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.

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The influence of digitonin upon the growth of carcinoma.

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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.

of the animals which received digitonin alone and two of those which received digitonin and lecithin died within a few hours after the treatment. Post-mortems showed that the heart had in each case stopped in extreme systole, the auricles being engorged. In subsequent treatments the dose of digitonin was reduced to 1 c.c. of a 0.5 per cent. suspension, and in the case of the animals which received lecithin, the concentration of the lecithin emulsion was increased to 2 per cent. In the course of these treatments two more of the animals which received digitonin and lecithin died, in each case post-mortem examination showed that the heart had stopped in extreme systole.

Treatments were given on the 20th, 22d, 25th, 27th, and 29th days after the inoculation.

It was found that twenty-four hours after the first treatment the tumors were soft and pulpy and also presented a discolored appearance. The tumors rapidly hardened again, however, and those which were treated with digitonin alone grew much more rapidly than the controls or the tumors which were treated with digitonin and lecithin together. This is shown by the following figures, the diameter of the tumors at the beginning of treatment being taken as 100 in each case.

	Diameter of the tumors.	
	20 days after inoculation.	25 days after inoculation.
Controls	100	114
Digitonin	100	146
Digitonin + lecithin	100	113

It is evident that lecithin antagonizes the action of digitonin in accelerating the growth of carcinoma. This we have confirmed by further experiments which will be reported in a later communication.

Thirty per cent. of the controls, thirty per cent. of the animals which had received digitonin alone, and twenty-five per cent. of the animals which received digitonin together with lecithin had undergone "spontaneous cure" 32 days after inoculation.

In order to ascertain whether or not the above effects were due to digitonin itself or to some impurity contained in Merck's preparation, we prepared pure digitonin by dissolving Merck's digitonin in alcohol, pouring this solution into a large volume of

water, collecting the precipitate, washing it in water and drying over H_2SO_4 at 36° C. The substance which was thus obtained was pure white. It is soluble in alcohol and forms unstable suspensions in water. One c.c. of a 1 per cent. suspension injected subcutaneously in rats produced no symptoms whatever, either local or general. A solution in alcohol precipitated cholesterol from alcoholic solution, and a suspension in water coagulated a suspension of cholesterol in N/100 sodium oleate.

Fourteen rats with carcinoma, which had been inoculated 20 days previously, were divided without selection into two lots, the one lot of 5 served as controls, the other of 9 received 1 c.c. of a 1 per cent. suspension of the above preparation of digitonin in m/6 NaCl, injected directly into the tumors on the 20th, 21st, 22d, 23d, 25th and 26th days after inoculation. As in the previous experiment, the earlier injections caused softening and discoloration of the tumors. The tumors soon hardened, however, and thereafter grew much more rapidly than the tumors in the controls, as the following figures reveal:

	20 days after inoculation	25 days after inoculation.	29 days after inoculation.
Controls	100	110	126
Digitonin	100	147	168

We interpret these results in the following way: When digitonin is injected into the tumor it first of all renders inactive the cholesterol then within the tumor and this results in softening and incipient degeneration of the tumor. The precipitated cholesterol is, however, replaced by cholesterol from the surrounding tissues and body-fluids, and the cholesterol bound by the digitonin is retained within the tumor and gradually liberated from its combination with the digitonin, with the result that cholesterol is concentrated within the tumor, and the growth of the tumor is thereby accelerated. The fact that lecithin prevents the acceleration of the growth of the tumor by digitonin is in harmony with the above interpretation and with our previous results showing that lecithin and cholesterol have opposite actions upon the growth of carcinoma.[1]

[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.