

## RESISTANCE TO SPONTANEOUS MOUSE CANCER INDUCED BY INJECTIONS OF OLEIC ACID.

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PLATE 14.

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We have previously shown that resistance to transplanted cancer in mice can be greatly increased by a previous injection of a small quantity of olive oil,<sup>1</sup> or such unsaturated fatty acids as oleic, linolic, and linolenic acids.<sup>2</sup> It is of interest to extend these observations by the study of the action of the fatty acids on the resistance of animals to their own spontaneously developed neoplasms.

In a preliminary experiment, mice with spontaneous cancers were treated with oleic acid without operative intervention. The result, though inconclusive, was sufficiently suggestive of influence upon the growths to lead to more elaborate experiments. In the studies to be presented in this communication, an attempt has been made to determine, first, the effect of oleic acid injections on the frequency of recurrence after operative removal of spontaneous mouse cancer, and second, the effect on the incidence of takes of autoplasmic cancer grafts. Oleic acid was used in these experiments for the reason that the range of doses effective against transplanted cancer is wider than in the other unsaturated fatty acids which have been tested.

### *Material.*

The mice for these experiments were taken from the tumor stock maintained at The Rockefeller Institute, a continuation of the Lathrop strains.

All the tumors included in this study occurred in the mammary

<sup>1</sup> Nakahara, W., *J. Exp. Med.*, 1922, xxxv, 493.

<sup>2</sup> Nakahara, W., *J. Exp. Med.*, 1924, xl, 363.

region of female mice. Histological examination showed about 55 per cent to be adenocarcinomas, and about 40 per cent alveolar carcinomas, both groups including a fair proportion of the cystic and hemorrhagic varieties. There was a single case each of squamous cell carcinoma, lymphoma, and adenocarcinoma sarcomatodes.

The age of mice at the time of operation varied between 10 and 28 months, with the average about  $17\frac{1}{2}$  months. The tumors, in the majority of instances, were of recent origin, but it is difficult and be certain of the duration of the disease.

#### *Prevention of Postoperative Recurrence.*

We have first tested the effect of oleic acid injections on the incidence of return of spontaneous mammary cancers in mice after an attempt at their complete removal by operation.

*Experiment 1.*—50 mice were operated on and their tumors removed as completely as possible. 48 hours later, the mice received an intraperitoneal injection of 0.5 cc. of 1 per cent emulsion of oleic acid. A second injection of the same amount was given 2 weeks later, and repeated thereafter at intervals of 1 to 2 months. The mice were kept under uniform living conditions and were observed as long as they lived. Careful autopsy was performed as soon after their death as possible.

For control, 25 mice were operated on and the tumors excised in the same manner as in the experimental series. These mice were given no injection, but were merely placed under the same living condition as the other group for observation. Autopsy was likewise performed at their death.

Mice that died within 6 weeks after operation were only included in the final consideration of results when they had shown a recurrence. It may be stated that under ordinary circumstances local recurrence usually appears within 6 weeks, if ever.

The technique of the operation was as follows: The animal was first anesthetized with ether, and the skin over and around the tumor was shaved and cleansed with a pledget of cotton soaked with alcohol. An incision was made with sharp scissors and all adherent skin was removed with the tumor. The loose skin was then retracted and the growth was separated from its surrounding tissues by blunt dissection with forceps. Any large blood vessels were ligated. After the removal of the tumor the wound was closed with a silk suture. It healed practically always by first intention.

The oleic acid emulsions were prepared either by the addition of a sufficient amount of hydrochloric acid to a solution of sodium oleate (Merck) or directly from oleic acid with the addition of sodium hydroxide to stabilize the emulsion.

TABLE I.  
*Effect of Oleic Acid on Postoperative Clinical Course.*  
*Experiment 1.*

	Treated.	Controls.
No. of mice.....	50	25
Local recurrence.....	10 (20.0 per cent).	15 (60.0 per cent).
New primary tumors.....	8 (16.0 " " ).	10 (40.0 " " ).
Metastases.....	11 (22.0 " " ).	11 (44.0 " " ).
Total recurrence of disease.....	19 (38.0 " " ).	22 (88.0 " " ).
Complete freedom from tumors.....	31 (62.0 " " ).	3 (12.0 " " ).

The results of this experiment are summarized in Table I, and are graphically represented in Text-fig. 1. The points noted are: local recurrence at the site of operation, development of new primary tumors at other locations, and macroscopic metastases encountered at autopsy.

Some of the internal tumors found at autopsy were of such a histological character that they must be regarded as primary tumors, rather than as metastases, but these have not been tabulated separately. With one exception the metastases were in the lungs.

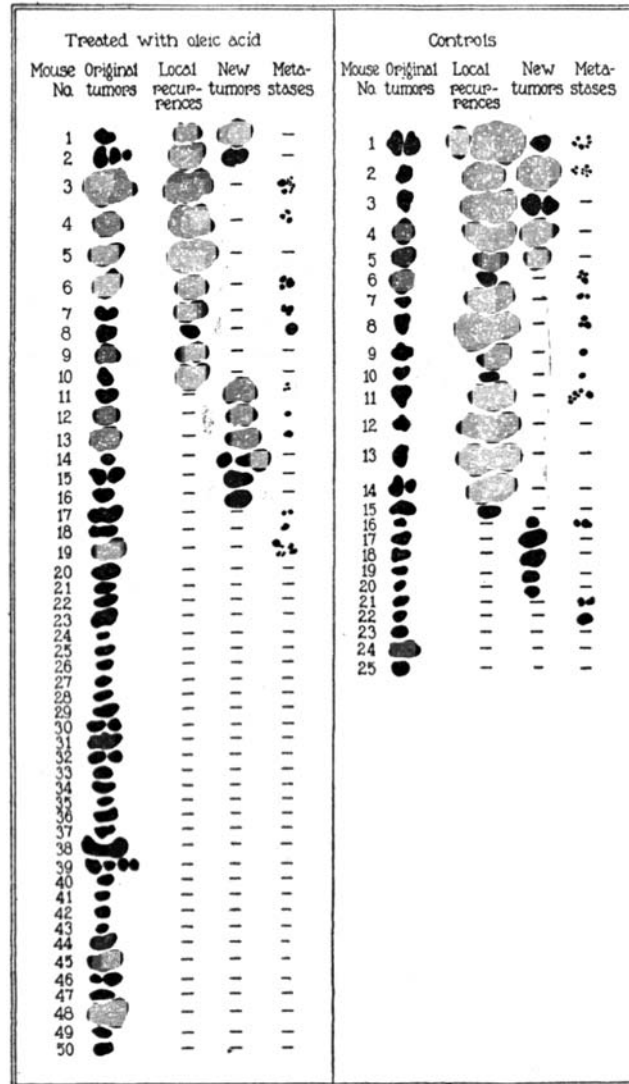
The average postoperative longevity in the treated mice was 147 days, and was 49 days longer than that in the control series (98 days). This difference seems significant in view of the similarity of the average ages of the two series of mice at the time of operation: 581 days for the treated series, and 560 days for the controls. The 49 days of life gained by the treated series of mice amount to nearly one-twelfth of a mouse's total duration of life, which is generally held to be about 2 years.

The postoperative clinical course of spontaneous mammary cancers in mice has been previously studied by Murray<sup>3</sup> and Haaland.<sup>4</sup> Murray observed local recurrences following attempted complete removal in 23 of 48 operated mice, and Haaland in 96 of 174. Murphy and Morton<sup>5</sup> also obtained approximately the same proportion.

<sup>3</sup> Murray, J. A., Third Scientific Report of the Imperial Cancer Research Fund, London, 1908, 69.

<sup>4</sup> Haaland, M., Fourth Scientific Report of the Imperial Cancer Research Fund, London, 1911, 1.

<sup>5</sup> Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.



TEXT-FIG. 1. Relative sizes of tumors in Experiment 1. The blackened masses represent the original tumors at the time of their excision, local recurrences, primary tumors subsequently developed, and the metastases found at autopsy.

The postoperative longevity in Murray's series "averaged 3 to 6 weeks in the later operations and in five cases was more than 100 days." Haaland reported the average to be about 15 weeks (105 days). These figures are in close agreement with our findings in the control series.'

*Suppression of Autoplastic Tumor Grafts.*

In order to confirm and extend the results of the above experiment, we have next tested the influence of oleic acid injections on the growth of autoplastic grafts of spontaneous tumors in mice.

*Experiment 2.*—54 mice with spontaneous mammary carcinomas were freed from their tumors as completely as possible by operation, and grafts of the neoplastic material were at once reimplanted in the subcutaneous tissue of the animals through a hollow needle. 48 hours later, these mice were injected intraperitoneally with 0.5 cc. of 1 per cent emulsion of oleic acid, and subsequent injections were given as in Experiment 1.

For control, 25 mice with similar mammary tumors were operated upon with the apparently complete removal of tumors and reimplantation of autologous tumor grafts as in the above experiment but no oleic acid was given.

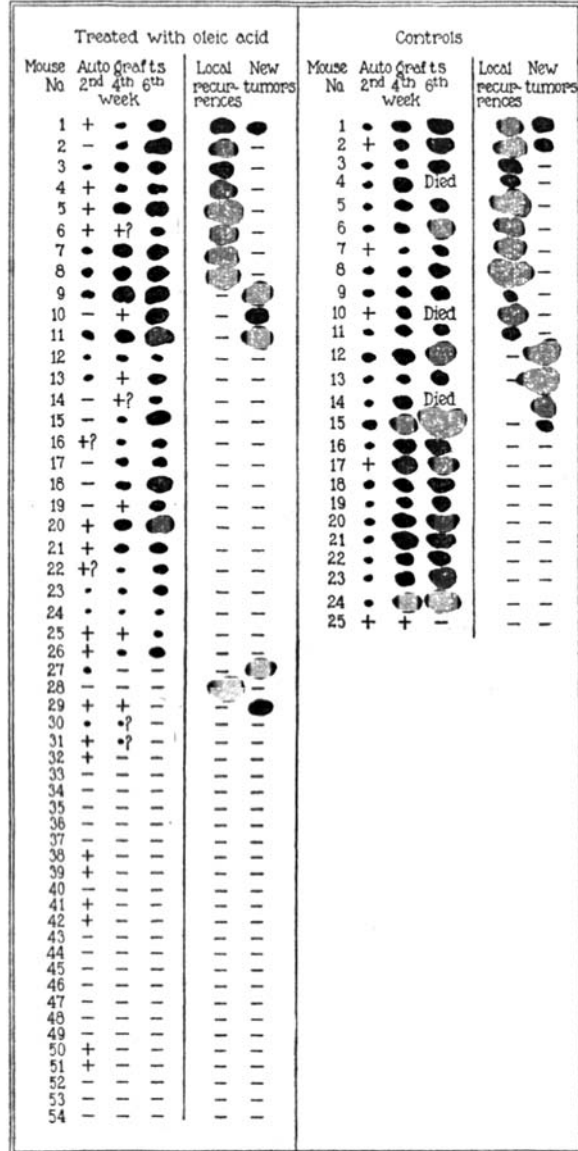
In this experiment no mouse was counted as negative that lived less than 6 weeks after operation. The majority lived much longer than this, and many are still living.

The results of the above experiment were as follows (see also Text-fig. 2):

TABLE II.  
*Effect of Oleic Acid on Autografts.*  
*Experiment 2.*

	Treated.	Controls.
No. of mice.....	54	25
Positive tumor grafts.....	26 (50.0 per cent).	24 (96.0 per cent).
Local recurrence.....	9 (16.6 " " ).	11 (44.0 " " ).
New tumors.....	6 (11.1 " " ).	6 (24.0 " " ).
Freedom from tumors.....	24 (44.4 " " ).	1 ( 4.0 " " ).

The above results are entirely in harmony with those of the preceding experiment, and show that the injections of oleic acid interfere with the growth of spontaneous mammary tumors in mice.



TEXT-FIG. 2. Relative sizes of tumors in Experiment 2, showing the rate of growth of autoplasmic cancer grafts during the 6 weeks immediately after operation, and the local recurrences and new primary tumors existing at the end of this time.

This fact is manifest, not only in the lessened incidence of local recurrence and of new primary tumors, but even more strikingly in the diminished frequency of takes of autoplasmic tumor grafts. It may again be pointed out that the results in the control series are in close agreement with those of previous investigators (Murray,<sup>3</sup> Haaland,<sup>4</sup> Rous,<sup>6</sup> and Murphy and Morton<sup>6</sup>).

*Modifications of Histological Reactions in Autoplasmic Tumor Grafting.*

It has long been established that an autoplasmic tumor graft practically always grows, and that a definite set of histological reactions is characteristically associated with this type of tissue grafting (Leo Loeb<sup>7</sup>). If the outcome of the autologous implantation is actually influenced by oleic acid, it seems probable that this treatment should produce some modifications in the accompanying histological reactions. The following experiment was carried out to determine this point.

*Experiment 3.*—30 mice with spontaneous mammary carcinomas were freed from their tumors by operation, and autologous tumor grafts were implanted in the subcutaneous tissue of the left groin. 15 of these mice were then injected with 0.5 cc. of 1 per cent oleic acid emulsion intraperitoneally, the remaining 15 animals being kept for controls.

3 mice from each group were killed 2, 4, 6, 8, and 10 days later, and the tumor grafts and surrounding tissues were removed and prepared for microscopical examination.

The grafts removed from the treated mice showed characteristic pictures. There was a striking reaction of connective tissue cells (fibroblasts), which not only accumulated around the graft to form a stroma, but also actively invaded the graft itself. This invasion usually commenced at one end of the graft, and grew in, replacing its necrotic parts. In later stages, the tumor tissue was seen to be split up into minute islands and imbedded in a dense mass of connective tissue (Fig. 1). Lymphocytic infiltration also appeared but not to such an extent as in typical immunity reactions to transplanted cancer. The vascular reaction appeared to be somewhat diminished.

<sup>6</sup> Rous, P., *J. Exp. Med.*, 1914, **xx**, 433.

<sup>7</sup> See, for a general discussion, Loeb, L., *Am. Naturalist*, 1920, liv, 45.

In contrast to the above findings, in the grafts taken from untreated mice, the islands of intact tumor cells tended to coalesce, and, by further cell proliferation, to form a more or less continuous layer of living tissue circumscribing the remaining necrotic part of the graft (Fig. 2). There was an adequate stroma formation, and also a marked proliferation of capillaries, but no evidence of connective tissue invasion into the deeper portions of the graft or of lymphocytic infiltration.

#### DISCUSSION.

Past experimentation on the systemic chemotherapy of cancer has been largely directed towards attempts to damage the cancer tissue by some toxic agent. These efforts have failed to yield any very definite results, owing, without doubt, to the difficulty, if not impossibility, of inflicting sufficient injury upon the neoplastic cells, without endangering the general health of the animal. With the exception of a small series of experiments on transplanted cancer by Koenigsfeld and Prausnitz,<sup>8</sup> Brancati,<sup>9</sup> and Lewin,<sup>10</sup> we are not aware of any serious attempt to discover a chemical agent that will produce in the animal body general changes which may militate against the proliferation of malignant cells. A possible starting point in research along this line is suggested by the investigation reported in the present paper.

An impression exists among cancer investigators that an animal cannot be made resistant to its own spontaneously developed tumor. The basis for this contention consists largely of the fact that the homologous tissue injection, so potent in its influence against subsequent implantations of homoplastic cancer grafts is entirely without effect when tested on spontaneously affected mice against their own tumors.<sup>4</sup> However, the so called cancer immunity exhibited against growths of foreign derivation is in all probability based on a process of sensitization to homologous tissue in general and has little to do with the animal's resistance to neoplastic growths as such, spontaneous or transplanted.

<sup>8</sup> Koenigsfeld, H., and Prausnitz, C., *Deutsch. med. Woch.*, 1913, xxxix, 2466.

<sup>9</sup> Brancati, R., *Tumori*, 1922-23, ix, 1.

<sup>10</sup> Lewin, C., *Med. Klin.*, 1922, xviii, 984.



Whether an animal can set up defensive processes against its own neoplasm or not is the more important consideration. If a defensive mechanism to the growth of spontaneous tumors exists, there would appear to be no reason why this mechanism cannot be stimulated by artificial means. As a matter of fact, the possibility has been established by Murphy and his associates working with small doses of x-rays<sup>5</sup> and with dry heat.<sup>11</sup>

Just how oleic acid produces an increased resistance is not at present evident. The general condition of the injected animals was excellent. In special there was no anemia induced. Histological examination of the site of autoplasmic grafting shows, however, that the activity of connective tissue and of lymphocytes is strikingly augmented in the treated mice. We refer especially to the early and active invasion of the autologous cancer graft by connective tissue cells, so frequently observed in the treated mice, and practically never seen in the case of normal autotransplantation. It would seem probable that a partial explanation at least of the effect of oleic acid on cancer growth may be found in these histological manifestations.

#### SUMMARY.

The experiments reported here show that the resistance of mice to spontaneous neoplastic diseases can be increased by means of oleic acid injections. This is demonstrated (1) by the greatly reduced incidence of local recurrences at the site of operation, and the lessened incidence (2) of primary tumors developing at other locations, (3) of metastases as encountered at autopsy, and (4) of takes of autoplasmic tumor grafts, while finally, (5) there is an appreciable increase of the average postoperative longevity.

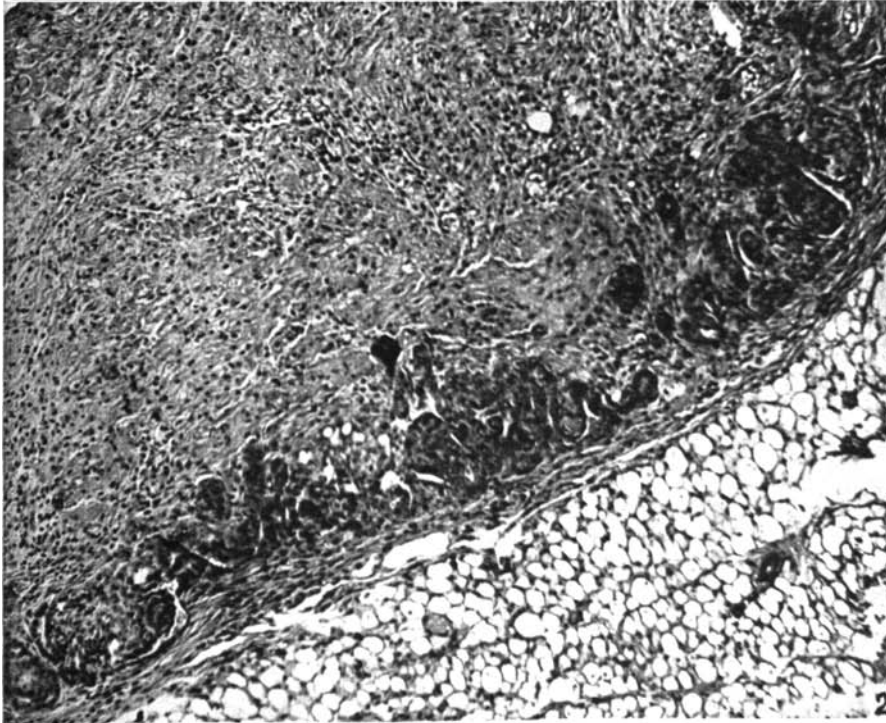
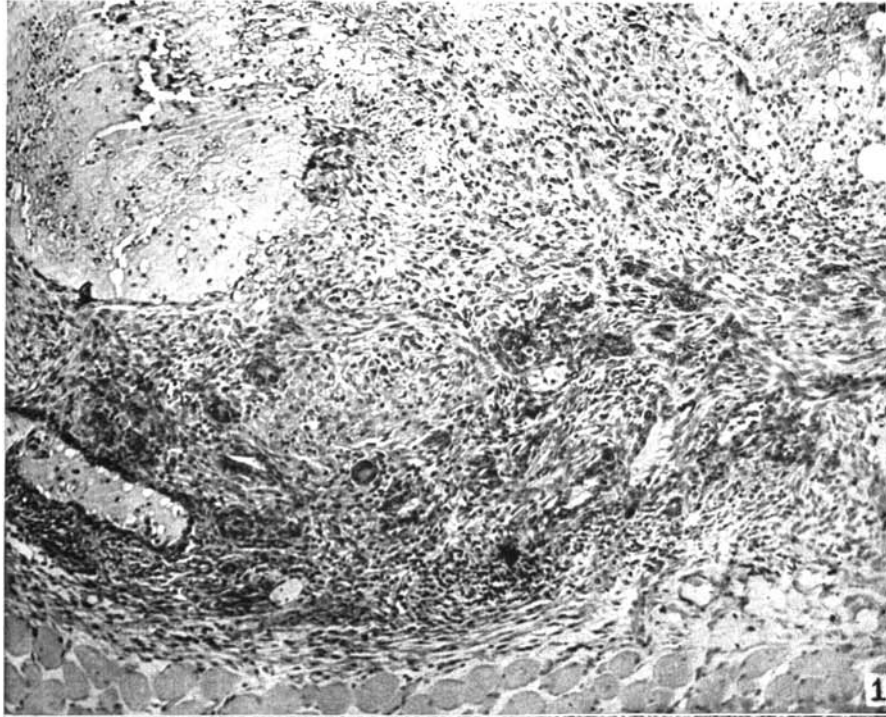
Supplementary to the above results is the finding that in the treated mice the reaction about the implanted cancer graft differs from that encountered in untreated animals.

<sup>11</sup> Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 31.

## EXPLANATION OF PLATE 14.

FIG. 1. 8 day autoplasmic cancer graft in mouse injected with oleic acid. The surviving cancer tissue is broken up into small islands and is imbedded in the midst of reactive connective tissue.

FIG. 2. 8 day autoplasmic cancer graft in an untreated mouse, showing a marginal zone of active growth.



(Nakahara: Resistance to spontaneous mouse cancer.)