SUMMARY

1. The inhibitory effects of a number of thiol reagents and other group-specific reagents on the cholinesterases of human blood have been studied.

2. Although the enzymes were moderately sensitive to arsenite and iodine, and were usually inhibited by other heavy metal reagents in sufficiently high concentration, they differed from typical —SH enzymes in being either much less sensitive or quite insensitive to most —SH reagents

tried. Further, arsenite inhibition was reduced by pH, not increased as would be expected if a thio-arsenite link had been formed.

3. It is concluded that these cholinesterases are in all probability not —SH enzymes and that there is also no evidence for the participation of metal ions, carbonyl groups or carboxylate groups in the active centre.

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The Effect of Dosage on Sexual Differences in the Iron Metabolism of Rats

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Both rats and human beings exhibit sexual differences in the metabolism of iron. Widdowson & McCance (1948) outlined the information available and showed that, given equal opportunities, female rats stored much more iron in their livers than males. They also showed that this was true of some but not of all other species of animals, and that in poultry it applied to the spleen as well as the liver. At that time it had been shown (Brenner, Brookes & Roberts, 1942) that female rats could store vitamin

A in their livers with much greater facility than males, and it was suggested by Widdowson & McCance that the storage of vitamin A might in some way be coupled with that of iron. More recently, Moore & Sharman (1950) and Moore, Sharman & Ward (1951) have pointed out that the level of dosage makes a great difference to the ease with which vitamin A is accumulated in the organs of the rat. At comparatively low intakes (40 i.u./day) the liver of the female contained over twice as

much vitamin A/g. as that of the male, whereas the kidney of the male contained over 5 times as much as that of the female. At higher levels of dosage (640–1280 i.u./day) these differences tended to disappear. This work suggested that varying the dose might affect storage of iron by the organs of male and female rats in a somewhat similar way, and drew attention to the fact that information about sexual differences in the storage of iron by the rat had so far been confined to the liver. The work now to be described was accordingly undertaken.

EXPERIMENTAL

Black and white hooded rats were employed for these experiments. The litters from sixteen females, which had been mated at the same time, were used. The litters were all born within 11 days. Forty-five males and forty-three females, two or three of each sex from each litter, were used for analysis at birth, and the number in all litters was then reduced to nine. At the end of 15 days, twelve males and twelve females, representing all sixteen litters, were taken for analysis. The remaining rats were weaned at 21 days. The oldest were maintained on the stock diet for 11 days until the youngest had been weaned. The average age at the end of this time was 28 days. Then the rats were paired according to age, weight and litter, so that there were two groups of males and two groups of females as similar as possible in all these respects. All were given unlimited amounts of the stock diet, consisting of a mixture of bread, whole wheat, oat flakes, bran, wheat germ, yeast, fishmeal and dried milk, with liver once a week and greens or carrots every alternate day. They were also given fresh whole milk every day. The stock diet contained approximately 3 mg. Fe/100 g. fresh weight. At the beginning of the experiment, when the rats were growing rapidly, they ate more food in proportion to their body weight than when they were older, and their food provided them with about 2.5 mg. Fe/100 g.

body wt./day. After the experiment had been running for 3 months and the rats were nearly fully grown the food supplied only 0.5 mg. Fe/100 g. body wt./day.

One group of each sex received additional iron as FeSO_4 . This was made up as 15% (w/v) aqueous $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ containing 30 mg. Fe/ml. The supply for each day was measured with a pipette and added to the fresh milk. The amount of additional iron given was equivalent to 3 mg. Fe/100 g. body wt./day throughout the experiment. The rats readily drank the milk containing the iron. A record was kept of any left over, but this was insignificant in amount. The rats were weighed weekly throughout the experiments. Twenty-two days after the administration of iron had begun, when the average age of the rats was 50 days, four males and four females were taken from each group. A similar number of rats was taken at the age of 100 and 150 days, and three of each sex were taken at 200 and 250 days. The rats were killed by a blow on the head. The livers, spleens and kidneys were removed, washed rapidly with distilled water, blotted on filter paper and weighed. The organs from all the rats in each group were pooled and homogenized with 3–50 times their weight of water. Samples of the homogenate were measured out for the determination of inorganic iron. This was estimated with 2,2'-dipyridyl (Shackleton & McCance, 1936). The solid matter was removed by centrifuging, and the intensity of the red colour was matched in a Spekker absorptiometer, using an Ilford green filter no. 604 (Harrison, 1952).

RESULTS

Table 1 shows the average weights of the animals at seven stages of growth, and the weights of the organs as percentages of the body weight. The rate of growth and the weights of the organs did not appear to be affected by varying the dosage of iron.

Fig. 1 shows the concentration of inorganic iron in the liver, spleen and kidney of the male and female rats at the normal (lower) levels of iron intake. The

Table 1. *Average weights of the bodies and organs of the male and female rats used for these experiments*

(Weights of organs are expressed as percentage of body weight.)

Age (days)	No. of animals in each group	Body wt. (g.)		Liver (as % body wt.)		Spleen (as % body wt.)		Kidneys (2) (as % body wt.)	
		Control	+ Fe	Control	+ Fe	Control	+ Fe	Control	+ Fe
		Males							
0	45	5.29	—	5.9	—	0.23	—	1.46	—
15	12	24.3	—	4.7	—	0.34	—	1.46	—
50	4	154	148	5.2	4.6	0.41	0.61	0.97	0.89
100	4	278	278	3.1	3.3	0.26	0.35	0.69	0.73
150	4	300	297	3.2	2.9	0.24	0.25	0.72	0.67
200	3	332	330	3.4	3.2	0.25	0.23	0.68	0.62
250	3	338	367	2.8	2.8	0.19	0.24	0.65	0.69
Females									
0	43	4.79	—	7.3	—	0.19	—	1.40	—
15	12	25.5	—	5.5	—	0.31	—	1.39	—
50	4	116	111	4.5	4.7	0.28	0.38	0.95	0.96
100	4	193	194	3.3	3.3	0.40	0.28	0.75	0.75
150	4	225	228	3.4	3.0	0.33	0.21	0.75	0.68
200	3	228	227	3.0	3.1	0.28	0.27	0.70	0.67
250	3	248	244	3.0	3.1	0.27	0.24	0.67	0.70

initial values were similar in all the organs, and the fall during the suckling period, already well known

Fig. 1 The effect of sex on the concentration of inorganic iron in the organs of rats.

to take place in the liver, clearly affected also the kidney and spleen. Thereafter the concentration of iron increased in all three organs, but to

very variable extents, and the percentages found in the female always exceeded those in the male tissues.

Table 2 shows the results of administering additional iron. Since the iron supplement only commenced after weaning, the earliest age at which any effects could be expected was 50 days, but by this time the additional iron given in the food had raised the concentration in the liver, spleen and kidney of both the male and female rats, and the differences increased with the age of the rats, although the results were somewhat variable. Increasing the intake of iron did not, however, at any age cause the concentration of iron in the male liver and spleen to exceed that in the female organs.

DISCUSSION

The effect of sex on the concentration of inorganic iron in the organs of the rat is evidently not confined to the liver. The kidney and spleen, and possibly other organs, are also involved. There are two ways in which this might be brought about: (1) since, as is now generally agreed, the amount of iron in the body is regulated by the rate of absorption rather than the rate of excretion (McCance & Widdowson, 1943, 1944), the effect of sex may be confined to the epithelium of the gut, or even to the pH or to some physicochemical equilibrium within the lumen. (2) The quantity of ferritin, or other iron-binding proteins, or both, may be much greater in the organs of the female than in those of the male, and consequently the equilibrium in the absorption-circulation-storage system may be more easily maintained in favour of the first. These present results provide no evidence for either hypothesis. They do, however, suggest that the storage of iron

Table 2. *Effect of the administration of additional iron on its concentration in the organs of male and female rats*

Age (days)	Inorganic Fe (mg./100 g. organ, fresh wt.)					
	Liver		Spleen		Kidneys	
	Control	+ Fe	Control	+ Fe	Control	+ Fe
Male rats						
0	10.7	—	6.1	—	6.6	—
15	1.6	—	0.8	—	2.0	—
50	4.5	8.2	7.2	12.9	2.3	3.2
100	9.7	21.4	39.7	68.0	5.7	10.8
150	10.8	15.6	44.2	52.7	6.1	8.8
200	15.2	28.0	66.1	110.0	7.2	10.7
250	15.2	27.3	54.0	102.0	5.2	8.3
Female rats						
0	8.1	—	4.5	—	7.8	—
15	1.6	—	1.5	—	1.7	—
50	10.0	18.5	10.0	16.0	2.1	3.1
100	28.6	50.2	56.3	103.0	7.3	Lost
150	44.5	81.7	112.0	160.0	8.3	10.3
200	42.2	60.0	110.0	118.0	9.6	11.0
250	43.3	49.8	106.0	138.0	11.9	14.9

and vitamin A are quite independent processes, for with the limited dosages of iron which have been employed the organs of the female have always contained higher concentrations of iron, and there has been no suggestion of a reversal of the female-male relationship. The vitamin A intake in this investigation was about 100 i.u./rat/day, and at this level the amount in the kidneys of the males probably exceeded very materially that in the corresponding organs of the females.

SUMMARY

1. Higher concentrations of iron were found in the livers, spleens and kidneys of female rats than in the corresponding organs of male rats.

2. The differences were greatest at and after the 100th day of life.

3. Increasing the intake of iron 2-6 times raised the concentrations found in all three organs, but did not alter the female-male relationship.

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