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The Effect of Starvation on the Pseudo-Cholinesterase Activity of the Liver and Serum of Rats

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In man the serum cholinesterase has been found to be low in many diseases affecting the cells of the liver and to rise again to normal levels with recovery, and the measurement of the activity in the serum has been suggested as a test of liver function (McArdle, 1940; Faber, 1943; Kaswin, 1945). McCance, Widdowson & Hutchinson (1948) showed that the serum possessed subnormal activity during undernutrition, and that normal activity returned with recovery. Waterlow (1950) took liver biopsies and simultaneous samples of serum from infants during and after a period of malnutrition, and found that the enzyme activities increased in both tissues as nutrition improved.

Some have assumed that the pseudo-cholinesterase found in the serum has been made by the liver and discharged into the circulation, but the experiments undertaken by others to prove this on animals have not been conclusive. This has partly been due to a tendency for each species and sex to respond to the same stimulus in a different way, but the difficulties have been enhanced by the variable amounts of 'true' cholinesterase found in the serum and organs of the domestic and laboratory mammals. Thus chloroform and carbon tetrachloride poisoning raised the cholinesterase in the serum of dogs (Steensholt & Venndt, 1945; Brauer & Root, 1947), but ligaturing the pancreatic duct, phosphorus poisoning, or undernutrition made no permanent impression on the serum activity (Hutchinson, 1951). Brauer & Root (1947) found that the cholinesterase activity of the liver in dogs was depressed after prolonged carbon tetrachloride poisoning. In rats, carbon tetrachloride lowered the serum cholinesterase in females but not significantly in males, and it reduced the activity in the livers of both sexes (Ellis, Sanders & Bodansky, 1947; Brauer & Root, 1946). Hutchinson (1951) observed that feeding half

rations for 3 months halved the weight of adult male rats, but did not lower the activity of the serum cholinesterase. On the other hand, Mendel, Hawkins & Nishikawara (1948) determined the serum cholinesterase of males before and after a 12-day fast and found that the serum activity fell by 50%. Milch (1950) also found a fall in activity after feeding a protein-free diet for 6 weeks. No similar experiments have been carried out on female rats, although other liver enzymes have been followed during starvation in both sexes (Miller, 1948).

No significant sex difference has been found in the serum cholinesterase activity of dogs (Brauer & Root, 1947), or of human beings except during pregnancy (Levine & Hoyt, 1949). In rats, however, the females have much more cholinesterase in their livers and sera than the males (Birkhäuser & Zeller, 1940; Sawyer & Everett, 1947). Both tissues contain true and pseudo-cholinesterase, but it is the latter which is responsible for the sexual variation (Mundell, 1944; Sawyer & Everett, 1946).

In the present study, the cholinesterase activity of the liver and the serum of male and female rats was followed during a 6-day period of starvation. At the same time, other changes in the composition of the liver cell during starvation were investigated by one of us (M.F.H.) and these will be reported in a later paper.

METHODS

Animals. Thirty-five male and thirty-five virgin female black and white hooded rats of the Lister strain, 3-4 months old, were used. Until the fast, they were fed on the stock diet consisting of bread, milk, whole wheat, wheat germ, yeast, fish meal, oatflakes and bran. The rats were weighed twice a week for 3 weeks before the fast to ensure that they were growing at a normal rate. The males and females were then divided into five comparable groups of seven rats, according to their mean weight for the 3 days before the fast,

Sex	Length of fast (days)	Body weight		Liver weight		'Protein N'		Cholinesterase units	
		Initial (g.)	At death (g.)	Total (g.)	Per 100 g. initial body wt. (g.)	Per g. liver (mg.)	Per 100 g. initial body wt. (mg.)	Per g. liver	Per 100 g. initial body wt.
Male	0	270 (24)	270 (24)	8.91 (1.49)	3.30 (0.40)	31.4	104	29	96
	1	272 (18)	256 (19)	7.03 (0.38)	2.59 (0.18)	38.4	100	32	83
	2	271 (21)	244 (19)	6.70 (0.54)	2.47 (0.08)	37.6	93	32	79
	4	271 (22)	229 (18)	5.80 (0.59)	2.14 (0.20)	37.6	80	30	64
	6	271 (23)	210 (20)	5.41 (0.58)	2.00 (0.10)	37.3	75	35	70
Female	0	208 (16)	208 (18)	6.20 (0.61)	2.98 (0.18)	32.0	95	111	330
	1	209 (17)	200 (15)	5.13 (0.50)	2.46 (0.21)	36.0	89	72	178
	2	209 (15)	189 (16)	4.67 (0.22)	2.25 (0.12)	37.0	83	49	110
	4	208 (15)	180 (15)	4.39 (0.28)	2.11 (0.13)	36.7	78	53	112
	6	208 (16)	167 (14)	4.44 (0.34)	2.13 (0.12)	33.4	71	45	96

female rats, the mean weights being 271 and 208 g., respectively. During the fast the body weights fell continuously, and after 6 days the males had lost 61 g. and the females 41 g., equivalent to a loss of 23 and 20% of their initial body weights, respectively. The livers of males averaged 8.91 g. in weight before the fast, of females 6.20 g. During the fast the liver weights fell by 3.50 and 1.76 g.; the livers lost weight most rapidly during the first 24 hr. of starvation.

Fig. 1. Effect of starvation on the serum cholinesterase of rats. ○—○, male; ●—●, female.

The 'protein N', expressed in mg./g. liver tissue, did not change much during the fast, although there was a slight rise during the first day which was probably due to the loss of glycogen. There was a striking sex difference in the cholinesterase activity of the liver. The activity per g. liver tissue was initially 3.8 times greater in the females than in the males. In the males the activity changed little during the fast, but in the females the cholinesterase fell rapidly during the course of the first 2 days to less than half its original value and thereafter changed little.

The total amounts of cholinesterase and 'protein N' in the livers have also been expressed per 100 g. initial body weight. These figures bring out the fact that the males had relatively, as well as absolutely, more liver protein than the females and that starvation reduced it by about the same extent in both sexes. In the males the cholinesterase fell with the 'protein N' to about 71% of its initial value, but in the females there was a much greater fall in activity.

In Fig. 1 are shown the concentrations and variations in the activity of cholinesterase of the pooled sera during the 6 days fast. It will be seen that the sex difference was even greater in the serum than in the liver, the serum of females being almost six times more active than that of males. During the fast, the mean serum cholinesterase in the females fell continuously from 41.2 to 22.9 units/ml., but in the males remained constant at 7.1 units/ml. The protein decreased gradually from 7.18 to 6.75 g./100 ml. in the males, and from 7.68 to 6.68 g./100 ml. in the females, and the serum albumen from 3.78 to 3.40 g./100 ml. in the males, and from 3.88 to 3.44 g./100 ml. in the females. There was no correlation with the serum cholinesterase.

DISCUSSION

These results, taken in conjunction with those of Birkhäuser & Zeller (1940), Sawyer & Everett (1946, 1947), Ellis *et al.* (1947) and Brauer & Root (1947) show that the liver of the female rat has a very high cholinesterase activity, that this activity is maintained by the ovarian hormones, and that the greater part of it disappears when the normal functions of the liver have been disturbed by poisoning or by fasting for 2 days. Initially, the liver of the male has a much lower cholinesterase activity, but by the end of the second day of fasting the difference has largely been eliminated. In the male rat, and in the female after the second day of starvation, the cholinesterase of the liver falls per 100 g. initial body weight, but it does not change much per mg. 'protein N' in the liver. In the female there would appear to be in the liver a considerable cholinesterase activity which is labile, sex-linked and in some way different from the small basal activity which is common to both sexes and is closely associated with the liver proteins.

The fall in the serum cholinesterase of the female rat is consistent with the hypothesis that the serum activity depends on the liberation of the enzyme from the liver. It resembles that obtained in human undernutrition and liver disease, but nothing is known at present about the hormonal control of the cholinesterase activity of the liver in man, or whether there is a large labile element in it. The results for male rats are in keeping with those of Hutchinson (1951) who worked with the same colony of rats, and it is possible that those of Mendel *et al.* (1948) and Milch (1950) who obtained a fall in the serum of males, may have been due to the use of a different strain. However, it is clear that the cholinesterase activities of the liver and serum in male rats are much more stable than in females, and, like the serum enzyme in the dog, are much less easily influenced by undernutrition.

SUMMARY

1. The amount of pseudo-cholinesterase in the livers of adult male rats decreased from 96 to 70 units/100 g. initial body weight during a 6-day fast, but the activity per g. liver changed very little, and the activity of the serum enzyme remained constant at 7.1 units/ml. serum.

2. The total pseudo-cholinesterase in the livers of

female rats amounted to 330 units/100 g. initial body weight. Two days' starvation reduced this figure to 110, and 6 days' starvation to 96. There was little change per g. liver after the second day. The activity in the serum fell from 41 to 23 units/ml. in the 6 days.

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Studies in Carotenogenesis

1. GENERAL CONDITIONS GOVERNING β -CAROTENE SYNTHESIS BY THE FUNGUS *PHYCOMYCES BLAKESLEEANUS* BURGEFF

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Information concerning carotenogenesis in both Phanerogams and Cryptogams is limited, and that which does exist is often far from unequivocal (see Goodwin, 1951, for a full discussion). In order to simplify the experimental approach as much as possible by eliminating photosynthesis, it was decided to investigate carotenogenesis in a fungus; *Phycomyces blakesleeanus* was an obvious choice, for it is easily cultured on simple aqueous media, requiring only aneurin as a growth factor (Schopfer, 1934), and produces considerable amounts of a single carotenoid, β -carotene (Schopfer & Jung, 1935; Karrer & Krause-Voith, 1947; Albrecht, 1948;

Bernhard, 1948) together with traces of α -carotene (Karrer & Krause-Voith, 1947; Albrecht, 1948; Bernhard, 1948) and, possibly, lycopene (Albrecht, 1948; Bernhard, 1948); no xanthophylls have ever been detected. *Phycomyces blakesleeanus* is a heterothallic fungus existing in two well defined strains, (+) and (−) (Blakeslee, 1904). According to Schopfer (1935), cultures of the (+) strain are generally yellower than those of the (−) strain, an observation which suggests that the (+) strain produces the greater amount of carotene.

The only quantitative information concerning carotenoid formation in *Phycomyces* is that obtained by Schopfer (1935); he records the carotene concentration as being about 0.09% on dry weight.

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