EXPERIMENTAL STUDIES OF FOOD SELECTIVE BEHAVIOR IN SQUIRREL MONKEYS FED ON RIBOFLAVIN DEFICIENT DIET

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Summary A squirrel monkey, if it needs a particular dietary component because of a metabolic disorder or because that food has been excluded from its diet, will develop a specific hunger for the food. In cases where specific hungers show up clearly, four behaviors can be demonstrated: (1) The monkey prefers the food it needs to other foods that are also available; (2) It usually ingests large amounts of the food to meet its particular physiological requirements; (3) The animal will tend to eat the needed food even while its stomach is full; (4) When vitamin B\textsubscript{2} is removed from its diet, a squirrel monkey will exhibit digestive disturbance, general weakness, a lack of vigor, and loss of weight.

Richter and Schmidt (6) found when rats were presented with several dishes of food, in each of which was one element, (i.e., salt, sugar, or fat) they would eat from each dish an amount correct for their needs. Further, the animals would develop a specific hunger for any food which might lead to a metabolic disorder if it was excluded from their diet. Davis (2) found young children made a good selection of vitamin sufficient foods over a period of time where adults had difficulty doing this. He concluded that acquired habits can override beneficial self-selection. Richter and Eckert (5) indicated that not all rats make beneficial selections equally well. They found wide individual differences between animals; even strain and species differentiation. Scott and Verney (7) showed that many rats fail to grow normally when allowed to select their own diets.

There are two general methods of studying food preferences: (a) The single stimulus method (Stellar and Hill, 8) is one in which the animal is presented with only one food at a time, usually in solution, and its rate of ingestion is measured. This ingestion rate is compared with that for a different food, or for a different concentration of the substance. Differences in ingestion rate are represented as differences in preference. (b) The two-stimulus method (Young, 9) is exemplified by the receiving two different foods in separate containers. The procedure is repeated a number of times, and the preference is indicated by the animal preferring
one food over the other. As long as the two foods are distinguishable, the animal shows a preference for that one of which it has been deprived. The forthcoming research utilized this method of study.

Riboflavin helps promote growth, strength, and vigor, as well as provide for general health (KILANDER, 3). Its best known function is that it combines with phosphoric acid and protein to form tissue respiratory enzymes which control some of the oxidations involved in the life processes of the tissue (LANFORD and SHERMAN, 4). When food is poor in riboflavin for any considerable length of time, digestive disturbances, nervous depression, general weakness, and a loss of weight are some of the results (ALLISON and MUNRO, 1).

The present study was conducted to find out if squirrel monkeys demonstrated self-selective behavior when fed on a riboflavin deficient diet, and, if such a diet would lead to digestive disturbances, weakness, and weight loss in the animals. The investigation was carried out in two separate experiments; each one devised to test a hypothesis related to the above.

In the first experiment the hypothesis to be tested was: Squirrel monkeys, after two weeks of feeding on a riboflavin deficient diet, will demonstrate specific hunger toward high riboflavin foods in preference for foods with low riboflavin content. In the second experiment, the hypothesis was as follows: Squirrel monkeys fed on a riboflavin deficient diet will show digestive disturbances, general weakness, lack of vigor, and a loss of weight, when compared with squirrel monkeys fed a balanced diet over the same time span.

EXPERIMENT 1

I. Method

a. Subjects. Six, male, squirrel monkeys approximately eight months of age were selected from the monkey colony at St. Procopius College, Lisle, Illinois. They were divided into two groups of three animals each. One group was designated as the experimental (group A); the other was designated as the control (group B). All of the animals in each group were comparable in size, weight, and general activity level.

b. Apparatus. Animals were housed and fed in individual cages. Water bottles on each cage were graduated to indicate the amount of liquid consumption of each subject. Daily readings were recorded. One stopwatch was used to record the monkeys' reaction time to the selective foods in minutes and seconds. A T-maze was used to provide the animals with a discriminatory task in the selection process.

c. Procedure. The entire investigation took place in a well-lighted room with light and heat controlled. Many kinds of foods with varying degrees of riboflavin were utilized. Table 1 indicates foods selected to provide proper vitamin content for a well-balanced diet.
FOOD SELECTIVE BEHAVIOR IN SQUIRREL MONKEYS FED

Table 1. Riboflavin content in foods used in this study (μg per 100 g).

| Food                             | Range within which average will probably be found |
|----------------------------------|--------------------------------------------------|
| Food of animal origin:           |                                                  |
| Beef muscle                      | 180–260                                          |
| Pork muscle                      | 225–255                                          |
| Kidney (cattle and swine)        | 1,700–2,200                                      |
| Liver (cattle and swine)         | 1,800–2,600                                      |
| Milk                             | 195–240                                          |
| Eggs                             | 280–420                                          |
| Eggs (white)                     | 150–300                                          |
| Egg yolk                         | 380–750                                          |
| Grain products:                  |                                                  |
| Wheat, entire                    | 100–200                                          |
| Wheat germ                       | 600–800                                          |
| Vegetables and fruits:           |                                                  |
| Banana                           | 45–80                                            |
| Broccoli                         | 200–500                                          |
| Cabbage                          | 65–135                                           |
| Kale                             | 400–600                                          |
| Lettuce                          | 100–240                                          |
| Orange (or juice)                | 28–62                                            |
| Spinach                          | 250–400                                          |
| Tomato                           | 37–63                                            |
| Turnip                           | 50–100                                           |

The experimental monkeys were fed on the foods listed in Table 1 for a period of one week. After this period, they were fed on oranges, tomatoes, and other foods with low riboflavin content. Operationally, these foods provided the animals with a riboflavin deficient diet for a period of two weeks. After the two week period, these Ss were tested for self-selection for a total of four consecutive days.

Testing took place within the T-maze. Each animal of the experimental group was placed in the starting box at the stem of the T. He was allowed to move in the apparatus to the choice point or junction between the stem and the cross-piece. In one end of the T was a food low in riboflavin (oranges, for example, 28–62 μg/100 g); in the other a food high in riboflavin (liver, 1,800–2,600 μg/100 g).

On each day of trials, the Ss were placed in the apparatus for a series of 10 consecutive trials. A trial was completed when the monkeys selected one arm of the T and approached the food in that arm. The animal was not allowed to eat the food until the final trial on the fourth day. After having "selected" the one food, the monkeys were allowed to explore the other end of the T having the opposite food. The selection and timed reactions, however, were based on the initial choice. On the fourth day and 10th trial, the Ss were allowed to eat
as much of the self-selected food as they preferred. In this way, the idea that the animals would eat the needed food even when their stomachs were full was operationalized. All of the Ss in each trial had to observe both ends of the T-maze for each food selection before moving from the choice point because each food was alternated between the ends of the apparatus in random order to help control for place learning, learning set, and habit-formation. For all of the Ss in group A, there was a consistency of performance of selecting the food high in riboflavin over the one with low riboflavin content.

The same procedure as took place for the experimental monkeys also took place for the controls. The only difference in procedure was that Ss in group B were not placed on the riboflavin deficient diet; instead they were fed a well-balanced diet of a variety of foods in Table 1. Any differences in performance in the discrimination task, therefore were attributed to the relative riboflavin deficiency.

2. Results

Figure 1 shows the mean number of times the experimental monkeys selected either the high or low riboflavin foods and the number of choices on each day. On the first day of trials, the riboflavin (high) was chosen almost a mean of eight times for the three animals, while the regular diet (low riboflavin content) was selected about three times. By the second day of discrimination, the choice of ribo-

![Diagram](image-url)
flavin was over a mean of 9. Riboflavin was the choice of these Ss on eight of 10 trials at the third day of trials (See group A).

On the first day of trials, the control Ss (group B) showed a preference for regular food about eight out of 10 times. This preference was about the same for the second day of trials, as regular food was selected by a mean number of seven out of 10 times. At the third day of trials, monkeys in the B group chose riboflavin only four times, while the regular diet had a mean of six. By the fourth day of trials, these Ss selected the regular diet almost nine times and the riboflavin only somewhat more than once.

In comparing upper and lower halves (Fig. 1), there were significant differences in the mean number of selections of high riboflavin foods ($t=12.38$, df=$4$, $p<.001$) and selections of foods for the regular diet ($t=9.82$, df=$4$, $p<.001$) between experimental monkeys (A) and the controls (B).

Results of group A in Fig. 2 indicate the mean times in minutes and seconds for the "selection" of the riboflavin and regular diet foods for the Ss in the experimental group. It can be seen that after the first day and continuing throughout the remaining days of trial, the approach of these animals was much quicker toward the riboflavin food. There was a significant difference ($t=2.96$, df=$4$, $p<.05$) in the time for choice in discriminating between a food high in riboflavin content as compared with one of low content. Not only was the response quicker...
as the days of trial increased, but the mean time suggests that the discrimination between the two foods (high or low riboflavin content) was quicker as well.

Figure 2 indicates the mean time comparing riboflavin and the regular diet on each day of the trials for group B (control Ss). They also moved more quickly toward the riboflavin food as compared with the regular \((t=3.48, df=4, p<.05)\). Although their speed was quicker to the former, their selection was less (see Fig. 1). This performance might suggest that riboflavin is an essential factor in the nutritional process, and, when it is somewhat deficient (as might be the case in some of the controls due to physiological functioning) the monkeys will choose the foods high in riboflavin to block the possibility of future drastic deficiencies in their diet. Because the controls were fed a well-balanced diet, this performance might not be explained away by saying they were indicating a preference for meat, as meats were already in their diet.

EXPERIMENT 2

Method

a. Subjects. Ss were six, male, squirrel monkeys different from those in experiment 1, but from the same monkey colony. They were similar in age to those in the first part. Again, the Ss were divided into two groups of three animals each: an experimental (group A) and a control (group B). Matched variables included size, weight, and general activity level.

b. Apparatus. The same as in experiment 1 with one additional item. A "vigor roller apparatus" was constructed to measure the vigor or lack of it in the Ss. It consisted of a wooden box on which had been attached eight metal rollers so that the animal kept moving but went nowhere. These rollers were attached to a single cylinder which turned more slowly than the rollers. It was attached to a turnable arm which recorded a single turn on an electrical counter. In this way, each time the cylinder turned once, one score was noted on the counter.

c. Procedure. The same procedure was followed for a riboflavin deficient diet for the experimental group and for a well-balanced diet for the control animals as in experiment 1. Instead of having to discriminate foods in a T-maze, however, these Ss were weighed as one facet of this part to determine if there might be any weight loss due to riboflavin deficiency, while the other test was one of vigor.

In the first series of measures, the animals in group A were weighed before being put on deprivation of riboflavin and also each of the 14 days during the deprivation period. Operationally, these weights were used to judge his growth or lack of growth due to riboflavin deficiency.

These Ss were also measured on the vigor apparatus. Each one was placed in the 2 ft. x 3 ft. box, with a glass window at one end and screening across the top, at the same time, under the same conditions, each day for a period of four days of trials. A counter was used to measure revolutions of the cylinder per 5 min period. Observations were made at the same times to note the general activity level of the
S. After the completion of the four days of tests, the S was allowed to eat foods with a high riboflavin content.

The control monkeys (fed on the well-balanced diet) were also weighed for their original weight as well as every day for the 14. As with the experimental animals, the controls were also tested on the vigor apparatus in the same manner as described above.

RESULTS

Figure 3 presents the weights of the monkeys in the experimental and control groups. Weights of the experimental animals ranged from a beginning weight of 459 g to an end weight of 440 g (mean weights). Weights of the control animals consisted of means from 629 to 620 g for the beginning and end weights (respectively) of the animals during the 14 day period. For the animals in the first group, the spread of mean weights was 19 g; for those in the second group, the spread of mean weights was 9 g. A significantly greater weight loss (t=2.82, df=4, p<.05) was exhibited by the experimental monkeys (See upper half of Fig. 3).

The mean number of cylinder revolutions per 5 min period is shown in Fig. 3, for both the experimental and control animals. For the Ss in group A, on the first day there was a mean of about 35 revolutions per 5 min; on the second day it went down to 20; on the third day it rose slightly to a mean of about 23; on the fourth it went down to about 16. For the Ss in group B, the first day was a mean of
about 43 revolutions per 5 min period; on the second day the mean went up to over 50; the third day it dipped down to a mean of about 44; the fourth day it went up to 55. When comparing the total performance of the two groups of Ss, the experimental monkeys demonstrated significantly less vigor ($t=3.38, df=4, p<.05$) than those animals in the control group (See lower half).

CONCLUSIONS AND DISCUSSION

Results based on the data presented in Figs. 1 to 2 indicated a difference in selections of foods with high riboflavin content as compared with foods of a low content (or regular diet) for squirrel monkeys fed a riboflavin deficient diet. It was found that the Ss not only chose the high riboflavin food more frequently than their control animals, but they chose them quicker as well. It might be concluded that riboflavin deprivation diets in squirrel monkeys induce them to select their own diets which do contain the riboflavin necessary for a well-balanced diet.

Results suggested that the hypothesis for the first experiment should be accepted. Data indicated squirrel monkeys, after two weeks of riboflavin deprivation diet, show signs of specific hungers (apart from general hungers) and thus tended to select foods leading toward a more balanced diet from other foods which are available to them. It can also be concluded that squirrel monkeys will (a) ingest large enough amounts of the needed food to meet their physiological needs, and (b) they will eat the needed foods even when their stomachs are full.

As can be noted from the results illustrated in Fig. 3 (top), riboflavin deficient diet can lead to a loss of body weight and inhibit growth. Figure 3 (bottom) showed the lack of vigor for the experimental animals as a result of riboflavin deficiency. Such results suggest the acceptance of the second hypothesis. After two weeks on a riboflavin deficient diet, squirrel monkeys did show signs of digestive disturbances, general weakness, lack of vigor, and loss of weight. The loss of vigor had a somewhat resurgence for a short period, but, there were general continued trends in the vigor as well as in the overall activity level of the animals, in the decreased performance direction with continued riboflavin deficiency.

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