SEX DIFFERENCES IN THE REQUIREMENTS OF CERTAIN FOOD FACTORS.*

I.—During Growth.

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The adult male in any species is generally recognised as being a larger and heavier animal than the adult female. Donaldson¹ gives the weight of a male rat as 279.9 gms. and of the female as 230.4 gms. at 365 days, presumably fully grown.

It also appears to be accepted that the metabolism of the male is different from that of the female. Possibly this is quite correct, for the functions of the two are widely divergent. Mitchell¹⁰ points out that the biological value of proteins for growth is probably quite a different problem from the biological value of protein for maintenance, and further that the conditions of gestation and lactation also call for separate investigation. In these respects the function of the female is different from that of the male, and therefore it might well be expected that the requirements of the two sexes should differ.

In spite of this, many nutrition workers use only one sex for their experiments, and there are no available data giving the separate requirements of each sex. Osborne and Mendel¹¹ describe some very interesting experiments in which they endeavoured to find the daily dosage of vitamin B needed to keep a rat in health and to provide for normal growth. These observers, unfortunately, used a large majority of male

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rats and the number of females employed was insufficient to allow of any conclusions in respect of sex; but it must be admitted that there is, even here, a suggestion that on a definite dosage, especially if this dosage be on the low side, the male rat does less well than the female. For example—series C, with a daily dosage of 50 mgms. and the rats at 40 gms. weight at the beginning of the experiment, the growth of the three males is described by Osborne and Mendel as "nearly normal," and the three females as "normal." In another experiment in the same series, when the rats were receiving less vitamin B, the growth of the three females is again given as "normal," and of the three male animals these observers say that the growth is "one nearly normal and two very slow."

Hewer* states that male rats appear to be more susceptible to the influence of thymus feeding than the female rats.

There is some evidence in the experiments to be described in this paper that the male rat requires more vitamin B than does the female, and experiments are now in progress which, it is hoped, will throw more light on this problem.

1. Experimental.

1. Animals.—The rats used in this research were piebald animals bred in the laboratory. The stock is now good and has been inbred for four years. The usual practice is to keep any young does which are up to standard weight and in good general condition at weaning (twenty-one days) and mate them with bucks of approximately the same age, similarly selected. Details of the care of the stock animals have been published previously. (Hartwell, Mottram and Mottram, "The Technique of Breeding Rats for Feeding Experiments," Biochem. Journ., 1923, 17). Twelve rats were used for each experiment, six ? and six ♂ and were kept in a large cage together. The experiment was started when the animals were three weeks and two days old (i.e. two days after weaning). They were weighed once a day for the first few weeks and afterwards once a week.

2. Foods.—The animals were given food ad lib.; all constituents were well mixed so that the rats could not pick out any one substance. The food was given as a moist paste.
Sex Differences and Food Factors

In hot weather the rats needed extra water to drink, but in cold weather they drank none.

The diets given were:

1. 30 gms. white bread, 3 gms. butter, 0.7 gm. salt mixture, 60 c.c. 3 per cent. extract of vitamin B.
2. 30 gms. brown bread, 3 gms. butter, 0.7 gm. salt mixture, 60 c.c. water.
3. 30 gms. white bread, 3 gms. butter, 0.7 gm. salt mixture, 60 c.c. water, 1.5 gms. food casein.
4. 30 gms. white bread, 3 gms. butter, 0.7 gm. salt mixture, 60 c.c. water, 1.5 gms. gluten.
5. 30 gms. white bread, 3 gms. butter, 0.7 gm. salt mixture, 84 c.c. water, 1.5 gms. gelatin.
6. 100 gms. oatmeal (medium), 500 c.c. water, 2.88 gms. salt mixture, 14 gms. butter.
   The oatmeal was cooked for one and a half hours in 400 c.c. water, then 100 c.c. more water were added together with the butter and salt mixture, and the whole well mixed.
7. 100 gms. boiled potato, 25 c.c. water, 1 gm. salt mixture, 5 gms. butter.

2. Results.

In fig. 1 the composite growth curves of the male and female animals are given. Since the sexes were kept together from the beginning of the experiment, breeding could not be controlled in any way, and therefore the first litters appeared at different times. Sometimes a doe produced her first family at eleven to twelve weeks of age, and at other times no young were born until the doe was fourteen weeks old. Therefore in fig. 1, the growth is shown for different periods because the curves are only given up to three weeks before the birth of the first litter. The suitability of these diets with regard to gestation and lactation will be discussed in a later paper.

(1) Diet of White Bread, Butter, and Salt Mixture.—The rate of growth was distinctly slow, and the females grew more rapidly than the males (as previously stated *).

(2) Diet of White Bread, Butter, Salt Mixture, and Wheat Germ Extract. Again the rate of growth was slow, but the males did better than the females, i.e. the relative rate of growth of the two sexes was normal. The addition of extra vitamin B to the diet, therefore, led to improved growth in the males, but made practically no difference to the female animals.

(3) Diet of White Bread, Butter, Salt Mixture and 3 per cent. Extract of Vitamin B from Marmite. The results are in agreement with, and confirm those described under the wheat germ experiment.
Diet of white bread, butter, and salt mixture.
- Diet of white bread, butter, salt mixture, and wheat germ extract.
- Diet of white bread, butter, salt mixture, and extract containing vitamin B, made from marmite.
- Diet of brown bread, butter, and salt mixture.
- Diet of boiled potato, butter, and salt mixture.

- Diet of kitchen scraps, and bread and milk. *Control Animals.*
- Diet of white bread, butter, salt mixture, and food casein.
- Diet of white bread, butter, salt mixture, and gluten.
- Diet of oatmeal (medium), butter, and salt mixture.
- Diet of white bread, butter, salt mixture, and gelatin.
Sex Differences and Food Factors

(4) Diet of Brown Bread, Butter, and Salt Mixture.—The females grew at approximately the same rate as those fed on diets 1, 2, and 3, and the males grew at about the same or a slightly greater rate than those receiving diets 2 and 3 (see fig. 2). Therefore on this brown bread diet, the relative growth of the two sexes was normal. The improvement in the male growth may be due to the presence of more vitamin B than is present in diets 2 and 3, or as suggested elsewhere, to the possibility of the germ protein having a composition which is a very excellent supplement to the endosperm protein. The small amount of germ present (embryo is 1.5 per cent. of the seed, Osborne and Mendel) may be used as an argument against this suggestion, yet the embryo protein is given by Osborne and Mendel as 4.4 per cent. of the total protein of the seed. The protein percentage in the white bread diet is approximately 10.48 per cent., and in the brown bread it is approximately 10.32 per cent., calculated as dry constituents. If the increase in the rate of growth of the male rat is entirely due to the extra amount of vitamin B in the brown bread, the requirements of the male with regard to vitamin B must be distinctly in excess of those of the female rat. It seems more likely that the better growth is due to the two factors in conjunction, i.e., increase in vitamin B content and improvement in the quality of the protein.

(5) Diet of Boiled Potatoes, Butter, and Salt Mixture.—The rate of growth of both sexes was extremely poor, which is in agreement with the experiments of McCollum, Simmonds and Parsons, although the diet used by these workers must have been rather better than that used by me, in that they dried the boiled potato, and their rats therefore might have been able to eat a greater bulk.

The males grew more rapidly than the females, thus giving the normal relative rates of growth of the sexes. A possible explanation, again, is that this
can be accounted for by the presence of large amounts of vitamin B in the potato, a fact demonstrated by several observers. Also, according to Karl Thomas, potato protein has a high biological value (Lusk 7).

(6) Diet of White Bread, Gluten, Butter, and Salt Mixture.—With this diet the rate of growth of the sexes was practically equal; in fig. 1 the male curve appears just the better, but the difference is so slight that it is hardly worthy of comment.

(7) Diet of White Bread, Gelatin, Butter, and Salt Mixture.—In this experiment, the growth curve of the males shows well above that of the females, the difference being that normally expected.

(8) Diet of White Bread, Food Casein, Butter, and Salt Mixture.—The females became pregnant early and therefore only a short curve is shown. It is sufficient, however, to demonstrate a normal result, i.e. the male growing more rapidly than the female.

Thus, diets 7 and 8 give normal results, while diet 6 does not. The difference in vitamin B content of the three diets cannot be put forward as an explanation in this case. Neither the gelatin, gluten, nor food casein were purified products, and therefore might all contain a little vitamin B, but it is hardly likely that an appreciable amount would be present in any of these substances. Also the food casein (which gave the best growth, practically equal to that of the control animals) has been used by the author to form 20 per cent. of a synthetic diet and the animals fed thereon exhibited the typical symptoms indicative of lack of vitamin B. All three diets contain about 20 per cent. of protein, and therefore the amount of protein cannot be put forward as a possible explanation of the divergent results, especially as some observers use only about 10 per cent. of protein to obtain maximal growth.

It is interesting that the addition of gelatin to the bread diet resulted in such a marked improvement in growth, because gelatin in itself is regarded merely as a protein sparer. It seemed possible, therefore, that the chemical composition of the proteins used might throw some light on the results. Attacking the problem from this standpoint, however, presents much difficulty, because the figures for protein analysis (as regards amino acids) differ considerably according to separate workers. Even the same worker obtains different results by different methods, e.g. Folin and Looney 8 find that the results obtained colorimetrically differ from those obtained gravimetrically. Wheat protein, however, contains very little or no lysine (Osborne and Mendel give 0.63, and Matthews, 8 0.00) while gelatin contains an appreciable amount (Osborne and Mendel, 5.9, Matthews, 2.75).

Thus a mixture of these proteins should theoretically be good, since gelatin can supply the amino acid lysine, necessary for growth and practically absent from wheat protein. Hence the gelatin and caseinogen diets are more complete from the point of view of good protein than the gluten diet.

On the whole, nutrition workers are inclined to use only one protein in an experimental diet, and this question of the supplementing of one protein by another is very much in its infancy. It is surely one of great importance to man, especially amongst the poorer classes, where one protein (e.g. that of potato, bread, or oatmeal) may form such a big proportion of the protein ration.
Sex Differences and Food Factors

(9) Diet of Oatmeal, Butter, and Salt Mixture.—With this diet, the growth was quite good, though by no means up to that of the control animals, which were fed on a good mixed diet. The females grew better than the males, but all the animals developed especially silky coats.

According to Eddy, the vitamin B content of oats is +++ while the report of the Medical Research Council gives ++; neither report gives the value of oatmeal in respect of this vitamin. According to Plimmer, kilned oatmeal contains no accessory food factors. No analyses of the amino acids in oatmeal protein can be found. From some as yet unpublished work of the author's, there seems a possibility that the vitamin B content of oatmeal is not great. However it is not possible to make any definite statements about this at present. The oatmeal diet is merely given here as an example of one on which the female rat grows more rapidly than the male.

3. Conclusions.

On certain diets quoted in this paper, the relative rates of growth of the male and female rats were reversed. During the first weeks of growth, the male animals only developed at the same rate as the females, in some cases even slower growth was noted. This was really a temporary effect, because after several months the male rats were the heavier. The exact period at which the males went ahead in respect of weight cannot be stated, because the females were breeding, and hence it was impossible to get the weight of the six female rats, non-pregnant, on the same day.

Therefore it seems obvious that during the period of greatest growth (in the rat the first twelve to fifteen weeks after birth), the male requires a different diet from the female, since she can show a better growth curve on a diet which is not supplying all food factors in maximal amounts.

When wheat germ extract, or extract from marmite containing vitamin B was added to the diet of bread, butter, and salt mixture, an improvement was noted in the growth of the males, while the female curves showed practically no improvement, therefore it appears that the male needs a greater supply of vitamin B than does the female to produce good growth. Experiments with synthetic diets are in progress to try to determine the extent of this difference. When gelatin was added to the diet of bread, butter, and salt mixture, the relative rate of growth of the two sexes was normal, i.e. the male heavier than the female, but the addition of gluten to the
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bread diet resulted in practically equal growth of males and females. From this it would seem that protein, also, is a more important factor to the male than to the female, because the females grew equally well on wheat flour + gluten and wheat flour + gelatin, while the males showed a distinct improvement on the latter diet. Thus the male seems more susceptible than the female to the amount of vitamin B in the diet and to the quality of the protein.

4. Summary.

1. On certain diets quoted in this paper, the male rat grows at about the same rate as the female, and does not show the more rapid growth which is usually exhibited by the male of the species. Finally the male becomes distinctly the heavier, but his growth is slow during the first weeks of life.

2. It is suggested that the growing male requires more vitamin B than the growing female, and also that he is more susceptible to the quality of the protein in the diet.

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5. References.

1 Donaldson (1924), The Rat.
8 Matthews, A. P. (1921), Physiological Chemistry.