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### An Attempt to Secure "Refection" in Rats.\*

LAFAYETTE B. MENDEL AND HUBERT B. VICKERY.

(With the Collaboration of Helen C. Cannon.)

*From the Laboratory of the Connecticut Agricultural Experiment Station and the Laboratory of Physiological Chemistry, Yale University, New Haven.*

Fridericia<sup>1</sup> reported to the XII International Physiological Congress at Stockholm a phenomenon of apparent acquired immunity to deficiency of vitamin B in rats receiving a diet devoid of this vitamin group, but presumed to be adequate in all other respects. He stated that "In spite of the deprivation of B-vitamin a few of the rats resumed their normal rate of growth, and at the same time a change occurred in their intestinal tract, manifesting itself by their feces turning white and bulky. Eating of feces was prevented (wiregauze bottom in cages). Control rats, also fed on a diet devoid of B-vitamin, behaved in the usual way (growth stopping after 1 to 3 weeks, death after 4 to 5 weeks)."

To the restoration phenomenon Fridericia gave the name "refection". Subsequently he<sup>2</sup> described its manifestations in detail including the characteristic high content of undigested uncooked rice starch in the "white" feces. The hypothesis was offered that multiplication of micro-organisms supplying vitamin B occurs in "refected" rats; hence the unexpected resumption of growth. By feeding the feces of "refected" individuals to other rats that were showing failure of growth on a B-free diet, "refection" could often

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<sup>1</sup> Fridericia, L. S., XII Internat. Physiol. Congress at Stockholm, Aug. 3-6, 1926; *Skandin. Arch.*, 1926, xlix.

<sup>2</sup> Fridericia, L. S., Freudenthal, P., Gudjonsson, S., Johansen, G., and Schoubye, N., *J. Hyg.*, 1927, xxvii, 70.

be induced and growth restored. The feces then always turned "white". Many additional surprising details have been recorded.

The "spontaneous cures" in rats reared on diets devoid of the vitamin B components have also been recorded by Roscoe<sup>3</sup> from the Lister Institute in London. However, "refection" did not occur there when the diet was cooked or the starch was replaced by a soluble carbohydrate. Workers<sup>4</sup> in the Cambridge Biochemical Laboratory likewise have reported that from time to time rats on a vitamin B-free diet would decline for a short time only, and then resume growth, and continue to grow at a nearly or quite normal rate. The customary diet there used contained rice starch and cane sugar as the carbohydrate components. In each case where the rats failed to show signs of vitamin B deficiency, the rice starch had been replaced by potato starch. Older animals were not "refected" as readily as young rats weighing 40-60 gm. Kon and Watchorn remark that apparently what is of occasional occurrence when the vitamin B-free diet contains rice starch, becomes almost the rule when potato starch is used. Raw arrowroot starch gave similar though less striking "refection" results. The protective action, in the Cambridge experiments, was largely destroyed by gentle cooking of the starch, and less so by extraction with alcohol.

In hundreds of experiments with white albino rats in the laboratories of both the Connecticut Agricultural Experiment Station and Yale University over a period of many years we have failed to note any comparable phenomenon of "spontaneous growth" with production of bulky white feces when the animals were living on diets rich in raw starch and devoid of the plurality of food factors formerly described as vitamin B. The customary ration, which varied from time to time in the proportions of the ingredients according to the purpose of the tests, had a composition essentially as follows: protein 18 to 35%, Osborne and Mendel salt mixture IV<sup>5</sup> 4%, fat 24%, and the remainder corn starch (Duryea brand). Cod liver oil was fed apart from this mixture, or else butter fat was included therein to supply fat-soluble vitamin. Latterly we have added small doses of irradiated ergosterol.

Years ago we observed that rats, maintained a long time on mixtures of isolated foodstuffs, often ate their own feces. Feeding small quantities of rat feces to animals showing nutritive decline sometimes brought about a favorable response in restoration of

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<sup>3</sup> Roscoe, Margaret Honora, *J. Hyg.*, 1927, xxvii, 103.

<sup>4</sup> Kon, S. K., and Watchorn, Elsie, *J. Hyg.*, 1928, xxvii, 321.

<sup>5</sup> Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 572.

weight.<sup>6</sup> After the development of the vitamin hypothesis in the study of nutrition the favorable effects of feces were attributed to the presence of residual vitamins therein, perhaps of bacterial origin. The possibility of such a source is now generally recognized<sup>7</sup> and has led to the use of special cage devices whereby access to the feces is prevented.

The significance of the "refection" phenomena reported abroad for all experiments involving biological examinations for vitamin B in foods or other chemical substances is obvious. We have considered it worth while to make observations on the possibility of "refection" in our laboratory by imitating somewhat closely the dietary conditions reported by Kon and Watchorn, in contrast to our own customary feeding procedures. Two food mixtures were prepared with the same ingredients except that in one (Ration C) the raw starch was a commercial corn starch (Duryea brand), whereas the other (Ration P) contained starch prepared in the laboratory from potatoes. These rations devoid of added sources of vitamin B (*i. e.*, the components in yeast) contained casein 18%, starch 54%, Osborne and Mendel salt mixture<sup>8</sup> 4%, butter fat 9%, lard 15%. Ten male rats weighing 50-60 gm. were placed in individual cages with a fresh supply of water daily. Six of the animals received Ration P. Of these, 4 promptly declined in weight and died within a short time in the characteristic way. Another lived somewhat longer without substantial gain in weight. The sixth rat, B6963, on the potato starch diet grew at a slow rate during 40 days, when a decline ensued. Subsequently the animal maintained its weight for more than 2 months. The feces were noticed after one week to be lighter in color than usual, but not "white". Four rats received Ration C. Of these all declined in weight and developed polyneuritic symptoms or died within the customary period. Two of them, when nearly moribund, were fed feces secured from Rat B6963. These animals survived for some time with slight if any gains in weight.

Dr. Gudjonsson of Professor Fridericia's laboratory observed Rat B6963 and its records during a visit here and expressed the

<sup>6</sup> Detailed bacteriological experiments with some of our animals were reported by Rettger, L. F., and Horton, G. D., *Centralbl. f. Bakteriol., I. Abt., Originale*, 1914, lxxiii, 362.

<sup>7</sup> Steenbock, H., Sell, Mariana T., and Nelson, E. M., *J. Biol. Chem.*, 1923, lv, 399; Dutcher, R. A., and Francis, Emma, *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 189; Bechdel, S. I., Honeywell, Hannah E., Dutcher, R. A., and Knutsen, M. H., *J. Biol. Chem.*, 1928, lxxx, 231; Smith, A. H., Cowgill, G. R., and Croll, Hilda M., *J. Biol. Chem.*, 1925, lxxv, 15.

opinion that there was no evidence of "refection". It has been suggested that potato starch at times may carry traces of vitamin B which would explain the delayed symptoms in one of the animals.

These results are indicative of the failures to observe in our laboratories phenomena comparable to "refection".

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## Effect of Thyroparathyroidectomy on the Action of Irradiated Ergosterol.

A. F. HESS, M. WEINSTOCK AND H. RIVKIN.

*From the Department of Pathology, College of Physicians and Surgeons, Columbia University.*

In a recent publication<sup>1</sup> we suggested that irradiated ergosterol increased the calcium concentration of the blood by acting on the parathyroid glands. This view was based on the following observation: Latent tetany, manifested by calcium concentration of about 6 mg. per 100 cc. of serum, was first brought about in a monkey by means of a low calcium diet. It was then found possible to raise the calcium level to about 11 mg. by means of large amounts of irradiated ergosterol given by mouth. However, after extirpation of the parathyroid glands, the calcium could not be elevated much above 7 mg. per 100 cc. of serum. Slight hypercalcemia was induced by means of injections of parathyroid extract (Collip).

Urechia and Popoviciu<sup>2</sup> have given irradiated ergosterol to dogs in which tetany had been induced by means of parathyroidectomy. They concluded that the tetany was not relieved by this means nor the calcium level raised. They do not state the exact composition of the dietary. Brougher<sup>3</sup> carried out similar experiments. He concluded that irradiated ergosterol "when given in conjunction with milk from the day of operation prevents the development of violent tetany."

During the past year we have carried out numerous extirpation experiments on monkeys and on dogs with the same object in view. In many instances the technic has been modified by giving irradiated ergosterol at the outset in order to establish the fact that hypercalcemia could be induced; levels of 13-16 mg. per 100 cc. of serum

<sup>1</sup> Hess, A. F., Lewis, J. M., and Rivkin, H., *J. Am. Med. Assn.*, 1928, xci, 783.

<sup>2</sup> Urechia, C. I., and Popoviciu, G., *Compt. rendu. de la Soc. Biol.*, 1928, xeviii, 405.

<sup>3</sup> Brougher, J. C., *Am. J. Physiol.*, 1928, lxxxvi, 538.