Summary.—1. Depressor and pressor substances arise after vaccinia infection in the blood-serum of rabbits. 2. Aging tends to eliminate the depressor substance and a pressor substance then comes in evidence.

Note.—A serum obtained from a rabbit after streptococcus infection, which had 12 months previously given a profound depressor reaction was also tested (3–18–13) and was found to give no reaction. 8 c.c. of a saline extraction of the adrenals of a 30 day vaccinia rabbit, gave (3–28–13) no reaction. This extraction was made in 20 c.c. of physiological saline solution and was kept 6 days in the ice-box. 7 c.c. of a saline extraction of the adrenals of a two day vaccinia rabbit gave (3–8–13) a fine rise followed by a marked fall. This extraction was made in 20 c.c. and was kept 24 hrs. in the ice-box, *i. e.*, since immediately after removal.

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92 (788)

Preliminary communication on the part played by cholesterol in determining the incidence of carcinoma.

By **T. BRAILSFORD ROBERTSON** and **THEODORE C. BURNETT.**

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

We have elsewhere shown[1] that cholesterol, when injected directly into rat carcinomas, causes a marked acceleration both of the primary and of the metastatic growth of the tumors.

This led us to form the opinion that cholesterol is probably a factor of importance in determining the incidence of carcinoma.

It has been shown by Dorée and Gardner, Ellis and Gardner, and others[2] that cholesterol is not synthesized by animals, the

[1] T. Brailsford Robertson and Theodore C. Burnett, PROC. SOC. EXPER. BIOL. AND MED., 10 (1912), p. 59; *Journal of Exper. Med.*, 17 (1913), p. 344.

[2] C. Dorée and J. A. Gardner, *Proc. Roy. Soc. London*, 80 *B* (1908), p. 227; 81 *B* (1909), G. W. Ellis and J. A. Gardner, *ibid.*, 81 *B* (1909), p. 129.

cholesterol in animal tissues being derived from their diet. This fact suggested the possibility that the incidence of carcinoma in inoculated animals might be diminished by feeding them for a considerable period prior to the inoculation upon a diet poor in cholesterol.

Accordingly twenty-two white rats, about two months old, were divided without exercising any selection into two lots. One lot of 15 were fed upon a diet composed exclusively of milk; the remainder were fed upon a mixed diet of oats and meat.

The content of cholesterol in milk, while by no means negligible, is extremely small in comparision with the content of cholesterol in other foodstuffs. Thus Tolmatscheff finds that human milk contains from 0.025 per cent. to 0.039 per cent. of cholesterol,[1] while Bömer and Kirsten find that the fats in cow's milk contain 0.5 per cent. cholesterol, corresponding to a content of less than 0.02 per cent. in the whole milk.[2] Meat, on the other hand, contains from 0.07 to 0.08 per cent. of cholesterol,[3] while the content of phytosterols in seeds is considerable.[4]

Both lots of animals thrived well, the milk-fed animals presenting an especially well-nourished appearance.

At the end of two months both lots of rats were inoculated in the axillary region with portions of a Flexner-Jobling carcinoma. The diet of each lot of rats was maintained unaltered. At the end of twenty days the proportion of successful inoculations in each batch of animals was determined, with the following results:

Milk diet: 10 out of 15 = 67 per cent.
Mixed diet: 7 out of 7 = 100 per cent.

Another batch of half-grown animals obtained at the same time from the same dealer, which were fed upon a mixed diet and inoculated with Flexner-Jobling carcinoma yielded the following results:

Mixed diet: 55 out of 64 = 86 per cent.

While the milk-fed animals yielded the lowest percentage of successful inoculations it is evident that the difference between

[1] Tolmatscheff, *Hoppe-Seyler's Med.-Chem. Unters.*, 2 (1867), p. 272.

[2] R. W. Raudnitz, *Ergeb. d. Physiol.*, 2, I (1903), p. 264.

[3] C. Dormeyer, *Arch. f. d. ges. Physiol.*, 65 (1896), p. 99; G. W. Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, 81 *B* (1909), p. 507.

[4] F. Czapek, "Biochemie der Pflanzen," Jena, 1905, Vol. I.

the milk-fed and the normal animals in this respect was not so marked as one would be inclined to anticipate if cholesterol were really a prime factor in determining the incidence of carcinoma, and if feeding for two months upon a diet unusually low in cholesterol really brought about any appreciable diminution in the cholesterol-content of the tissues.

That cholesterol is an important factor in determining the incidence of carcinoma can hardly be doubted in view of our previous results, cited above, and of the fact, recently discovered by Wacker, that the cholesterol content of the fatty deposits in the subcutaneous tissues and mesenteries of persons who have carcinoma is no less than 66 per cent. greater than the cholesterol content of the fatty deposits in normal persons.[1] An increase in the cholesterol content of reserve-fats was also observed by Wacker in aged persons and in persons afflicted with tuberculosis or diabetes.

Our failure to observe a more striking difference between the incidence of carcinoma in milk-fed and in normal rats we are inclined to attribute to the fact which has been demonstrated by Ellis and Gardner[2] that cholesterol is strictly conserved in the animal economy, since the cholesterol which is excreted in the bile is reabsorbed in the intestine and does not appear in the feces, while, on the other hand, cholesterol contained in the food is in large part absorbed. Hence removing cholesterol from the diet of an animal does not lead to an appreciable removal of cholesterol from the tissues. More conclusive results might be anticipated if animals were fed from the time of birth upon a cholesterol-free diet, since the tissues of newly-born animals contain relatively little cholesterol.[3] Experiments in this direction are being undertaken.

In conclusion we wish to point out that since the cholesterol in the diet is partly absorbed, while the cholesterol content of the body is strictly conserved, it follows that animals must tend to accumulate cholesterol. We believe that this explains the excessive cholesterol content of the reserve fats in aged persons which

[1] L. Wacker, *Zeit. f. physiol. Chem* , 80 (1912), p. 383.

[2] G. W. Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, 81 *B* (1909), p. 505.

[3] G. W.Ellis and J. A. Gardner, *Proc. Roy. Soc. London*, 84 *B* (1912), p. 461.

has been observed by Wacker, and that this in its turn is the reason for the well-known increase in the incidence of carcinoma with advancing age.

93 (789)

The influence of digitonin upon the growth of carcinoma.

By T. BRAILSFORD ROBERTSON and THEODORE C. BURNETT.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

It has been shown by Windaus[1] that digitonin combines with cholesterol to form a very insoluble and pharmacologically inactive compound. In view of the marked influence of cholesterol in accelerating the growth of carcinoma we have thought it of importance to ascertain the influence of digitonin upon the growth of Flexner-Jobling carcinoma in rats.

The digitonin employed was Merck's, stated to have no physiological action upon the heart. By heating the preparation to boiling in m/6 NaCl solution a soapy-looking fine suspension is formed which settles out in the course of several hours. We injected the digitonin, suspended either in m/6 NaCl, or in m/6 NaCl containing 1 per cent. of lecithin, directly into the tumors.

One hundred and sixty-six white rats were inoculated with Flexner-Jobling carcinoma in the axillary region. The number of successful inoculations, determined after 20 days, was 64, or 39 per cent.

On the 20th day after inoculation these animals were sorted, without selection, into three batches, of which one (consisting of 12 animals) served as controls, another (12 animals) received injections of digitonin, and the third (40 animals) received injections of digitonin together with lecithin.

We began by administering 1 c.c. of a 1 per cent. suspension of digitonin, suspended in m/6 NaCl and in m/6 NaCl + 1 per cent. lecithin respectively. The animals which received digitonin without lecithin evinced symptoms of severe local irritation, and one

[1] A. Windaus, *Ber. d. d. Chem. Ges.*, 42, I (1909), p. 238; *Zeit. f. physiol. Chem.*, 65 (1910), p. 110; M. T. Fraser and J. A. Gardner, *Proc. Roy. Soc. London*, 82 *B* (1910), p. 559.