EFFECT OF FATTY ACIDS ON THE RESISTANCE OF MICE TO TRANSPLANTED CANCER.*

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Soon after the discovery of the fact that resistance against transplanted cancer can be produced in animals by preliminary injections of homologous living cells,[1] it was found that neither serum nor plasma has such an immunizing action, nor are killed cells potent in bringing it about.[2] These facts have lead to a general conclusion that "some subtle product of metabolism of the living cell" is essential in producing immunity, and that cell-free substances are ineffective as "antigens."[3] Nevertheless, I have been able to show recently that a state of increased resistance, in every way comparable to that induced by living cells, can be engendered in mice by the injection of a suitable quantity of olive oil.[4] This work had for its starting point the observation that a lymphoid cell reaction follows the injection of fatty oil,[5]—a reaction essentially similar to that which ensues after an injection of homologous living cells.[6,7] Pursuing the investigation, I found it

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[1] Bashford, E. F., *Brit. Med. J.*, 1906, ii, 209. Bashford, E. F., Murray, J. A., and Cramer, W., *Proc. Roy. Soc. London, Series B*, 1907, lxxix, 180. Schöne, G., Münch. med. Woch., 1906, liii, 2517. Ehrlich, P., *Arb. k. Inst. exp. Therap. Frankf. a. M.*, 1906, i, 75.

[2] Bashford, E. F., Murray, J. A., and Cramer, W., Third Scientific Report, Imperial Cancer Research Fund, London, 1908, 333, 367. Lewin, C., *Z. Krebsforsch.*, 1907–08, vi, 308. Haaland, M., *Proc. Roy. Soc. London, Series B*, 1909–10, lxxxii, 293.

[3] For the review of literature see Woglom, W. H., Studies in cancer and allied subjects. The study of experimental cancer, a review, New York, 1913, i.

[4] Nakahara, W., *J. Exp. Med.*, 1922, xxxv, 493.

[5] Bergel, S., *Berl. klin. Woch.*, 1919, lvi, 915.

[6] da Fano, C., *Z. Immunitätsforsch., Orig.*, 1910, v, 1.

[7] Murphy, Jas. B., and Nakahara, W., *J. Exp. Med.*, 1920, xxxi, 1.

possible, by a judicious use of olive oil, to induce a general stimulation of lymphoid tissue such as occurs in mice which have been rendered resistant to cancer inoculation by injections of living cells. Shortly after the appearance of my communication, Bierich[8] reported that an increased resistance to cancer transplantation could be induced by means of an unsaturated fatty acid, the name of which he failed to give.

In experiments to be reported in the present paper, I have attempted to throw a further light on the effect of fatty acids on the resistance to transplanted cancer by testing the influence of the following substances: sodium oleate, sodium palmitate, sodium stearate, oleic acid, linolic acid, and linolenic acid.

Experimental Methods.

The soaps were made up in 1 per cent solutions with distilled water and were administered in the form of a single intraperitoneal injection. Fatty acids were similarly injected in 1 per cent emulsion. In general these latter were emulsified with distilled water to which an appropriate amount of sodium hydroxide was added so as to make the emulsion fairly stable. In the case of oleic acid, the emulsion was sometimes prepared from a sodium oleate solution by adding a sufficient amount of hydrochloric acid. The oleic acid emulsion thus obtained was found not to differ in its action from the one prepared directly from oleic acid.

In testing the resistance to transplanted cancer of mice thus injected, an interval of 10 days was allowed to elapse between the injection and the implantation of cancer (Bashford Adenocarcinoma No. 63.) In each experiment, as control, a suitable number of untreated normal mice of the same breed were implanted at the same time with the same tumor. The implantation was made through a hollow needle, according to the established technique, into the subcutaneous tissue of the left groin, and the rate of growth of tumors was charted weekly thereafter. All the mice used were young adults of about the same size, weighing from 17 to 20 gm. each.

[8] Bierich, R., Leeuwenhoek-Vereeniging, 1922, i, 14.

Immunizing Action of Soaps.

My first task was to compare the action of unsaturated and saturated fatty acids. This I have attempted to do by testing sodium salts of oleic acid (unsaturated), palmitic acid, and stearic acid (saturated).

Sodium Oleate. Experiments 1 to 5.—Sodium oleate (Merck) was injected in 2 to 6 mg. amounts (0.2 to 0.6 cc. of 1 per cent solution). The incidence of resistance to the transplanted cancer exhibited by the treated mice as contrasted with that of the normal controls is shown in Table I.

TABLE I.

Effect of Sodium Oleate on Resistance to Implanted Cancer. Experiments 1 to 5.

Experiment No.	Amount of sodium oleate.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	mg.				
1	2	7	2 (28.5 per cent).	9	1 (11.1 per cent).
2	4	12	5 (41.6 " ").	9	1 (11.1 " ").
3	6	8	5 (62.5 " ").	9	2 (22.2 " ").
4	6	12	4 (33.3 " ").	10	0 (0.0 " ").
5	6	16	9 (56.2 " ").	10	1 (10.0 " ").
Average for 6 mg.....		36	18 (50.0 per cent).	29	3 (10.3 per cent).

The above results demonstrate that injections of sodium oleate in amounts of 4 to 6 mg. each produce a material increase in the resistance of mice to subsequent cancer transplantation. All the mice were in good physical condition, though an amount of sodium oleate larger than that used in these experiments, if given in a single injection, produced ill effects on their general health.

Sodium Palmitate. Experiments 6 to 11.—Sodium palmitate (Merck) was injected into mice in the form of 1 per cent solution in amounts varying from 2 to 12 mg. Cancer implants were made 10 days after the soap injection. The results of six experiments were as follows (Table II):

TABLE II.

Effect of Sodium Palmitate on Resistance to Implanted Cancer. Experiments 6 to 11.

Experiment No.	Amount of sodium palmitate.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	mg.				
6	2	8	0 (0.0 per cent).	9	1 (11.1 per cent).
7	4	10	2 (20.0 " ").	9	1 (11.1 " ").
8	6	10	3 (30.0 " ").	9	2 (22.2 " ").
9	8	10	2 (20.0 " ").	9	2 (22.2 " ").
10	10	9	1 (11.1 " ").	8	2 (25.0 " ").
11	12	10	2 (20.0 " ").	8	2 (25.0 " ").
Average.....		57	10 (17.5 per cent).	52	10 (19.2 per cent).

It is evident that sodium palmitate, unlike sodium oleate, does not exert an immunizing action on mice against transplanted cancer, at least when given in the amounts employed in the above experiments.

Sodium Stearate. Experiments 12 to 17.—Various amounts of sodium stearate (Merck) were injected into mice in the form of 1 per cent solution and the resistance of the animals subsequently tested. Table III gives the results.

TABLE III.

Effect of Sodium Stearate on Resistance to Implanted Cancer. Experiments 12 to 17.

Experiment No.	Amount of sodium stearate.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	mg.				
12	2	10	2 (20.0 per cent).	7	1 (14.2 per cent).
13	4	7	1 (14.2 " ").	7	1 (14.2 " ").
14	6	10	2 (20.0 " ").	11	2 (18.1 " ").
15	8	10	1 (10.0 " ").	11	2 (18.1 " ").
16	10	8	2 (25.0 " ").	9	2 (22.2 " ").
17	12	6	1 (16.6 " ").	9	2 (22.2 " ").
Average.....		51	9 (17.6 per cent).	54	10 (18.5 per cent).

As in the case of sodium palmitate, no perceptible difference can be noted in the incidence of resistance in mice treated with sodium stearate and that of untreated controls.

Immunizing Action of Unsaturated Fatty Acids.

The foregoing experiments show that sodium oleate has a marked immunizing action on mice against transplanted cancer, and that sodium palmitate and sodium stearate are wholly without such effect. These observations, coupled with our previous results with olive oil[4] lead one to question whether oleic acid may not be responsible for the positive findings. In order to test this point and to determine, furthermore, the influence of unsaturation in the reaction, we have tested three unsaturated fatty acids, representatives of three different chemical series; *viz*, oleic acid, linolic acid, and linolenic acid.

Oleic Acid. Experiments 18 to 23.—A 1 per cent emulsion of oleic acid was injected intraperitoneally into mice in doses varying from 0.4 to 0.8 cc. each (0.004 to 0.008 cc. of oleic acid). In Experiments 18, 20, and 23, the emulsion used was prepared from sodium oleate (Merck) in the manner already described. The emulsion used for other experiments was made directly from oleic acid (Eimer and Amend). The cancer implantation was carried out 10 days after the injection of the emulsion. The results are summarized in Table IV.

TABLE IV.

Effect of Oleic Acid on Resistance to Implanted Cancer. Experiments 18 to 23.

Experiment No.	Amount of oleic acid.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	cc.				
18	0.004	9	3 (33.3 per cent).	6	1 (16.6 per cent).
19	0.004	10	8 (80.0 " ").	10	2 (20.0 " ").
20	0.005	10	7 (70.0 " ").	12	2 (16.6 " ").
21	0.006	8	6 (75.0 " ").	6	1 (16.6 " ").
22	0.006	11	5 (45.4 " ").	10	0 (0.0 " ").
23	0.008	13	8 (61.5 " ").	11	4 (36.3 " ").
Average.....		61	37 (60.6 per cent).	55	10 (18.1 per cent).

The results of these experiments provide conclusive evidence that oleic acid is highly potent in increasing the resistance of mice to transplanted cancer. It is noteworthy that this action of oleic acid was obtained with all of the various amounts employed.

Linolic Acid. Experiments 24 to 30.—A 1 per cent linolic acid (Kahlbaum) emulsion was injected into mice in amounts varying from 0.2 to 0.8 cc. each (0.002 to 0.008 cc. of linolic acid). The resistance to transplanted cancer shown by the treated mice as contrasted to that of untreated controls is recorded in Table V.

TABLE V.

Effect of Linolic Acid on Resistance to Implanted Cancer. Experiments 24 to 30.

Experiment No.	Amount of linolic acid.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	cc.				
24	0.002	10	3 (30.0 per cent).	10	4 (40.0 per cent).
25	0.004	10	5 (50.0 per cent).	10	2 (20.0 per cent).
26	0.004	10	5 (50.0 " ").	10	2 (20.0 " ").
27	0.005	10	6 (60.0 " ").	12	2 (16.6 " ").
28	0.006	10	6 (60.0 " ").	10	2 (20.0 " ").
29	0.006	10	5 (50.0 " ").	10	2 (20.0 " ").
Average for 0.004 to 0.006 cc.....		50	27 (54.0 per cent).	52	10 (19.2 per cent).
30	0.008	7	3 (42.8 per cent).	11	3 (27.2 per cent).

It is evident from the above figures that linolic acid is capable of producing increased resistance to cancer inoculation, and that the optimum doses are from 0.004 to 0.006 cc. each.

Linolenic Acid. Experiments 31 to 37.—Linolenic acid (Kahlbaum) was injected into mice in the form of a 1 per cent emulsion in varying amounts. The outcome of cancer implantation 10 days after the linolenic acid injection was as follows (Table VI):

TABLE VI.

Effect of Linolenic Acid on Resistance to Implanted Cancer. Experiments 31 to 37.

Experiment No.	Amount of linolenic acid.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	cc.				
31	0.002	10	5 (50.0 per cent).	10	4 (40.0 per cent).
32	0.004	10	10 (100.0 per cent).	10	4 (40.0 per cent).
33	0.004	10	6 (60.0 " ").	10	2 (20.0 " ").
34	0.005	10	4 (40.0 " ").	12	2 (16.6 " ").
Average for 0.004 to 0.005 cc.....		30	20 (66.6 per cent).	32	8 (25.0 per cent).
35	0.006	10	2 (20.0 per cent).	11	4 (36.3 per cent).
36	0.006	6	1 (16.6 " ").	10	2 (20.0 " ").
37	0.008	9	3 (33.3 " ").	11	4 (36.3 " ").

The above result warrants the conclusion that linolenic acid shares, with other unsaturated fatty acids already tested, the immunizing action against subsequent cancer inoculation. The range of optimum doses here is apparently somewhat less than in the case of linolic acid and it is decidedly less than that of oleic acid.

Influence upon Previously Implanted Cancer Grafts.

With the immunizing action of unsaturated fatty acids against subsequent cancer inoculation established, attention was next turned to the possible effect of these substances on the growth of cancer grafts already implanted.

Sodium Oleate. Experiments 38 to 41.—Mice were implanted with Bashford adenocarcinoma in the usual manner. They were divided into two groups, and the mice in one group were injected with 0.5 cc. of 1 per cent solution of sodium oleate 2 or 48 hours after the implantations. The other group remained untreated and served as control. The results of four similar experiments are summarized in Table VII.

TABLE VII.

Effect of Sodium Oleate on Cancer Grafts Previously Implanted. Experiments 38 to 41.

Experiment No.	Interval between tumor inoculation and the soap injection.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	hrs.				
38	2	15	7 (46.6 per cent).	10	3 (30.0 per cent).
39	2	7	3 (42.8 " ").	10	2 (20.0 " ").
40	48	20	3 (15.0 " ").	10	0 (0.0 " ").
41	48	8	1 (12.5 " ").	8	0 (0.0 " ").
Average.....		50	14 (28.0 per cent).	38	5 (13.1 per cent).

Oleic Acid. Experiments 42 to 44.—Similar experiments were carried out to test the effect of oleic acid, injected in 0.5 cc. of 1 per cent emulsion following the cancer implantation. Table VIII summarizes the result.

TABLE VIII.

Effect of Oleic Acid on Cancer Grafts Previously Implanted. Experiments 42 to 44.

Experiment No.	Interval between tumor inoculation and oleic acid injection.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	hrs.				
42	2	15	6 (40.0 per cent).	10	3 (30.0 per cent).
43	48	7	2 (28.5 " ").	7	1 (14.2 " ").
44	48	9	4 (44.4 " ").	10	2 (20.0 " ").
Average.....		31	12 (38.7 per cent).	27	6 (22.2 per cent).

Linolic Acid. Experiments 45 to 47.—The effect of 0.5 cc. of 1 per cent emulsion of linolic acid was similarly tested in three experiments (Table IX).

TABLE IX.

Effect of Linolic Acid on Cancer Grafts Previously Implanted. Experiments 45 to 47.

Experiment No.	Interval between tumor inoculation and linolic acid injection.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	hrs.				
45	2	10	3 (30.0 per cent).	10	2 (20.0 per cent).
46	48	9	3 (33.3 " ").	7	1 (14.2 " ").
47	48	18	4 (22.2 " ").	10	1 (10.0 " ").
Average.....		37	10 (27.0 per cent).	27	4 (14.8 per cent).

Linolenic Acid. Experiments 48 to 50.—In these experiments the effect of 0.5 cc. of 1 per cent emulsion of linolenic acid was tested as in the preceding experiments (Table X).

TABLE X.

Effect of Linolenic Acid on Cancer Grafts Previously Implanted. Experiments 48 to 50.

Experiment No.	Interval between tumor inoculation and linolenic acid injection.	Treated mice.		Controls.	
		Number.	Resistant mice.	Number.	Resistant mice.
	hrs.				
48	2	10	2 (20.0 per cent).	10	2 (20.0 per cent).
49	48	8	3 (37.5 " ").	7	1 (14.2 " ").
50	48	20	2 (10.0 " ").	10	1 (10.0 " ").
Average.....		38	7 (18.4 per cent).	27	4 (14.8 per cent).

It is evident from the above results that no marked effect can be produced by any of these means on cancer grafts already implanted. There was a slightly lower per cent of tumor development in the treated mice than in the controls, but the difference by itself was too small to be considered significant. However, in treated mice, there were always two or three out of every eight or nine tumors that grew extremely slowly, or almost not at all. Upon microscopical examination these tumors were found to consist of small, discrete masses of

neoplastic cells surrounded by a dense layer of connective tissue with intense lymphocytic infiltration. No such retardation of growth occurred in tumors of the control series, all growing progressively. These findings suggest, rather than prove, that the fatty acids do exert some influence but that this is not adequate to modify markedly the development of a rapidly growing cancer such as the one used in these experiments. It may be recalled that all the hitherto discovered methods of inducing general resistance against subsequent cancer grafting fail when tested against grafts already *in situ*. This is probably because of the great rapidity with which most of the transplantable cancers grow, as also because the artificially induced state of resistance develops only after a latent period of several days.[1,4,9]

DISCUSSION.

The experiments reported in the present paper show conclusively that unsaturated fatty acids such as oleic, linolic, and linolenic acids have a distinct immunizing action against transplanted cancer in mice, and that this action is apparently not shared by saturated fatty acids. Of the unsaturated fatty acids used, oleic acid shows the widest range in the amounts capable of producing the desired effect, while the range with the other acids is more limited. There is no apparent difference in the activity of these unsaturated fatty acids in the optimum amounts. Whether the immunity-producing quality of fats can be estimated on the basis of their iodine values remains to be determined. In an unpublished observation it was noted that cocoanut oil which has an extremely low iodine value produces no resistance to cancer, and at the same time that cod liver oil, in spite of a very high iodine value, is equally impotent in this regard. However, it must be remembered that in dealing with such complex mixtures as these fats, factors other than fatty acids must be taken into consideration in interpreting biological effects.

A problem of broad interest suggested by these results is that of the possible part played by unsaturated fatty acids in cancer immunity induced by means other than injection of them. In addition to the

[9] Woglom, W. H., *J. Exp. Med.*, 1912, xvi, 629. Murphy, Jas. B., Nakahara, W., and Sturm, E., *J. Exp. Med.*, 1921, xxxiii, 423.

older method of living tissue injection,[1] it has been possible to induce immunity by exposing mice to suitable doses of x-radiation[10, 11] or to intense dry heat.[12] It seems not unreasonable to suspect that these varied treatments may be at one in producing disturbances that involve some changes in the unsaturated fatty acid.

The possibility that the cancer immunity induced by unsaturated fatty acids may be consequent on increased activity of the lymphoid tissue deserves consideration. The close relationship existing between fat metabolism and the lymphoid tissue,[13] under both physiological and pathological conditions, and the clearly demonstrated association of lymphoid stimulation with cancer immunity[7, 11,12,14] are in favor of this view. As a matter of fact, we have found that injections of sodium oleate in amounts sufficient to produce immunity bring about a marked increase in the number of karyokinetic figures in the lymphoid tissue, indicating an increased proliferative activity of this tissue.

SUMMARY.

Sodium oleate, oleic acid, linolic acid, and linolenic acid injected into mice in suitable amounts induce a material increase in the resistance against subsequent transplantation of cancer grafts, although they fail to exert so marked an influence on cancer grafts already in place.

Sodium palmitate and sodium stearate, on the other hand, do not produce immunity, at least in the amounts employed in the present study.

[10] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800. Russ, S., Chambers, H., Scott, G. M., and Mottram, J. C., *Lancet*, 1919, i, 692.

[11] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, xxxiii, 429.

[12] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 25.

[13] Schäfer, E. A., *Internat. Monatschr. Anat. u. Histol.*, 1885, ii, 6. Bergel, S., *Ergebn. inn. Med. u. Kinderheilk.*, 1921, xx, 36.

[14] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204. Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1.