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The Time Factor in the Production of Gastric Lesions on Low Calcium Diets.

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Gastric lesions develop in rats kept for 4 weeks on a low calcium diet. They occur exclusively in the antrum, the non-acid, mucus-secreting region of the glandular portion of the rat's stomach. The restoration to normal dietary calcium levels prevents the lesions.[1] The microscopic appearance of various gastric lesions together with a discussion of the normal histology of the rat's stomach has been given by Berg.[2] The fundus and rumen show defects, each characteristic for its location, on dietary deficiencies which have not as yet been definitely identified.[3,4]

Most of our experiments were based on a set 28-day period. There have, however, been indications that time is a distinct factor in determining the end results, and we have now extended the duration of experiments. The diet was essentially the same as previously described[1] (0.017% calcium and 0.41% phosphorus). Two groups of 10 and 12 animals respectively, matched as to weight, sex, and litter origin, were started simultaneously. The members of the first group were killed after 4 weeks; the others were left on the diet as long as 8 weeks.

Fig. 1 shows representative photographs of the stretched stomachs preserved in formalin.

As between the two groups, the mean number of lesions per rat did not differ essentially (22 vs. 24). The defects after 4 weeks appeared as well defined single lesions 1-3 mm in diameter. On the longer experiment, they appeared larger (2-5 mm) and many were confluent and irregular in shape. The central craters, also, were larger and more irregular and the degree of hyperplasia appeared greater. At the end of the 8-weeks experiment, many more craters were filled with blood clots than at the end of 4 weeks. In the former, the gastric contents always contained visible blood, whereas in the latter, gross evidence of bleeding was less frequent. In both groups, the lesions remained superficial, *i.e.*, did not penetrate beyond the submucosa. One of the objects of the longer experiment was to ascertain whether or not with

1 Zucker, T. F., and Berg, B. N., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 34.

2 Berg, B. N., *Am. J. Path.*, 1942, **18**, 49.

3 Sharpless, G. R., *Federation Proc.*, 1942, **1**, 192.

4 Schiödt, E., *Acta med. scand.*, 1934, **84**, 456.

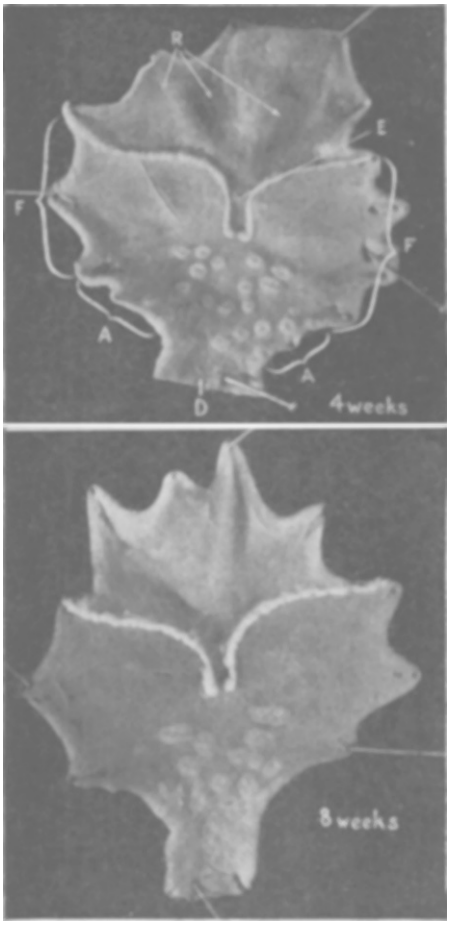

FIG. 1.

Rats' stomachs opened along the greater curvature, stretched out and preserved in formalin. Above the dividing ridge is the rumen (R) and below is the glandular portion consisting of fundus (F) and antrum (A). The lesions are restricted to the antral area. The division between antrum and fundus, while not so obvious in the reproduction, is easily distinguished in the specimen by the greater transparency of the former. Below the pylorus a small segment of duodenum (D) is shown. The esophagus (E) enters at the dividing ridge near the juncture of the three types of epithelial coats.

extended time the lesions would perceptibly approach the penetrating type.

It has been stated that 2 different deficient diets—deficiencies of calcium and B-complex respectively—will in 4 weeks' time lead to epithelial lesions, each characteristic of a definite region of the glandular portion of the stomach. Separate etiologies are indicated. It might of course also be the case that, with any common cause, the rate at which the process goes on may differ for the two types of epithelium found in the antrum and fundus respectively. Since the animals of the longer experiment were in large part exposed to the diet until practically moribund, and no fundus changes were produced, it seems unlikely that calcium deficiency *per se* will ever produce them.

Discussion. In the dog, Martin[5] has shown that prolonged marked calcium deficiency may produce hemorrhage in any part of the body, particularly the gastro-intestinal tract. Eppright and Smith[6] mentioned nasal hemorrhage incidental to calcium deficiency in rats. Boelter and Greenberg,[7] observing rats on low calcium diets, attributed paralysis of the hind legs to hemorrhage into the spinal canal and also record muscle hemorrhage. Light and Frey,[8] on the basis of rabbit experiments, believed that such paralysis is primarily due to fracture of vertebræ. We have seen loss of hind leg function (paralysis?) and muscle hemorrhage occasionally in the shorter experiments and found these symptoms quite pronounced as the 8-week experimental period neared its end. About half the animals were either moribund or dead before the full 56 days had elapsed.

Muscle hemorrhage in the lower extremities occurred with or without fractures in that region. This confirms the existence of a hemorrhagic tendency independent of fracture trauma. The circumscribed hemorrhagic areas in the gastric antrum may be secondary to necrosis. However, the existence of a more generalized hemorrhagic tendency should not be overlooked, nor should primary vascular changes be discounted entirely as initial causative local factors. We agree with Quick[9] that lowered blood calcium under such conditions (about 6 mg per 100 cc) cannot be looked

5 Martin, G. J., *Growth*, 1937, **1**, 175.

6 Eppright, E. S., and Smith, A. H., *J. Nutrition*, 1937, **14**, 21.

7 Boelter, M. D. D., and Greenberg, D. M., *J. Nutrition*, 1941, **21**, 61.

8 Light, R. F., and Frey, C. N., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 256.

9 Quick, A. J., *The Hemorrhagic Diseases . . .*, Thomas, 1942, p. 27 ff.

upon as leading to a lessened coagulability which in analogy with Vitamin K phenomena could be held to be a factor in the hemorrhage described.

Summary. Keeping rats for longer periods of time on low calcium diets accentuates the ulcerative as well as the hyperplastic and hemorrhagic character of the antrum lesions as against the conditions produced in 4 weeks. It also brings out a generalized tendency to bleeding. No perceptible change, however, is produced with regard to penetration, the lesions remaining superficial.

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Tumor-bearing Chicks Hatched from Eggs Inoculated with Mouse Carcinoma.*

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Taylor, McAfee, and Taylor[1] state that chicks may hatch from eggs inoculated into the yolk with mouse mammary carcinoma.

Using a slightly modified Taylor technic,[2,3] fertile eggs were inoculated on the 5th day of incubation with minced, or pressed mammary carcinoma of strain A mice. All materials used came from tumors arising originally in this strain and having a variable number of transfers in the strain. Mouse transfers were done using sterile technic.

Tumor-bearing mice were killed with ether and the skin sterilized with iodine. Separate instruments were used for skin dissection and manipulation of the tumor. Pressed tumors were forced through muslin supported by a drilled, brass plate. Minced tumors were cut with very sharp fine scissors without addition of fluid. In both cases Tyrode solution was then added to give a suspension of about the same density as that used by Taylor.

The shell over the air sac was wetted with 70% alcohol and after drying was cut through with a round dental burr, turned by a high speed electric hand tool, care being used not to cut through the shell membrane. The membrane was wetted with alcohol and allowed to dry. The membrane was then burned through with a hot, tapering probe large enough to fit tightly into the hole in the shell, thus sterilizing the shell against which the needle subsequently touches.

The suspension was inoculated into the yolk using a tuberculin syringe and a No. 20 needle 1¼ inches in length. The amount of suspension injected varied from 0.1 to 0.6 cc.

All procedures were carried out aseptically. The hole in the shell was sealed with a vaseline-paraffin mixture. Eggs were incubated at 38°C.

We have had some early embryo deaths not due to contamination. Whether these are due to mechanical causes or some effect of the inoculum is not clear at this time.

The tumors available to us have not thus far produced as high percentage of "takes" as originally reported by Taylor.[2]

The ultimate embryo mortality is high. The causes will be considered in a separate report.

The embryos which die are small for their age.

The chicks which hatch do so late. They appear to be less vigorous than control chicks. The general tendency has been toward improvement after hatching. Some have appeared quite normal by the third day.

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1 Taylor, D. R., McAfee, M., and Taylor, A., *Cancer Research*, 1943, **3**, 542.

2 Taylor, A., Thacker, J., and Pennington, D., *Science*, 1942, **96**, 342.

3 Taylor, A., Hungate, R. E., and Taylor, D. R., *Cancer Research*, 1943, **3**, 537.