Abnormal Serum Protein in Parenchymatous Liver Diseases

SEVERAL colloidal serum reactions have been proposed during recent years for revealing damage of the liver parenchyme: the cephalin-test (Hanger), the colloidal gold and thymol tests (Maclagan) and the colloidal benzoin and shellac tests[1]. Most authors suppose that increased gamma-globulin forms the basis of these tests, but it has been suggested that beta-globulin may be responsible, too.

In fractionation experiments with variable concentrations of alcohol at low temperature (5–10° C.), we found that normal sera caused precipitation only in alcohol concentrations higher than 20 per cent; sera of hepatic cases, on the other hand, gave precipitations in 5–10 per cent alcohol. No precipitation occurs when alcohol is diluted with 0·9–10 per cent sodium chloride or when twice-distilled water is added without alcohol, or when the *p*H of the diluted alcohol is less than 5·5.

The protein fraction precipitated with 10 per cent alcohol is insoluble in water, but is readily soluble in 0·9 per cent sodium chloride; it is partly precipitated with 30 per cent saturated, and completely precipitated with 50 per cent saturated, ammonium sulphate. Saturated sodium chloride solution precipitates the greater part of it. The iso-electric point is about *p*H 5·5. The tryptophane content varies between 3·0 and 3·8 per cent. The serum concentration of this fraction, present in all cases of parenchymatous hepatic damage, amounts to 1–3 per cent, forming a considerable part (30–50 per cent) of the total globulin.

The purified fraction gives positive colloidal gold, benzoin and Takata tests of the same intensity as does the corresponding quantity of serum; with the remaining, non-precipitable protein, colloidal tests give negative results. The effect of the isolated fraction on thymol turbidity is somewhat less than that caused by the corresponding quantity of serum. In Weltmann's coagulation test, on the other hand, the isolated fraction is coagulated in presence of 0·1 per mille calcium chloride; the higher values (0·2–0·4 per mille calcium chloride) obtained with total serum are the consequence of some unknown factor hindering coagulation.

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[1] Fischer and Wiltner, *Acta. med. Scand.* (in the press).

Increased Muscle Phosphorylase Activity in the Starved Rat

IT was shown earlier that glucose uptake in the isolated rat diaphragm was reduced after a period of non-carbohydrate, high-fat diet[1], and that a spontaneous regulation of food intake took place in animals shifted from such a diet to a high-carbohydrate diet[2]. These physiological changes occurring after a period of carbohydrate deprivation, together with many other observations after starvation or fat-feeding by other investigators, suggest that the kind of foodstuff taken by the animal (or human subject) determines in some way the metabolic pattern.

It is known that a decrease in insulin content of the pancreas is found after starvation or following a fat diet[3]. Many observations seem, however, to point to the fact that the change in the metabolic pattern can take place independently of the hormonal status, for example, the dependence of the glucose-tolerance curve[4,5] and the rate of depletion of glycogen stores[6] on the previous carbohydrate intake in the hypophysectomized animal. It is conceivable that a difference in cellular enzymatic activity is found in different metabolic conditions.

To investigate this problem we have undertaken a study of muscle phosphorylase activity in fed animals, in starved animals, and in animals on diets containing different amounts of carbohydrate. The results obtained in fasting animals will be given briefly here.

Phosphorylase activity was studied by the method of Cori, Cori and Green[7], determining the liberation of phosphate during the process

glucose-1-phosphate → glycogen + phosphate.

A crude extract of rat muscle was made after freezing with liquid air. The extract was filtered, adjusted to *p*H 6·1, and dialysed against running tap water. The dialysed extract was diluted with a weak solution of cysteine and added to tubes containing glucose-1-phosphate, glycogen and cysteine-glycerophosphate buffer. To some tubes adenylic acid was added. The tubes were agitated for one hour at 31° C. in a Warburg bath. After treatment with trichloracetic acid and filtering, the phosphate formed during the incubation period was determined as the difference between a zero value and a 60-min. value. The protein content of the enzyme extract was estimated by the biuret method, standardized against human serum. In each experiment the muscle phosphorylase activity was observed in one fed and one fasted animal.

The effect of fasting is shown in the accompanying table. The activity, both with and without adenylic acid, is consistently higher in the fasted animals.

Muscle phosphorylase activity in fed and fasted rats

	Fed			Fasted			
Number of experiment	Phosphorus liberated (γ) 1*	2†	Protein in reaction mixture (mgm.)	Phosphorus liberated (γ) 1*	2†	Protein in reaction mixture (mgm.)	Duration of fast (days)
1	132	256	—	708	862	—	2
2	80	304	5·8	304	278	6·2	3
3	34	56	5·6	174	214	5·4	2
4	170	540	5·2	618	632	5·2	3
5	33	75	5·8	284	412	5·4	1
6	12	170	6·4	198	282	5·8	5
7	54	246	6·6	374	744	6·6	2
8	44	146	5·6	164	298	6·0	2

*Without added adenylic acid. †Adenylic acid added.

We are not suggesting, at present, any definite explanation for the higher phosphorylase activity in the starved animals. Studies are now being conducted on the phosphorylase activity during refeeding after fasting, in animals on diets containing different amounts of carbohydrate, and in animals shifted over from a low- or non-carbohydrate diet to a diet containing large amounts of carbohydrate (starvation diabetes). It is proposed to investigate other enzyme systems in intermediary carbohydrate metabolism, for example, the hexokinase system and the system of oxidative phosphorylation in animals on different dietary regimes.

The results obtained appear to indicate profound changes in the pattern of intermediary carbohydrate