LVIII. THE SELF-SELECTION OF FOOD CONSTITUENTS BY THE RAT.

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OSBORNE and MENDEL [1918] and Mitchell and Mendel [1921] have described experiments in which rats and mice were offered the choice between adequate and inadequate food mixtures. The latter authors summarise their results as follows. "The outcome of this investigation shows in general that in their choice of foods, even between 'synthetic' mixtures which appear to the senses to be essentially alike, rats and mice make selections which are as a rule advantageous for their nutritive condition."

The experiments described below were planned with the aim of ascertaining whether rats would be able to face successfully a similar but probably more complex problem, namely that of choosing for themselves a suitably balanced diet when given access to isolated food components.

In order to simplify the problem as far as possible fats were not made an item of choice for the rats¹. Similarly a fixed supply of vitamins was given, so the rats were only called upon to choose between three variables—proteins, carbohydrates and minerals.

EXPERIMENTAL.

A litter of six albino rats of Wistar origin, consisting of 3 males and 3 females, was used for the experiment. The animals were weaned at 3 weeks, and kept for 1 week on our stock ration (a slightly modified Steenbock [1923] diet) before being transferred to the experimental diet. Two animals of each sex were placed upon the "free choice" regimen, consisting of caseinogen, sucrose and salt mixture (McCollum's 185) offered in separate cups. The remaining male and female served as controls and were given the same food constituents in the form of a mixture, composed of 75 parts sucrose, 25 parts caseinogen, and 4 parts salt mixture. This diet is known to give good growth when supplemented with cod-liver oil and a suitable concentrate of the B vitamins [Evans and Lepkovsky, 1929].

The caseinogen used in the experiment was prepared in the laboratory from crude commercial "casein" by repeated washing with acidulated water in wooden tubs. It contained 86.5 % protein ($N \times 6.25$) on the dry basis.

Each of the six rats received daily 2 drops of cod-liver oil fed by hand,

¹ Synthetic diets almost free from fat are very well utilised by rats and the amount of fatty substance present in ordinary washed caseinogen and in cod-liver oil is more than sufficient to rule out the possibility of the fat-deficiency disease recently described by Burr and Burr [1929, 1930].

and 0.25 cc. of a watery extract of brewer's yeast [Osborne and Wakeman, 1919] equivalent to 0.5 g. of dry yeast (nitrogen content—8 mg. per dose).

The rats were kept in individual round cages on screens and the nutrients were offered in small glass cups fitted with metal hoods to prevent scattering. This however, especially in the case of the salt mixture and dry caseinogen, could not be entirely avoided, but Petri dishes placed under the cages beneath each food cup permitted a satisfactory separation and recovery of the scattered food constituents and a quantitative estimation of the amounts ingested. The food consumption was recorded daily, except on Sundays. On Saturdays a double portion of the nutrients was weighed out in the food cups, and the food intake for 2 days was measured every Monday. Double rations of the vitamins were also given on Saturdays. The food cups containing the various constituents were always placed in the cages in the same order. They were reversed once during the experiment. Towards the close of the experiment the sugar was replaced by rice starch.

The experiment lasted 10 weeks (69 days). In the course of this time the experimental rats Nos. 116 and 117 died after 54 and 61 days respectively, the first with symptoms of peritonitis, while the cause of death of the second was not ascertained.

Weekly averages of the food consumption of the four experimental rats and a chart comparing the average food intake of the experimental and control rats as well as growth curves of the experimental animals and an average growth curve of the two controls are given in Figs. 1, 2 and 3. All the figures are calculated on a water-free basis, the values for protein refer to 100 % protein ($N \times 6.25$). The non-protein solids in caseinogen were included in the "sugar."

At first glance the figures disclose two salient features: firstly the protein intake of the experimental rats is very low, being in every case less than 10 % (with the exception of the premortal periods of rats Nos. 116 and 117 and of one period for rat No. 115), and secondly their growth increase is small. In fact, if the ability to grow is taken as a measure of the success of the experiment, then it must be said that, on the whole, the rats were unable to select a mixture which would surpass or equal in efficiency the prepared mixture based on conventional standards. On a quantitative or caloric basis the proportions selected by the rats are also far from being economic.

Table I gives the total intake of protein, sugar (and later rice starch), salts and Calories (calculated) for the experimental rats and for the controls.

From these figures the number of Calories consumed per g. increase of weight has been calculated for each rat.

Rat	Calories per g. increase
114	49.6
115	31.1
116	65.4
117	43.2
Average for two controls	22.2

Fig. 1. Weekly food intake of rats 114 and 115.

At ↓ sugar changed to rice starch.

Fig. 2. Weekly food intake of rats 116 and 117.

At ↓ the sugar was changed to rice starch.

When the first seven weekly periods during which all the experimental rats remained alive are taken as a basis, the following average figures are obtained:

	Consumption Calories	Initial weight g.	Weight increase g.	Consumption Calories per g. increase
Control rats	1568	48	82	19.1
Experimental rats	1438	49	26	55.3

A calculation for the last six periods has also been made for rats 114 and 115. It may well be assumed that at this time the rats had become well accustomed to the experimental conditions. Here the figures are more in favour of the experimental rats:

	Consumption: Calories	Initial weight g.	Weight increase g.	Consumption Calories per g. increase
Rat 114	1289	40	43	30
Rat 115	1679	70	59	28.5
Control rats	1891	89	78	24.2

Fig. 3. Comparison of weekly food intake of experimental and control rats.

The left half of each rectangle represents the average food intake of the experimental rats, the right one that of the controls.

The ↓ arrows represent the death of an experimental rat.

The ↘ arrow represents a change from sugar to rice starch.

It must be remembered however that the control animals were heavier than either of the experimental ones and that, according to Hitchcock [1927], "The caloric cost of a gram increase in weight is inversely proportional to the square root of the percentage increase in weight." This of course would

imply that the heavier controls were handicapped when compared with the lighter experimental animals.

It is clearly visible from the curves in Fig. 4 that the animals allowed the choice of food actually consumed more Calories per unit of weight than their

Table I.

Consumption of	Total g.	Daily g.	%	Total Calories	Calories daily
Rat 114. No. of days on experiment: 69; initial weight: 46 g. weight increase: 38 g.					
Protein	26.9	0.39	5.8	110	1.6
Sugar*	432.4	6.27	93.1	1773	25.7
Salts	5.1	0.07	1.1	—	—
Total	464.4	6.73	100.0	1883	27.3
Rat 115. No. of days on experiment: 69; initial weight: 48 g. weight increase: 81 g.					
Protein	48.4	0.70	7.6	198	2.9
Sugar*	565.7	8.20	89.1	2319	33.6
Salts	20.9	0.30	3.3	—	—
Total	635.0	9.20	100.0	2517	36.5
Rat 116†. No. of days on experiment: 49; initial weight: 54 g. weight increase: 22 g.					
Protein	22.7	0.46	6.2	93	1.9
Sugar*	328.1	6.70	90.1	1345	27.4
Salts	13.3	0.27	3.7	—	—
Total	364.1	7.43	100.0	1438	29.3
Rat 117†. No. of days on experiment: 56; initial weight: 47 g. weight increase: 40 g.					
Protein	31.0	0.55	7.2	127	2.3
Sugar*	390.5	6.97	90.2	1601	28.6
Salts	11.3	0.20	2.6	—	—
Total	432.8	7.72	100.0	1728	30.9
AVERAGE FOR CONTROL RATS.					
No. of days on experiment: 69; initial weight: 48 g. weight increase: 118 g.					
Protein	131.7	1.91	19.8	540	7.8
Sugar*	508.3	7.37	76.6	2084	30.2
Salts	24.1	0.35	3.6	—	—
Total	664.1	9.63	100.0	2624	38.0

* And, later, rice starch.

† The last incomplete premortal period is omitted.

Fig. 4. Consumption of Calories per 100 g. of weight.
Weekly averages.

The curve for the experimental rats represents an average for four rats for the first seven weekly periods, for three for the eighth period, and for two for the ninth and tenth periods.

control mates when equal weights are compared, while their growth was at the same time far inferior.

The results are much more favourable for the experimental rats when their weight increments are evaluated on the basis of protein intake. The weight increase per g. of protein ingested can easily be calculated from the figures given in Table I with the following result.

Rat	Weight increase per g. protein
114	1.41
115	1.67
116	0.97
117	1.27

The average protein intake for the four experimental rats for the first 7 weekly periods is 23.5 g. (6.5 % of the total food intake), while the weight increase for the same period amounts to 26 g., a ratio of 1.08. For the last six periods (42 days) the values are:

Rat No.	Protein consumed P (g.)	% of total food intake	Weight increase W (g.)	Ratio W/P
114	21.9	6.7	43	1.96
115	33.1	7.6	59	1.78

It is obviously not possible to compare the experimental rats with the controls in this respect, as the food of the latter contained a rather large percentage of protein, probably well over the requirements for growth at "normal" rate.

On the other hand the values obtained above are nothing else but the biological value of the food protein, caseinogen in this case, calculated according to the method of Osborne, Mendel and Ferry [1919] and may be compared with data found in the literature for caseinogen.

These authors obtained a value for caseinogen of 1.45 at a 9.3 % level in an experiment of 4 weeks' duration and in another experiment a ratio of 1.76 (11 weeks) and 1.77 (4 weeks) at a 9.2 % level. Hoagland and Snider [1926] found a value of 1.98 for caseinogen at a 10.0 % level in a 30 days' experiment. These values refer to levels higher than those encountered in this experiment and, as the maximal values for caseinogen are found, according to Osborne, Mendel and Ferry [1919], at the 12 % level and decrease in both directions, the present figures are at least as good as those reported in previous observations. This finding certainly confirms the obvious supposition that the low level of protein intake was the first limiting factor in the diet freely selected by the experimental rats, but does not supply any additional information as to the reason for this rather surprising choice. It would seem however reasonable to assume, judging from these results, that the poorer growth of the experimental animals was not due to other causes connected with the free selection of their food, such as daily variation of the ratio of various constituents and so on.

As contrasted with the low intake of protein, the voluntary consumption by the "free-choice" rats of the mineral components of the diet in the form

of salt mixture did not differ greatly from the accepted standards, with the exception of rat No. 114. This animal consumed on the average only 1.1 % salts or one-third as much as the remaining three experimental rats. It does not seem however that this low intake of minerals limited the growth of rat No. 114. The protein was here probably the first limiting factor.

Owing to the low voluntary ingestion of protein, sugar formed over 90 % of the diet of the experimental rats (90.6 % as an average for the first 7 weekly periods). It is well known that rats eat sugar very readily and it was thought possible that the factor of taste might have induced the rats to satisfy their caloric requirements almost entirely with a highly palatable carbohydrate to the detriment of the more or less insipid protein. The sugar was therefore replaced by rice starch at the beginning of the 8th weekly period. This change did not however exert the slightest influence on the intake of protein, the only noticeable result being a short temporary drop in the consumption of the carbohydrate.

As already stated the order in which the food cups were placed in the cages of the experimental rats was changed once in the course of the experiment. This had no noticeable effect on the intake of the various food constituents, even on the day of the change.

DISCUSSION.

Perhaps the most noteworthy fact in the above experiment is the voluntary limitation by the experimental rats of their protein intake well below the standards accepted as normal or necessary for the rat. At first sight this result might certainly be interpreted as giving "natural" evidence in favour of low-protein diets and as an objective argument confirming the views of certain students of nutrition. Any such reasoning however would lose its point in face of the poor growth and performance of rats voluntarily subsisting on such diets. The inferiority of the experimental animals as compared with controls consuming a diet richer in protein makes an explanation of their choice highly interesting and desirable but at the same time very difficult. A simple explanation could be offered on the assumption that caseinogen *per se* is distasteful to the rats and that the dislike for this nutrient causes them to limit the intake. It must be remembered however that, when given no choice, the control rats ingested without difficulty adequate amounts of a diet containing 3 times as much caseinogen, and that Osborne and Mendel [1921, 1, 2], Osborne *et al.* [1927], Reader and Drummond [1924-5] and others found quite satisfactory food consumption on diets consisting almost entirely of caseinogen. It is also known that, in search for necessary nutrients, animals subsisting on deficient or faulty diets will consume substances which they would not touch under normal conditions, and which in all probability are distasteful; for example the coprophagy of rats fed on "synthetic" diets, an almost constant phenomenon in all laboratories, the pica or bone-eating disease of cattle and many other examples of depraved appetite. Though a particular

dislike for caseinogen cannot easily be ruled out, it does not seem to afford a sufficient explanation for the rather noteworthy failure of the experimental rats.

Mitchell and Mendel [1921], when offering rats the choice between two diets, one consisting of ground whole corn, with a protein ($N \times 6.25$) content of 9.5 %, the other of meat meal, prepared "by thoroughly drying lean round-steak and then grinding to a meal" and containing 79 % protein ($N \times 6.25$), while a salt mixture was freely accessible in both cases, observed that "contrary to what might have been expected the younger animals selected a smaller proportion of protein in their diets than did the older ones. On the other hand none of the rats chose an excessively 'high-protein' diet although an abundance of the meat meal was always available."

One of the younger animals (rat 21) referred to by Mitchell and Mendel consumed a diet containing only 12 % of Calories in the form of protein; in other words it hardly touched the meat meal. The other (rat 22) consumed 19 % of protein Calories. Both these values are well above the levels ingested by the rats in the present experiment, and, moreover, the rats of Mitchell and Mendel achieved much better growth, rat 21 gaining approximately 85 g. and rat 22 approximately 110 g. in 8 weeks. It must be remembered however that the lowest possible protein intake was for these rats higher than the level voluntarily selected by the animals in the present experiment. Whether the results obtained by Mitchell and Mendel give a truthful picture of the protein requirements of young growing rats or whether they were to any degree complicated or caused by an unequal distribution of vitamins, especially vitamin B, in the two natural foods employed by these authors remains to be answered. Taken as they are they nevertheless point in the same direction as the rather extreme results set forth in this paper.

When the present experiments were planned it was thought that their outcome might throw some light on the much debated question of the optimal level of protein intake and also on its variation in relation to growth (change in nutritive ratio). It seems however that any definite conclusions regarding the "natural" or "instinctive" (whatever these words may imply) protein requirements of young growing rats must be withheld until further experiments with different suitable proteins are instituted.

It has been taken for granted that free choice of regimen is a reliable guide for animals living under normal conditions. It certainly is well worth while to ascertain whether nature is not at a loss in conditions so highly artificial as described in the present paper.

SUMMARY.

1. Young growing rats, offered sucrose (later changed to rice starch), caseinogen and a salt mixture in separate dishes and allowed to make their own choice of these food constituents, consumed on an average only 6.5 % of protein.

2. Changing the sucrose to rice starch did not affect the intake of protein.
3. Two of the experimental rats died in the course of the experiment.
4. Litter mate controls which were given a ready-mixed diet consisting of the same ingredients and containing about 20 % of protein made much greater gains in weight.
5. All rats received equal vitamin supplies, fed separately by hand.

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