Since Rous and Murphy's discovery of an extracellular etiological agent in a chicken sarcoma the search for something similar in neoplasms of the mouse and rat has been eagerly prosecuted.

The ease with which such a principle can be demonstrated in many chicken sarcomas and in the leukemia of fowls, in contrast with the failure to find one in tumors of the mouse and rat and in mouse leukemia, may mean that the etiological agent is more tenaciously held by mammalian than by avian cells, or so labile as to be destroyed by even the most gentle methods of extraction. Or it may mean that new growths of the mouse and rat are not the product of an extracellular agent at all.

Those who incline toward the belief that a majority of neoplasms will some day be proved to have a viral origin insist that the evidence against their view is wholly negative, and hence only partially convincing. Their entire argument need not be reviewed here, since it has been so ably presented in the past few years by Gye and Purdy (1), by Andrewes (2), and by Rous (3), but a few of the most telling points may be rehearsed with profit.

In the first place, the Rous sarcoma is sometimes encountered in a phase when it cannot be transmitted as usual by a cell-free extract, but only by means of intact grafts, and in which it therefore resembles the transplantable neoplasms of the mouse and rat. Secondly, Foulds (4) has described a dibenzanthracene sarcoma of the fowl which could be propagated only by grafts, though in the rabbit its extracts elicited antisera that neutralized active filtrates of the Rous sarcoma. This experiment, thoroughly controlled as it was, supports some analogous observations by Andrewes (5), and the two in conjunction can only mean that non-filterability does not guarantee the absence of an extracellular etiological agent. Thirdly, virus can seldom be obtained from the Shope papilloma when it is growing in domestic rabbits, yet the presence of antibodies in these hosts has been demonstrated by Shope (6) and by Kidd, Beard, and Rous (7). Here, again, the assumption must be that virus exists in the neoplasm even though its extracts do not invariably reproduce the tumor.

Such observations as these justify the continued search for an extracellular etiological agent in other mammalian new growths as fresh methods of investigation present themselves. The following paragraphs will record an attempt to demonstrate a virus through modification of an experiment recently described by Rous and Kidd (8).

These authors found that fulminant carcinosis was induced in tar warts on the ears of rabbits about three weeks after Shope papilloma virus had been injected into a leg vein, a discovery which may provide a test for certain viruses. In any case, the phenomenon is not an isolated one, Lacassagne and Nyka (9)
having duplicated it with benzpyrene tumors while Andrewes, Ahlström, Foulds, and Gye (10) and Andrewes and Ahlström (11) transformed tar granuloma in the rabbit into sarcoma by injecting the Shope fibroma virus.

Mouse sarcoma 37 was selected for the experiments about to be described because it is the one mouse tumor, of all those propagated during the past few decades, which has most often seemed just on the point of declaring a viral origin. Thus it is the growth which Gye (12) at first appeared to have transmitted by means of a cell-free filtrate and with which Sittenfield and Johnson (13) thought they had repeated his success; the growth which Blumenthal and Auler (14) said they had propagated by blood cells soaked in its extract; the growth which Tinozzi (15) asserted that he had transferred, in the proved absence of placental metastases, by the implantation of embryos from mice bearing it; and the growth, finally, in which Cramer (16) found the strongest suggestion of an extracellular agent. After it had been repeatedly frozen at a temperature below — 20° C. the successful inoculations averaged 35 per cent, and even after 4 or 8 exposures to liquid air at a temperature below — 80° C. the transmissibility of this growth was not abolished. Frozen sarcoma which produced tumors in a high proportion of the mice inoculated with it lost this power when washed several times with physiological saline solution, a procedure which is known not to affect the living cell, and the death of all cells was further suggested by a failure to cultivate frozen tumor in vitro.

At first sight the outcome would appear to indicate transmission without the intervention of living cells, an explanation previously considered by Koose and Lemmel (17) on similar evidence, but Cramer was unwilling to accept such an interpretation without more definite proof. If failure to grow in vitro were a certain sign of cell death, it would be necessary to admit that the tumors elicited by frozen and thawed sarcoma were not the product of living cells. On the other hand, he thought it conceivable that sarcoma cells might be so damaged by freezing as not to revive under the highly artificial conditions of culture in vitro, yet come to life when placed amid the more favorable surroundings provided by the living animal.

Those investigators who have more recently discussed the problem are unanimous in the belief that all tumor cells are not invariably killed by freezing. Thus Gye and Purdy (18), after having examined early stages of frozen sarcoma 37, came to the conclusion that a few cells survived, a view shared by Auler and Hohenadel (19), by Rössle (20), and by Hörner (21), and it has been suggested that the refractory elements are the "small cells" of Borst (22), structures consisting of a negligible rim of cytoplasm about a minute nucleus, and thought by him to represent a highly resistant cell analogous to the bacterial spore.

Klinke (23), who exposed various sarcomas or carcinomas of the mouse, rat, or rabbit to liquid nitrogen at a temperature of about — 196° C., was unable to cultivate them in vitro, though all would grow in vivo. The smaller the amount of unfrozen tumor necessary to assure successful inoculation the greater was the resistance to cold, and Klinke expressed the conviction that some tumor cells are able to resist freezing.

The most recent contribution to this question is the paper by Breedis and Furth (24), who found that exposure to — 70° C. did not abolish the trans-
plantability of either mouse sarcoma or mouse carcinoma. They regarded as highly improbable the supposition that the transfer of neoplasms after they have been frozen is due to a virus present within them, for the reason that cells irradiated with 4,000 $\gamma$ while in the frozen state were found to be entirely inactivated, though this amount does not injure viruses at room temperature and would not be likely to do so at $-70^\circ$ C.

**EXPERIMENTAL**

With the idea that a virus in mouse sarcoma 37, if, indeed, it contain one, might disclose its presence by stimulating benzpyrene reactions as the Shope papilloma and fibroma viruses initiate the malignant change in tar warts and tar granulomas respectively, finely mashed tumor was inoculated into mice bearing benzpyrene lesions at various stages from mere thickenings up to frank neoplasms. Intravenous injection, which would have imitated more closely the procedure of Rous and Kidd, was precluded by the possibility that a virus, if present, would be destroyed during preparation of the necessary extract, and freshly ground tumor paste was therefore introduced into the peritoneal cavity.

*Experiment 1:* Into 6 mice with benzpyrene lesions, 0.25 c.c. of sarcoma 37 was injected intraperitoneally, while 6 others with similar lesions were reserved as untreated controls.

*Experiment 2:* Six mice with benzpyrene lesions received 0.50 c.c. of sarcoma 37 intraperitoneally, 5 with similar lesions being set aside as controls.

In the two following experiments the tumor mash was rapidly frozen 6 times with alcohol and solid carbon dioxide at $-70^\circ$ C. and thawed at $37^\circ$ C. between freezings. The freezing required about five minutes, the thawing about ten. Here the purpose was to destroy as many of the tumor cells as possible in order to set free any agent that might not have been released under the conditions of the first two experiments.

*Experiment 3:* Eight mice with benzpyrene lesions were inoculated intraperitoneally with 0.15 c.c. of frozen tumor mash and subcutaneously, on the side opposite these thickenings, with 0.05 c.c. An equal number with similar lesions was kept as untreated controls.

*Experiment 4:* Ten mice with benzpyrene lesions received each 0.05 c.c. of frozen tumor mash subcutaneously on the opposite side, while 9 with similar thickenings were set aside as controls.

When the 30 treated mice in these 4 experiments were compared with their 28 untreated controls it was clearly apparent that in not a single instance was a benzpyrene lesion stimulated by the injection of sarcoma 37, frozen or unfrozen, at different sites, or in various doses, though the animals lived a sufficiently long time for such an effect to declare itself. Enough cells survived the freezing and thawing to elicit abdominal or subcutaneous tumors in some of the animals.

The tests indicate that this neoplasm contains no agent which reacts upon hyperplastic tissues in the mouse as do the Shope papilloma and fibroma viruses in the rabbit.

**SUMMARY**

No extracellular etiological agent could be demonstrated in mouse sarcoma 37 by the method employed.
SARCOMA 37 AND VIRUS PROBLEM

References