Choice of Diet by Rats Deficient in Members of the Vitamin B Complex

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There is a conflict of opinion about the ability of animals to select for themselves the nutrients best suited to meet their needs. Earlier experiments have been carried out under a variety of conditions and with a variety of species. On the whole, experiments involving selection of the B-vitamins by rats have been the best designed and are therefore of greater value than others.

The work of Harris, Clay, Hargreaves & Ward (1933) showed that a rat, on a diet deficient in thiamine and offered the choice of two diets, one devoid of thiamine and the other containing not more than a bare adequacy of it, and in some distinctive form, such as Marmite or yeast, will almost invariably select the latter diet. On the other hand, a normal rat will eat the two diets indiscriminately. They also found that diets containing vitamins not from a natural source, but as minute additions of highly potent concentrates and without the addition of flavouring agents, e.g. Bovril or cocoa, were not selected even by deficient rats. Richter, Holt & Barelare (1937) described a craving of thiamine-deficient rats for thiamine solutions and yeast; later, however, Richter & Hawkes (1940–1) demonstrated marked appetites for thiamine, riboflavin, nicotinic acid and pyridoxin solutions even in normal rats. Scott & Quint (1946*b*) found that rats, previously fed a diet deficient in thiamine, riboflavin or pyridoxin, showed appetites for the missing vitamin, but that normal rats did not. These workers also found that appetites for salts of pantothenic acid could not be developed in pantothenate-deficient rats unless the diets were labelled with a distinctive flavour, and even then some animals failed to select the vitamin. Richter *et al.* (1937) detected a craving in riboflavin-deficient rats for riboflavin, but Jukes (1938) was unable to detect a similar craving in riboflavin-deficient chickens.

General theories on appetite behaviour and instinct have, nevertheless, been formed on the basis of these conflicting results. The experiment described here was designed to find if normal and deficient rats, when offered the choice of two diets identical in all respects except for their vitamin B-complex content, would select the one containing the necessary quantities of the vitamin in preference to that containing none. The opportunity was also taken to investigate the effect on the selection of diets of variations in the position of the feeding vessel itself. Scott & Quint (1946*a*) have already indicated that rats may show a preference for particular positions of the feeding pot in the cage, and it was considered worth while to investigate this point under our conditions.

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EXPERIMENTAL

Diets. The compositions of the diets used in this experiment are shown in Table 1.

Management of rats. Five litters, each of six hooded 'Lister' rats, were divided into five groups in such a way that the groups were similar in sex and litter-mate distribution. Groups 2, 3 and 4 each contained six animals, whereas groups 1 and 5 contained eight and four, respectively. When the rats weighed approximately 100 g they were placed in individual cages measuring 10 × 10 × 5 in. and with a fine-mesh portable mat as floor. Each animal was offered 15 or 30 g of the appropriate diet, depending

Table 1. *Composition of experimental diets*

Diet no. 16		Diet no. 37 The same as diet 16 with the following:	mg/100 g diet
Casein (vitamin-free) (g)	230	Thiamine	0·3
Glucose (g)	330	Pyridoxin hydrochloride	0·3
Margarine (g)	150	Riboflavin	0·3
McCollum salts 185 (g)	50	Ca pantothenate	2
Maize starch (g)	400	Nicotinic acid	4
Radiostoleum (vitamins A and D) (ml.)	3	*i*-Inositol	10
		p-Aminobenzoic acid	10

on its body-weight, and the difference between this amount and the daily residue was taken as the amount consumed. All the feeding pots were identical in shape and colour. Beneath the meshed floor of each cage was placed a sheet of paper to collect any food scattered by a rat, but this seldom amounted to an appreciable quantity. All the animals were weighed daily and received tap water without stint.

Experimental treatments

Group 1. The eight animals were all given the choice between diets 16 and 37. In order that they would react to the diets and not to the pots containing them or to their positions in the cage, the following precautions were taken. On the 1st day, diet 16 was placed in pot A at the front of the cage while diet 37 was in pot B at the back; on the 2nd day, diet 16 was in pot A at the back and diet 37 in pot B at the front. The relative positions of both pots and diets were altered daily in the following way:

Day	Diet 16	Diet 37
1	A front	B back
2	A back	B front
3	B back	A front
4	B front	A back

Group 2. These six animals were offered diet 16 in two containers each. When they showed obvious clinical signs of a general vitamin B deficiency, one of the containers was replaced by another containing diet 37.

Group 3. These six animals were treated exactly as those in group 1, except that the positions of the pots were kept the same and only the positions of the diets alternated.

Group 4. These six animals were given exactly the same treatment as group 1, except that here the positions of the food remained the same and the pots alternated.

Group 5. These four animals were fed diet 37 only. The body-weight increases of this group provided the necessary check on the efficiency of mixing the vitamin supplement through the bulk of diet 37.

RESULTS AND DISCUSSION

The results have been analysed in two different ways: (1) a comparison is made between the behaviour of the deprived rats and that of the normal rats in the same choice situation; and (2) a comparison is made between the behaviour of the deprived rats given a choice of diets 16 or 37 and that of the same rats offered diet 16 only.

Comparison of groups 1, 2, 3 *and* 4. The percentage of the total diet consumed in the form of diet 37 is taken as the measure of diet preference. With group 2 this refers only to the trials in which the choice of diet lay between diets 16 and 37. As will be

Table 2. *Percentage of total food consumed represented by diet* 37 *for all rats in groups* 1, 2, 3 *and* 4. *Within group* 2 *this refers only to the trials in which diet* 16 *or diet* 37 *was offered.*

Group no.							
1		2		3		4	
Rat no.		Rat no.		Rat no.		Rat no.	
2	56·1	7	76·3	25	54·6	28	46·0
3	62·7	10	58·5	26	58·4	29	42·2
4	67·3	11	79·8	27	55·0	30	46·0
5	61·9	19	81·6	31	59·2	34	50·9
14	61·5	20	71·6	32	54·3	35	51·6
16	59·2	21	73·2	33	54·8	36	60·2
17	62·2						
18	63·8						
Mean	61·84		73·52		56·05		49·48

seen from the results, the rats showed marked preference for diet 37 and regained body-weight. When they reached approximately 200 g they were again offered only diet 16. Again they lost weight and showed signs of vitamin B deficiency, and again one pot of the diet 16 was replaced by one of diet 37. Once more the rats showed the marked preference for diet 37 and again increased in body-weight to 200 g. The detailed results of this analysis are shown in Table 2. An analysis of variance indicates that the groups differed significantly in their preferences ($P < 0{\cdot}001$). The main contributor was group 2; in other words, the rats deficient in the vitamin B complex showed a marked preference for the diet containing adequate quantities of the vitamin B complex. However, group 4 also deviated, though in the opposite direction, and an analysis excluding group 2 shows significant differences; therefore we can conclude that the rats showed a preference for a feeding place as well as for a diet, and that in the group 4 condition the bias introduced by place was favourable to diet 16. Since the percentage of the total food consumption represented by diet 37 is 62 and 56 in groups 1 and 3, respectively, it may be concluded that even the normal rat shows

a slight preference for an adequate over a vitamin-deficient diet, although this preference is not nearly so obvious as that of the deprived rats in group 2.

Behaviour of group 2. It is possible to compare the two situations: the choice of diet 16 or diet 37 and the choice of diet 16 or diet 16 for each individual rat in group 2. The 16–16 trials give a measure of the feeding variability in the null case. The difference in weight of food consumed between the pots each day is expressed as a percentage of the total food consumed that day. This obviously varies from zero, when the same amount of food is consumed from each pot, to 100, when all the food taken is from one pot. In order to compare the behaviour when a choice of diet is presented, it is assumed that the null index is symmetrically distributed from +100 to −100. It is further possible to assume that the distribution will be roughly normal in form, and the variability can therefore be described in terms of the standard deviation.

Table 3. *Standard deviation of variability index for each rat in group 2 when given the choice diet 16 or diet 16*

Rat no.	7	10	11	19	20	21
No. of 16–16 trials	70	21	74	64	64	67
Standard deviation of variability index	42·44	23·52	44·84	31·78	27·31	28·07

This is not strictly correct, as most of the observed distributions are too platykurtic, but this does not invalidate the use of the standard deviation as a standard measure for the 16 versus 37 performance. The calculated standard deviations are shown in Table 3. For the 16 versus 37 choice the same measure can be used, but with the positive sign when the weight of diet 37 consumed is in excess of the weight of diet 16. This can then be expressed in terms of standard deviation. If standard units of deviation are taken, the following categories can be made, being equally probable intervals on the normal distribution:

A above +0·85
B +0·26 to +0·84
C −0·25 to +0·25
D −0·84 to −0·26
E below −0·85

This enables a tabular comparison of the results to be made. As the sign is unknown in the 16–16 comparison, the observed distribution is assumed to be symmetrical. Table 4 gives the results of this analysis.

With changes of the order of magnitude shown, it is not necessary to make any further statistical calculations. If rat no. 21 is taken as being the least typical (except for the special case of rat no. 10, for which see below), we have $\chi^2 = 74{\cdot}6$, with 4 degrees of freedom. This being well beyond the range of the usual tables, the probability of the changes being due to chance must be of the order of one in a million. Even if the assumption of a symmetrical distribution is unfounded (and it is difficult to see what one could put in its place), the differences observed are so great as to be well beyond the bounds of chance.

Thus it is true to say that rats deficient in the vitamin B complex prefer a diet containing those vitamins to one that does not and that, as their behaviour is consistent, they must be able to differentiate between the diets.

Rat no. 10 of group 2 is of interest, for it was the only individual in this group that failed to show a preference for diet 37. It tended more than the others to eat nearly equal quantities from each pot when given the choice of diet 16 or diet 16. When given the choice of diet 16 or diet 37 it tended to eat nearly all from one pot, and so alternated its diet.

It was found that a group of twelve normal human beings failed to distinguish between these two diets either by smell or taste. The diets differed by 0·27 g of a mixed vitamin supplement in 1 kg of diet.

Table 4. *Standard deviations of the observed choice, diet* 16 *or diet* 16, *the expected choice, diet* 16 *or diet* 37, *and the observed choice, diet* 16 *or diet* 37. *A, B, C, D, E are standard units of choice with equally probable intervals of the normal distribution*

Rat no.	Choice	37+ A	B	C	D	37− E
7	Observed 37–16	21	4	0	0	0
	Expected 37–16 based on 16–16	5·5	4·1	5·7	4·1	5·5
	Observed 16–16	15·5	11·5	16	11·5	15·5
10	Observed 37–16	16	4	3	2	14
	Expected 37–16 based on 16–16	9·2	8·3	3·7	8·3	9·2
	Observed 16–16	5	4·5	2	4·5	5
11	Observed 37–16	25	4	3	0	1
	Expected 37–16 based on 16–16	6·0	5·5	8·9	5·5	6·0
	Observed 16–16	13·5	12·5	20	12·5	13·5
19	Observed 37–16	27	2	0	0	0
	Expected 37–16 based on 16–16	6·1	5·6	5·4	5·6	6·1
	Observed 16–16	13·5	12·5	12·0	12·5	13·5
20	Observed 37–16	29	4	1	0	5
	Expected 37–16 based on 16–16	7·3	7·9	8·5	7·9	7·3
	Observed 16–16	12	13	14	13	12
21	Observed 37–16	27	1	2	3	1
	Expected 37–16 based on 16–16	6·8	6·8	6·6	6·8	6·8
	Observed 16–16	13·5	13·5	13	13·5	13·5

SUMMARY

1. Rats deficient in the vitamin B complex showed a very marked preference for a diet containing an adequate quantity of the vitamin-B complex over one deficient in these vitamins but otherwise similar in every respect.

2. Rats not deficient in the vitamin B complex showed a less obvious but similar preference.

3. It is confirmed that in experiments on choice of diet by rats, the position of the diet in a cage may influence its selection.

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