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Vitamin studies. X. Feeding technique in vitamin studies.*

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The work described in this paper is presented with the view of re-emphasizing the importance of the use of false screen bottomed cages in vitamin studies when rats are used as the experimental animals. Steenbock, Sell and Nelson¹ have pointed out that rats which do not have access to their own feces require a larger proportion of vitamin B in the basal ration than those rats which ingest variable amounts of excretory material daily. In our studies of vitamin B in the past (using diets deficient in this vitamin), we have often been unable to account for the lack of uniformity in the development of animals from the same litters on identical diets. In fact certain animals grew satisfactorily for many weeks on a vitamin B deficient ration. In watching the animals over relatively long periods it was observed that those animals whose food intakes and growth curves were most satisfactory were in the habit of ingesting relatively large amounts of excretory material; for example we have noted a few animals which would consume as many as 15 to 20 pieces of feces in the space of eight or ten hours.

In order to study the matter more thoroughly 63 rats were placed on experiment in individual cages. All animals received a basal ration consisting of casein 18, salts 3, agar 2, dextrin 77 and 5 drops of cod liver oil daily. Iodine was furnished in the drinking water. A number of the animals were placed in cages without screens while the remainder were distributed in cages containing false bottoms made of galvanized wire screens (3 meshes to the inch). The false bottoms were constructed about 1 1/4 inches above the cage pans in order that the particles of feces would fall through the screens to the pans below

¹ Steenbock, H., Sell, M. T., and Jones, J. H., *J. Biol. Chem.*, 1923, *lv*, 399.

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where the rats could not reach them. As indicated in the chart, weekly records were taken of the amount of food eaten, but it was impossible to keep an accurate record of the weight of fecal material ingested. In order, however, that we might have a fairly definite idea of the feces intake, certain rats were placed on screens, and feces from definite sources were fed. In some cases the feces were collected from rats which were on diets rich in vitamin B, while in other cases feces were collected from rats on diets containing little or none of this accessory food substance.

Some of the animals were placed on screens from the start and later the screens were removed in order to provide ready access to the fecal particles. Other rats were allowed access to feces from the start and later the screens were introduced.

In order to conserve space we have chosen a few of the most striking curves, placing them in a single chart for comparison. Certain curves were chosen, not because they are necessarily representative, but rather to show to what extremes animal experiments may go when seemingly unimportant details in technique are neglected.

The curve given for rat 392 is representative of all animals which were on screens without access to feces from the beginning of the experiment. It is evident that there is little, if any, vitamin B in the basal ration. When vitamin-rich feces from another rat were fed to rat 392 (see arrow) the response in food intake and growth was immediate. Although rats 292, 293, 296 and 299 received the same ration as rat 392, it is interesting to note that they were able to grow at a fairly satisfactory rate for from five to seven months, in spite of the fact that the ration was deficient in vitamin B. When screens were introduced in the cages containing rats 292 and 293, the fall in the food intake and body weight was very marked. On the 230th day vitamin-rich feces, obtained from rats on a vitamin-rich diet, were placed in dishes and offered to rats 292 and 293. The increase in food intake and body weight is ample proof that the feces possessed a stimulatory effect.

The curves of rats 299 and 301 tell the same story up to the point where the screens were introduced. When vitamin-rich feces were fed, the responses were very marked while the reverse was true when vitamin-poor feces were fed (see curve for

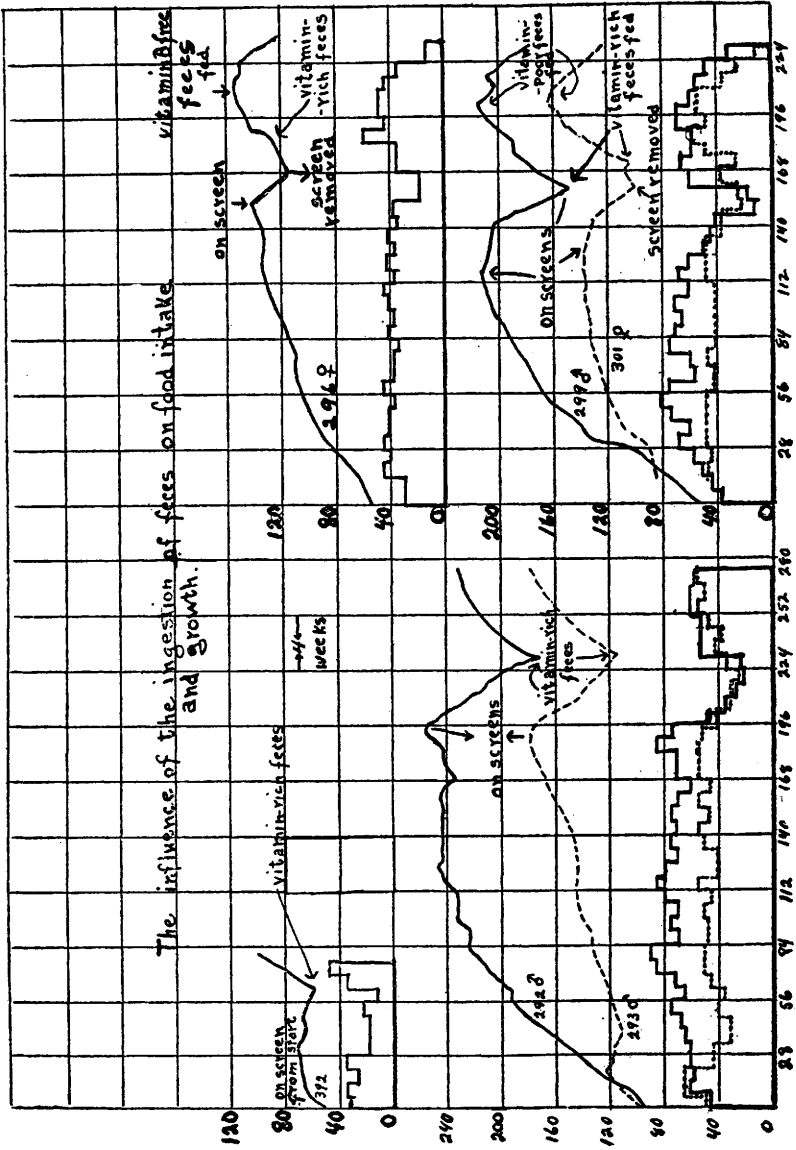


Chart showing the department of albino rats on diets deficient in vitamin B, with and without the use of false screen bottoms in the cages. Those rats which consumed large quantities of excreta were able to grow well—due, apparently, to the tissue vitamin stored in the body previous to the feeding trials. It would appear that this food hormone can be used over and over again. When rats are placed on screens appetite and body weight fall almost immediately. Rats cannot store vitamin B for long periods. This is indicated by the fact that the feces of the rats (on screens) have lost this vitamin within two weeks time.

rats 296, 299 and 301). The parallelism between food intake and increase in body weight is interesting and emphasizes the necessity of possessing data on food intake of individual animals. This, in our opinion, has considerable advantage over the "group feeding" method inasmuch as it often aids in the interpretation of data. For example we have found it advisable to exclude all animals in any experimental group that have shown sub-normal appetites when compared with their litter mates receiving the same ration. This would be impossible if the feeding was conducted with several animals in a cage.

Curves 292, 293, 299 and 301 emphasize the point just mentioned. Rats may be reared in the same environment and on the same rations and yet deport themselves differently, depending upon the amount of food that the animal normally desires to ingest. In other words, they will grow in proportion to the natural appetite that they possess.

We have also been able to corroborate the finding of Steenbock with reference to the storage of vitamin B. It is quite evident that the rat is able to store sufficient vitamin in the tissues to carry the animal over several months, provided a large proportion of the feces are eaten. In other words it would seem that the stimulatory substance can be stored, utilized, voided, re-ingested, and the cycle continued for a considerable time, indicating that the demand for vitamin B is urgent but that the quantity needed is not large. On the other hand, if rats are placed on screens for two weeks and then the screen removed, allowing the rat to commence feces eating at that point, the feces seem to have lost their stimulatory properties. This would indicate that the rat does not possess the power to store the vitamin for long periods.

CONCLUSIONS.

Rats are prone, if they have the opportunity, to consume varying quantities of excreta, the ingestion of which seems to keep up a sufficient supply of some stimulatory material (probably vitamin B) to vitiate certain types of experimental work. Just how serious the work of the past has been affected it is difficult to tell. Certainly it will be necessary to repeat certain types of work.

The importance of food intake records for individual animals has been emphasized.

Evidence is cited to show that the rat is limited in its ability to store vitamin B.

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The influence of the liver on the chemical regulation of the heart.

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The liver was the first tissue to which the function of internal secretion was ascribed by the genius of Claude Bernard. The liver is further the place of the most manifold and intense chemical processes in the animal body and no single cellular unit is in possession of so many enzymes as the liver cell. Yet as to internal secretion of the liver our knowledge is very scanty and modern text-books of endocrinology make little mention of the liver in this connection.

Dr. Takahashi and myself have made a series of experiments on the question as to whether the liver contributes towards the chemical regulation of the heart-beat. We have employed methods which we have been using for related questions since the year 1917 in studying the possible chemical basis of the action of antagonistic nerves, such as those regulating the beat of the heart. We used frog's heart as this is very sensitive to slight changes in chemical conditions and allows easy investigation under controlled conditions without any other influences hampering the results. Either a normal Ringer's solution, or Ringer's solution mixed with blood, was perfused from a small collecting vessel by way of the vena cava interior through the heart and returned from the aorta to the collecting vessel. We used this method in order to be able to work with small quantities of the fluid and to collect any substances which may be formed during the course of the experiment. The beat of the heart is recorded by aid of a suspension lever. Both vagi are laid