probably makes the precision of the two methods of about the same order. The very much simpler technique of the dye-laking procedure, and the shorter time required, should make the method of particular value if plasma concentrations are required to be estimated in the field with limited laboratory facilities.

Specificity of the dye-laking method

A number of the possible metabolic fission products of suramin have been subjected to the analytical procedure described above and the results are listed in Table 2.

These results indicate that the dye-laking reaction occurs with those fragments which contain the naphthylaminetrisulphonic acid group and also the bis-urea derivatives. It may be assumed from the results with (VII) that amides and the simpler amino-acids do not give the reaction. It is interesting to note that the order of suramin equivalents of this series of compounds approaches very closely to their order of persistence in the blood stream as found by Spinks (1948).

Table 1 indicates that the results obtained by the two methods are of the same order, the dye-laking method giving slightly the higher figures. This suggests the absence from the plasma of appreciable amounts of simple amino-acids and amides without the naphthylaminetrisulphonic acid group, since these would cause the dye-laking method to give lower apparent plasma concentrations. On the other hand appreciable amounts of naphthylaminetrisulphonic acid would cause the dye-laking method to

give a higher apparent concentration, since according to Spinks (1948) the azo dye developed from this compound is weakly coloured. It is possible that the observed higher results with the dye-laking method are due to this cause. Spinks has also shown that persistence for a prolonged period in the blood after intravenous injection is only exhibited by symmetrical polyamides of high molecular weight, which contain naphthylaminepolysulphonic acids as end groups, and that after 10 hr. the amounts of the other products of hydrolysis of suramin in the blood are negligible. Moreover, since all the degradation products examined have no trypanocidal activity, it is very likely that after this period both methods are measuring true suramin concentration and any divergence between them is due to experimental error.

SUMMARY

1. A method for estimating suramin in aqueous solution or in serum and plasma is described. It utilizes the colour change which is observed when suramin is added to a styrylquinoline dye, 2-*p*-dimethylaminostyryl-6-acetamidoquinoline methochloride.

2. The method is of about the same precision as the hydrolysis method of Dangerfield *et al.* (1938) and rather more sensitive; it involves a much simpler and more rapid technique. The two methods yield similar values for the suramin content of the blood of a rabbit injected with suramin.

3. The behaviour of hydrolysis products of suramin has been investigated and the specificity of the method is discussed.

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Sexual Differences in the Storage and Metabolism of Iron

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It is now an accepted fact that hormones and/or vitamins have important parts to play in the exchanges and functions of sodium, chlorine, potassium, calcium and iodine, but their role in the metabolism of iron has not been so conclusively demonstrated or so generally recognized. Bunge (1889) suggested that young female animals put aside reserves of iron for the future needs of reproduction, and that the deviation of iron from the

circulation to the storage organs at puberty might explain the high incidence of chlorosis in adolescent girls. In more recent years further evidence has been produced that the metabolism of iron is controlled, to some extent at any rate, by the sex hormones. Women, for instance, tend to have lower concentrations of circulating haemoglobin than do men, apart altogether from the obvious effects which menstruation must have upon their bodily require-

ments (Widdowson & McCance, 1936, 1942; Barer & Fowler, 1936). There is also good evidence that they have less inorganic iron in the serum (Vahlquist, 1941). Pregnancy depletes the iron stores of female rats (Smythe & Miller, 1929; Steenbock, Semb & Van Donk, 1936), and Mitchell (1932) showed that if males and females were given a diet which was deficient in iron the males became anaemic more quickly than the females. Smith & Otis (1937) found that, given the same diet, female rats produced more haemoglobin/g. of available iron than did males, and Steenbock *et al.* (1936) that they accumulated more iron in their livers. Hubbell & Rose (1937) and Smith & Otis (1940) showed that the whole bodies of female rats contained more iron/g. than those of males given similar amounts of iron in their diets; Smith & Otis (1940) further demonstrated that, at a low level of intake, the total amount of iron in the bodies of the females was greater than that in the bodies of the males, despite the fact that the females were smaller animals. Steenbock *et al.* (1936), moreover, showed that castration decreased a female's capacity to store iron, and that injections of follicular hormone into immature females stimulated them to store more iron than their litter mates. This work was reported very briefly and no confirmation or extension of it has been found. It is evident, however, that both in the rat and in man there are quantitative differences in the way in which the two sexes use the iron available to them.

The experiments now to be described were undertaken to confirm some of these findings and to discover to what extent they were true of other species. The work formed part of a more general study of the chemistry of growth and development. A short communication has already been made to the Journées thérapeutiques de Paris (McCance & Widdowson, 1946).

METHODS

Rats, mice, rabbits, guinea pigs, poultry, perch and eels have been used for these experiments. The rats were an inbred black and white strain, and were reared on a stock diet consisting of bread, oats, bran, fish meal, milk and greenstuff. The mice, which were highly inbred, were given similar food, and the rabbits and guinea pigs their usual diets. The mice, rabbits and guinea pigs were obtained from the Pathological Laboratory, Cambridge. The cockerels and pullets were specially reared for the experiment by Chivers and Sons Ltd. They were Light Sussex, and the numbers of females and males were the same. The two sexes received exactly the same diet. The perch and eels were obtained from the Freshwater Biological Station, Wray Castle, Windermere. They had not been reared in captivity.

Total iron was determined after dry ashing by the thioglycolic acid method (McCance, Widdowson & Shackleton,

1936). Inorganic iron in the liver was estimated by the modification of Hill's (1930) method described by Shackleton & McCance (1936).

RESULTS

The effects of age, sex, and reproduction on the concentration of inorganic iron in the livers of rats

Seventy-eight male and 99 female animals were used for these experiments, and the results are shown graphically in Fig. 1. It will be seen that the livers of

Fig. 1. Concentration of inorganic iron in the livers of male and female rats.

newborn animals contained between 14 and 15 mg. of inorganic iron/100 g. of wet tissue. At the end of a fortnight the inorganic iron had fallen to less than 1 mg./100 g. of liver. This was an expression of the deficiency of iron to which all mammals are liable towards the close of the suckling period. In rats this is not usually accompanied by anaemia, nor does it affect one sex more than another. By the time the young rats were 50 days old the concentration of inorganic iron in the livers of both sexes had risen to about 4 mg./100 g., but with the onset of sexual development the females began to lay down iron in their livers much faster than did the males, and by the time the animals were 90 days old and sexually mature, the female livers contained 17 mg. whereas the male livers contained but 5 mg./100 g. At 200 days the iron in the female livers had reached a level of 34 mg. and in the male livers 11 mg./100 g., so the difference had continued to increase. At this age some of the female animals were mated and at the end of pregnancy the liver iron was found to have fallen to little more than 8 mg./100 g. No increase took place during lactation, but by the time the surviving mothers had reached the age of 320 days, their livers contained 18 mg./100 g. This was a greater concentration of iron than was found in male livers at 320 days, but a lower concentration than was found in the livers of the unmated females.

It is evident that the stores of iron in the livers were drawn upon freely to supply the needs of the foetuses, and the present results can be used to show the extent to which they did so. The liver of an unmated female rat 200 days old contained about 3.8 mg. of inorganic iron. By the end of pregnancy this may be considered to have fallen to 1.1 mg., a difference of 2.7 mg. The bodies of the newborn rats each contained about 0.36 mg. of iron, and, assuming a litter to consist of 8 young animals, this suggests that during pregnancy each mother had to provide 2.8 mg. of iron for her progeny. This is very close to the amount which disappeared from the liver, and suggests that most of the iron in the newborn animals may have come from that source. It would have been interesting to have studied the effects of a second pregnancy, but there were not sufficient animals to enable this to be done.

The effects of castration and sex hormones upon the concentration of inorganic iron in the livers of rats

Eighteen male and 24 female rats were used for these experiments, and they were all killed at the age of 90 days, which is about the time at which rats become sexually mature. The males were divided into three groups. The animals in group 1 were allowed to grow normally, those in group 2 were castrated at the age of 46 days; those in group 3 were also castrated at the same age, but were subsequently treated with subcutaneous injections of stilboestrol; 0.3 mg. of stilboestrol dissolved in arachis oil was injected each day for 3 days, the injection was stopped for 2 days, given for the next 3 days and so on. The females were divided into four groups. The animals in group 1 were allowed to grow normally, and those in group 2 were spayed at the age of 46 days. Those in groups 3 and 4 were also spayed at the same time, but the animals in group 3 were subsequently given stilboestrol by injection, 0.3 mg. a day for 3 days, none for 2 days, the injection continued for 3 days and so on. In this way it was

possible to establish a regular oestrous cycle, which was followed by means of vaginal smears. The animals in group 4 were given 0.25 mg. of testosterone each day, also by subcutaneous injection.

The livers of each group were pooled for analysis, and the results are given in Table 1. It will be seen that removing the gonads made the two sexes grow at the same rate, equalized the weights of their livers, and the concentration of inorganic iron in them. The administration of stilboestrol to the castrated males did not increase the percentage of iron in their livers, but it did increase slightly the percentage of iron in the livers of the spayed females. Testosterone did not appear to decrease the percentage of iron in the livers of such animals, or if it did the effect was small. Changes might have been produced by administering larger doses of stilboestrol and testosterone to the male and female castrates respectively, but the present results, taken at their face value, suggest that the male and female hormones may normally act in conjunction with factors present only in bodies of the same sex, for there can be no doubt about the effects of the natural hormones; there are, moreover, the findings of Steenbock *et al.* (1936), to which reference has already been made, that the injection of follicular hormones into immature females increased their capacity to store iron in their livers.

The effects of sexual development on the concentration of inorganic iron in the livers of other animals

Mice. Twelve adult male and 18 unmated female animals were killed. Their livers were removed and those of each sex were pooled for analysis. 1.08 mg. of inorganic iron was found in each 100 g. of male liver and nearly three times as much (3.08 mg./100 g.) in the female organ.

Guinea pigs. Six adult male and 6 adult unmated female animals were killed and the livers were worked up for analysis as already described. The male livers contained 4.9 mg. and the female 4.1 mg. of inorganic iron/100 g.

Table 1. *Effect of castration and of sex hormones on the concentration of iron in the livers of sexually mature rats (90 days)*

				Inorganic Fe in liver	
	Body wt. (g.)	Liver wt. (g.)	Liver as % body wt.	Total/rat (mg.)	mg./100 g.
Males					
No treatment	296	12.7	4.3	0.88	6.9
Castrated	258	9.5	3.7	1.05	11.1
Castrated, and stilboestrol	241	9.4	3.9	0.96	10.2
Females					
No treatment	199	7.3	3.7	1.32	18.2
Spayed	258	10.3	4.0	1.29	12.5
Spayed, and stilboestrol	222	8.8	4.0	1.36	15.5
Spayed, and testosterone	242	9.8	4.0	1.06	10.7

Rabbits. Five male and 6 female animals were killed at the age of 17–25 weeks. The liver of each animal was analyzed separately. Those of the males were found to contain an average of 10.3 mg. and the female livers an average of 8.2 mg. of inorganic iron/100 g.

Eels. Twelve silver males and 11 silver females were caught on their way to the sea; their average weights were 75 and 347 g. respectively. The livers of each sex were pooled for analysis. Those of the males held 28.2 mg. and those of the females 83.5 mg. of inorganic iron/100 g. This figure for inorganic iron in the livers of silver females, although extremely high, is about the value which might have been anticipated from the findings of McCance (1944) for the total iron in the livers of sexually mature females.

Perch. The livers of 11 immature males and of 11 immature females were pooled for analysis. The male fish averaged 24 g. and the females 19 g. in weight. The livers of the former contained 7.5 and of the latter 7.6 mg. of inorganic iron/100 g. Twenty-four male fish and 14 females were caught in May when they were ripe. The male and female fish then weighed 23 and 41 g. respectively. Analysis revealed 9.6 mg. of inorganic iron/100 g. in the male livers and 9.5 mg./100 g. in the female livers. These figures show that the development of the sperm and ova did not take place at the expense of iron stored in the liver.

Poultry. Twelve cockerels and 12 pullets were killed when they were 20 weeks old. Their livers were analyzed separately, and the results are given in Table 2. The average for the cockerels was 8.7 mg.

Table 2. *Concentration of inorganic iron in the livers of cockerels and pullets*

Bird no.	Liver inorganic Fe (mg./100 g.)	
	Cockerels	Pullets
1	8.7	10.5
2	7.0	37.3
3	6.1	20.3
4	11.1	12.8
5	7.0	10.7
6	8.4	20.2
7	9.6	11.5
8	10.3	12.2
9	9.9	10.3
10	13.4	7.0
11	6.0	10.1
12	6.4	13.4
Average	8.7	14.7

and for the pullets 14.7 mg./100 g. The difference was statistically significant ($t = 2.49$, $P = 0.05-0.02$). The male spleens contained 4.7 mg. and the female spleens 8.5 mg. of inorganic iron/100 g., so sex appeared to affect the storage of iron in this organ also.

DISCUSSION

The results obtained with rats have confirmed those of previous workers, and there can be no doubt at all that sex has an important influence on the storage of iron in this animal. The same is probably true of mice, but the concentrations of iron are very much lower in this species, so much so that amounts stored can scarcely be of any practical value to either sex. Since, however, sex does not seem to influence the storage of iron in either guinea pigs or rabbits, it is clear that even within the mammalian order these sex differences do not always appear, and they can certainly not be taken to hold for any given species until their presence has been experimentally verified. It will be exceedingly difficult to do this for the human species. While, therefore, these differences can never be ranked among the great biological generalizations, it is stimulating to have found them so easily in each of the three vertebrate orders which have been investigated. It is clear that a great deal more work will have to be done to find out how the sex hormones bring about these effects, and why they do not always do so, for the sex hormones of the rat must be very similar to those of the rabbit or indeed of any other vertebrate. What can the second factor be which enables the sex hormones to exert their effects, or which prevents them doing so? It may be unwise to formulate theories at this stage, but it can do no harm to point out that the female rat has been shown to store vitamin A more readily than the male under identical conditions of feeding and whether carotene or vitamin A is acting as the source of supply. Yet males have higher blood levels (Brenner, Brookes & Roberts, 1942). During periods of depletion, moreover, male rats were found to lose their stores of vitamin A more readily than female rats (Popper & Brenner, 1942). Is this association of iron and vitamin A wholly fortuitous? Few biological discoveries can be fitted into their proper place in the framework of knowledge until some time after they have been made, and they often turn out to have a much more general application than was at first supposed.

SUMMARY

1. With the onset of sexual development, female rats have been found to accumulate iron in their livers at a much greater rate than males.

2. Removal of the gonads from male and female rats made the males store more and the females less iron in their livers, so that the concentrations were equal for the two sexes. The administration of stilboestrol had no effect on the castrated males, but it caused a slight increase in the iron storage of the spayed females.

3. The livers of sexually mature female rats, mice, hens and eels have been shown to have a greater concentration of iron in them than the livers of male animals at a corresponding stage of development.

4. Guinea pigs, rabbits and perch were not found to exhibit these sex differences.

5. Pregnancy rapidly reduced the percentage of

iron in the livers of female rats and the reaccumulation was comparatively slow.

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A Synthesis of *dl*-Hydnocarpic Acid

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Hydnocarpic acid in its dextro-form is isolated from several oils (e.g. oil of *Hydnocarpus wightiana*) used in the treatment of leprosy. The structure (I)

assigned to hydnocarpic acid rests on the results of permanganate oxidation (Barrowcliff & Power, 1907), the synthesis of dihydrohydnocarpic acid

(Noller & Adams, 1926a; Bokil & Nargund, 1937), the homologation to and from chaulmoogric acid (II) (Stanley & Adams, 1929; Buu-Hoi, 1944) and a synthesis of *dl*-hydnocarpic acid by a route involving several changes in a cyclopentane ring (Bokil & Nargund, 1941).

The synthesis of *dl*-chaulmoogric acid (II), at the Cunion Leper Colony (Perkins & Cruz, 1927b) is now a classic, and it was thought worth while to apply the same general method (the Robinson long-chain synthesis, Robinson & Robinson, 1925) to the synthesis of hydnocarpic acid.