AN ATTEMPT TO INDUCE A SPECIFIC IMMUNITY TO TRANSPLANTABLE NEOPLASMS

LUDWIK GROSS, M.D.¹

(From the Research Laboratories of the Pasteur Institute in Paris, Department of Prof. Dr. A. Besredka)

From the numerous experimental studies on acquired immunity to transplantable tumors one unquestionable fact emerges, namely that animals in which such neoplasms have regressed spontaneously are resistant to future inoculations with the same tumor strain (Clowes; Gaylord, Clowes and Baeslack; Sticker; Lewin; Rous and Murphy; Pearce and Brown). The spontaneous regression of tumors implanted subcutaneously or intramuscularly is, however, inconstant and unpredictable. With some tumors, as for example rabbit carcinoma and sarcoma of the dog, it occurs not infrequently; with others, such as mouse and chicken sarcoma, it is exceptional. If it were possible to induce voluntarily a tumor which would regularly undergo regression, the problem of the production of acquired immunity would be materially advanced.

Accordingly an attempt has been made in cooperation with Professor Besredka to produce a regressible tumor by the intracutaneous inoculation of tumor cells in mice, rabbits, and chickens.

EXPERIMENTS ON MICE

The experiments on mice were carried out with the Ehrlich sarcoma, which gives 100 per cent of takes and is invariably fatal. In a series of preliminary experiments mice were given injections of normal liver, spleen, lung, kidney, brain, or mouse embryo cell emulsions, but no immunity resulted and animals receiving subsequent inoculations of sarcomatous material died without exception. Similarly injections of heterologous neoplasms and of heated, inactivated sarcoma tissue or emulsions inactivated by prolonged agitation or the addition of beef bile or antiseptics were without effect. Nor is resistance induced by a growing tumor. Re-inoculation of sarcoma-bearing mice is invariably followed by the development of a tumor independent of the earlier growth.

Several hundred mice were inoculated intradermally, by a 0.5 mm. needle, with small doses—0.02 c.c. of a 10 per cent emulsion—of finely minced sarcoma tissue. In about 10 per cent of those successfully inoculated the skin tumor attained a diameter of 0.5–0.8 cm., then flattened, and after three to five weeks disappeared. In the remaining animals metastases occurred and death ensued.

In an attempt to increase the incidence of regression the technic of inoculation was modified. The freshly shaved skin was merely pricked with a fine

¹The work of the author was aided in 1936–37 by a grant from the Ella Sachs-Plotz Foundation for the Advancement of Scientific Investigation.
needle (0.4 mm.) that had been dipped in a 10 per cent sarcoma cell emulsion, special pains being taken to avoid piercing the skin. Among 80 mice inoculated by this technic, 60 developed typical intracutaneous sarcomas. Of these, 12 regressed spontaneously. The remaining animals died of multiple metastases.

Following disappearance of the intradermal tumor in both series, the animal was without exception immune to reinoculation—intradermal, subcutaneous, intracutaneous, or intraperitoneal. Mice which failed to react to the first intracutaneous inoculation were not naturally resistant nor did they become immune, for a subsequent inoculation was successful.

Immunity was induced only by spontaneous regression of the inoculated tumor. In 39 animals intracutaneous sarcomas were removed by the electrocautery five to fifteen days after inoculation. In 11 of these metastases appeared and in the other 28 reinoculation was successful.

The explanation of the difference in effect between subcutaneous and intracutaneous inoculations is not clear. That the skin offers an unfavorable soil for the development of the sarcoma cells seems unlikely, since intracutaneous inoculation of larger doses—0.03 c.c. of a 30 per cent emulsion—is followed by fatal skin sarcoma with multiple metastases. Nor can regression be attributed to accidental infection, since the tumors simply dry up and disappear. Ulcerated tumors as a rule persist and give rise to metastases.

The induction of regressible tumors in mice by intracutaneous inoculation is particularly difficult because of the narrow margin of difference between an effective and an ineffective dose. If too small an amount is injected no tumor develops and the animal is not immunized. If, on the other hand, too large a dose is given a fatal tumor appears. For this reason, and in view of the results in rabbits and chickens, the immunizing of mice by intracutaneous tumor inoculation is chiefly of theoretical interest.

**Experiments on Rabbits**

Brown and Pearce have shown that intratesticular inoculation of rabbit carcinoma is followed in a high percentage of cases by fatal metastases, but that subcutaneous, intramuscular, or intradermal inoculations of the same neoplasm as a rule produce regressing tumors. Animals recovering following intramuscular, intracutaneous, or subcutaneous inoculation develop an immunity which is sufficient to protect them from subsequent inoculation by the same route or into the testicle.

Besredka, Magat, Besnard and Laval were able, by intracutaneous inoculation of rabbit carcinoma, regularly to induce regressing tumors followed by a lasting immunity. The immunity thus obtained was general, for the immunized animals resisted not only subsequent intracutaneous inoculation but intratesticular and intraocular inoculations as well.

Further experiments have been conducted in collaboration with Besredka to determine whether an equally favorable result may be obtained by other routes of inoculation. Two hundred and thirty-six rabbits were used in these experiments: 68 were inoculated in the testicle, and of these 58 died of metastases and 12, or 18 per cent, recovered; 68 were inoculated subcutaneously,
and of these 25 died of metastases and 43, or 63 per cent, recovered. One hundred were inoculated intracutaneously and of this group only 6 died while 94 showed spontaneous regression of the tumor.

In all the surviving animals a lasting general immunity developed, from which it appears that spontaneous regression of a carcinoma, situated in any tissue, is followed by the development of immunity. The surest method, however, of producing immunity in rabbits, or in other words of producing regressing growths, is obviously by intracutaneous inoculation.

Since neither normal spleen nor brain emulsion, whether injected intracutaneously or subcutaneously, protected against subsequent intratesticular inoculation of rabbit carcinoma, the immunity induced by the spontaneous regression of the neoplasm may be characterized not only as lasting and general but also as specific. Further studies carried out by Besredka and the writer showed that passive immunity cannot be conferred upon normal rabbits either by the serum or organ-cell emulsions of immunized animals, or by parabiosis.

Experiments in Chickens

Chickens receiving 0.2 c.c. of a 10 to 20 per cent sarcoma cell emulsion developed tumors and died whether the inoculation was made subcutaneously or intracutaneously. If, however, a very small dose was given—0.01 c.c. of a 0.2 to 3.0 per cent cell emulsion—a difference was observed between the two routes of inoculation. Either may fail, but of the two, intracutaneous inoculation is followed by a higher percentage of takes. In no instance, however, was there any evidence of natural resistance, for reinoculations after a negative result were always successful.

Regardless of the size of the dose, tumors developing after subcutaneous inoculation were fatal. In animals inoculated intracutaneously with very small doses, however, regression of the tumor was sometimes observed after three to five weeks. In one experiment involving 7 chickens in which intracutaneous tumors appeared, 2 recovered. In another group of 3 regression was observed in 2 within three to four weeks. All the chickens in which the inoculated tumors regressed were thereafter resistant to reinoculation even of large doses, by any of the usual routes.

Summary

Neoplasms induced in mice, rabbits, and chickens by intracutaneous inoculation of emulsions of the Ehrlich sarcoma, Brown-Pearce rabbit carcinoma, and Rous sarcoma respectively, sometimes regressed spontaneously and the animals thereafter displayed a specific generalized immunity to inoculation with those tumors, which, however, was not transferable by blood serum or organ emulsions to other animals. Regression following subcutaneous inoculation of similar doses was unusual.

2 Fuller details pertaining to the experiments on chickens will be published shortly in the Ann. de l'Inst. Pasteur.

3 In some instances, instead of injecting the sarcoma cell emulsion we merely pricked the skin of the chicken with a very fine needle (0.4 mm.) which had previously been dipped in a 3 per cent to 10 per cent sarcoma cell emulsion, being careful not to pierce the skin.
NOTE: I wish to acknowledge a debt of gratitude to Miss Hortense C. Lee for her kindly assistance in the English translation of this paper.

REFERENCES