolium chloride to any substantial degree; slight production of formazan was observed with vitamin C (Table 1). Several of the reagents which did not reduce neotetrazolium chloride directly, were found to reduce menadione, which then led to the formation of formazan. For example, cysteine reduced menadione with a consequent formation of formazan on incubation, in spite of the fact that cysteine (and other similar compounds, for example, glutathione) combines readily with menadione under the conditions used to yield 3-substituted naphthaquinones. Under the conditions used (in *p*H 7·4 phosphate buffer: versene) the photochemical reduction of neotetrazolium chloride catalysed by cysteine with either riboflavin or riboflavin phosphate[5] was negligible.