sodium hydroxide so that the organic matter and a part of the silicic acid go into solution, while the residue now left is soluble in 5% hydrochloric acid and contains calcium, iron and silicic acid. It does not give a test for cholesterol.

The organic fraction of the substance appears to be comparatively small and it cannot be stated to what extent a more complex substance was disintegrated by the electro-dialysis.

Unlike the present substance, colloidal silica is negatively charged and migrates to the anode. The possibility of a gravity drift was guarded against in the present experiments. No distinct particles of the substance recovered in the present experiments could be seen when magnified 1000 diameters, and it can be recovered by electrodialysis through a collodion membrane which does not permit protein to pass. These facts and the general behavior of this substance make it seem most unlikely that it consists of organic matter adsorbed on colloidal silica.

While working with fairly large amounts of raw material, the amounts of substance recovered, especially from blood and urine, have been very small and larger amounts will be required to determine the more exact nature of this substance and its biological significance.

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The Intestinal Flora of Rachitic Rats Before and After Treatment with Ultra-violet Rays.

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Zucker and Matzner[1] showed that when rats develop rickets as the result of being placed on a standard rickets-inducing ration, high in calcium and low in phosphorus, the feces become more alkaline and that on the administration of cod liver oil the reaction veers quickly to the acid side. These observations have been amply substantiated. Grayzel and Miller[2] showed later that the reaction of the intestinal contents of dogs is acid throughout almost the entire

[1] Zucker, T. F., and Matzner, M. J., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 186.

[2] Grayzel, D. M., and Miller, E. G., *J. Biol. Chem.*, 1928, **76**, 423.

length of the gut, but that this reaction tends to become alkaline when rickets is brought about. As in the case of the rat the reaction reverts to normal on the administration of cod liver oil or treatment with ultra-violet irradiation.

Our investigation was carried out on white rats weighing approximately 50 gm., *i. e.*, about 4 weeks of age. Its main purpose was to ascertain whether rickets leads to a change in the flora of the intestinal tract and whether, after the animal is treated with ultra-violet rays and healing of the rachitic lesion is brought about, there was any associated modification of the flora. The rats were fed the rickets-inducing ration of McCollum and some were irradiated with a mercury vapor lamp. Immediately after the animals were killed by trauma they were autopsied and segments of the intestinal tract were tied off; that of the small intestine extending from the duodenum to the lower ileum and that of the large intestine extending from somewhat below the caecum to the beginning of the rectum. The content of each of these segments was squeezed out and the H-ion reaction obtained as soon as possible by a colorimetric method (Clark and Lub indicators). The material was then suspended to a standard density in normal saline solution, gram-stained films prepared and cultures made by methods adequate to determine quantitatively and qualitatively the character of the flora. A technic described by one of us[3] was followed in part. Whether a flora was acid- or alkali-producing was decided on the basis of the known tendencies of predominant types isolated and also of the reaction, after a 3 or 4 days' incubation, of the surface-seeded lactose brom-cresol purple agar plates.

The accompanying table gives the data in regard to 3 bacteriological tests of this kind. The first pair of rats (17529 and 17527) were given a "normal diet", one which was complete in every respect. This diet contains a high percentage of carbohydrate. The whole yellow corn (57%) and the whole dried milk (25%) contain the particular substances, lactose and dextrin, which shift the intestinal flora to a purely fermentative type, more or less dominated by *L. acidophilus*. In surveying the test of these 2 normal animals, one of which was untreated and the other subjected to ultra-violet radiation, it will be noted that there was no distinctive difference between the 2 animals either in the acidity or in the type of intestinal flora, except that *B. coli* was more numerous both in the small and large intestine of the irradiated animal than in the untreated. This

[3] Torrey, J. C., *J. Infect. Dis.*, 1926, **39**, 351.

TABLE I.

Intestinal Flora of Normal, Rachitic and Irradiated Rats.

Rat No.	Diet	Antirachitic irradiation	Radiographs	Source of material cultured	pH	Flora
17529	Normal	None	Normal	Small intestine	6.8	Gm. + predom. *L. acidophilus* ++* *B. coli* + Yeasts + Proteolysis — Acid-producing
				Large intestine	6.9	Gm. — sl. predom. *L. acidoph.* ++++ Yeasts ++ *B. coli* + *Staph. albus* + Proteolysis — Acid-producing
17527	"	Irradiated	"	Small intestine	6.6	Gm. + predom. *L. acidophilus* ++ *B. coli* + Yeasts + g + cocci + sl. Proteolysis — Acid-producing
				Large intestine	7.1	Gm. — sl. predom. *L. acidoph.* +++ *B. coli* ++ Enterococci ++ Proteolysis — Acid-producing
17210	Rickets-inducing	None	Marked rickets	Small intestine	7.2	Gm. + predom. Streptococcus +++ *Staph. albus* ++ *B. coli* + Subtiloid types + *L. acidophilus* + Proteolysis ++ Alkali-producing
				Large intestine	7.2	Gm. + and — about equal Types similar to small intestine *B. coli* ++ *B. proteus* + Proteolysis + Alkali-producing
17213	"	Irradiated	Marked healing	Small intestine	6.5	Gm. + and — about equal *L. acidoph.* +++ *B. coli* ++ *B. proteus* ++ Proteolysis ++ Alkali-producing (?)

* Number of + signs indicates approximately the relative numbers of bacterial types, as shown by culture, for *each* specimen.

TABLE I (Continued)

Rat No.	Diet	Antirachitic irradiation	Radiographs	Source of material cultured	pH	Flora
17213	Rickets inducing	Irradiated	Marked healing	Large intestine	6.8	Gm. — predom. (strong) *B. coli* ++++ Streptococcus + *L. acidophilus* + Proteolysis + Acid-producing
17679	Rickets inducing	None	Marked rickets	Small intestine	6.9	Gm. + predom. Nearly sterile Streptococcus + Yeasts + *L. acidophilus* + Proteolysis —
				Large intestine	7.7	Gm. — predom. Enteroc. and Streptoc. ++++ Coliform alk. +++ Coliform acid + Yeasts + Proteolysis + Alkali-producing
17732	"	Irradiated	Marked healing	Small intestine	6.8	Gm. + predom. Streptoc. and enteroc. +++ *L. acidophilus* + Proteolysis + Sl. acid-producing
				Large intestine	7.0	Gm. — predom. *B. coli*, acid, ++++ Streptococcus + *L. acidophilus* + Proteolysis + Acid-producing

point is worthy of mention because in each pair of rats the same phenomenon was noted, particularly as regards the large intestine.

In the rachitic rats (Nos. 17210, 17213, 17679, 17732) we find a marked distinction between those which had advanced rickets and those which showed marked healing of the epiphyses as the result of ultra-violet rays. First, there is the difference in the reaction of the content of the small and large intestine, particularly of the latter. The McCollum diet stimulated the development of a more complex type of flora than did the normal (Bill's) diet. Not only was there a greater variety of bacteria in both sections of the intestines but in the untreated rachitic rats the alkali-producing tendency of the flora was marked. This was particularly true for the large

intestine. The bacterial types involved seemed to be principally alkali-producing coliform bacilli, *B. proteus* and subtiloid types. Irradiation apparently tended to suppress these types and to encourage the overgrowth of acid-producing *B. coli.* The proteolysis referred to in the table was that caused by spore-bearing anerobes. It will be noted that irradiation apparently had no effect on these organisms.

Irradiation seemed to increase the number of viable bacteria both in the small and large intestine. The exposures in the several tests were of varying intensities, the object being to give a sufficient intensity to bring about marked healing.

A test carried out along the same lines, but one in which calcification at the epiphyses was induced to a lesser degree, furnished information as to whether the change in reaction or in the flora was primary. This experiment may be summarized by the statement that a definite change to the acid side was brought about in the intestinal contents at both levels. In the untreated animal the small intestine showed a pH of 7.2 and the large intestine a pH of 7.7, whereas in the irradiated animal the corresponding values were 6.8 and 6.9. However, in spite of the fact that the contents had been rendered acid as the result of irradiation, there was but slight distinction in the nature of the flora. Enterococci and streptococci predominated strongly within the intestinal canal of the treated as well as of the untreated animal. The sole difference was that the former had rather more *B. coli* in the large intestine. From this experiment and the fact that the flora of the small intestine would seem to be too scanty to play a rôle, we infer that the change in reaction towards acidity, which follows ultra-violet irradiation, is the primary phenomenon and that it is due to an alteration in metabolism and is not the product of bacterial activity. In other words, the acid reaction led to a change of the flora and the flora did not seem to bring about the acid reaction, but may have rendered it more pronounced.

The question arises as to whether similar bacterial alterations occur in connection with the development of infantile rickets and osteomalacia and what effect such changes might have on metabolism, on nutrition and on the intensity of the rachitic process. In infants there is a tendency toward alkalinity of the feces during rickets and likewise a tendency for the reaction to become more acid in the course of the cure of the disorder.

This study will be extended to include other animals and experiments will also be carried out to ascertain the effect on the intestinal

flora of other antirachitic agents, such as cod liver oil and viosterol. It seemed best to begin with a test of the effect of ultra-violet irradiation, as this measure did not necessitate the introduction of any curative substance into the intestinal canal.

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Hemoglobin Regeneration in the Anemic Albino Rat with Dietary Supplements of Spinach, Apricot and Liver.

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Waddell, Elvehjem, Steenbock and Hart[1] showed that anemic rats on a milk diet receiving a supplement of 0.5 mg. iron daily failed to materially increase their hemoglobin levels. Acid extracts of ash residue from liver, lettuce and corn when fed in amounts sufficient to supply 0.5 mg. iron daily, however, induced hemoglobin regeneration. Subsequently, Hart, Steenbock, Waddell and Elvehjem[2] presented evidence that the response elicited by vegetable and meat tissue ash is due to the presence of copper in small but sufficient amounts to act as a supplement to the iron. They also stated that their experiments failed to demonstrate the existence of an organic factor necessary for hemoglobin synthesis. Later work by Elvehjem, Steenbock and Hart[3] failed to substantiate the claim of Drabkin and Miller[4] that glutamic acid may serve to stimulate hemoglobin regeneration in anemic rats on a diet of whole milk with additional iron.

Myers and Beard[5, 6] report hemoglobin regeneration with but 0.25 mg. iron unsupplemented by other metals when fed with a milk diet. Larger doses, and supplemental metals as copper, manganese, arsenic, etc., gave more rapid hemoglobin regeneration.

This paper reports results obtained in experiments on rats with

[1] Waddell, J., Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1928, **77**, 777.

[2] Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

[3] Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197.

[4] Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531.

[5] Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

[6] Myers, V. C., and Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 89.