THE PRODUCTION OF TRANSPLANTABLE CARCINOMA AND SARCOMA IN GUINEA-PIGS BY INJECTIONS OF THOROTRAST

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Roussy, Oberling and Guérin (13, 14) produced sarcomata in rats by injecting the preparation of thorium dioxide used in radiography under the name of thorotrast. Selbie (15, 16) confirmed their observations and found that thorotrast had a similar but slower carcinogenic action in mice. In the present experiments thorotrast was injected into the mammae of guinea-pigs and, after about three years, produced a carcinoma and three sarcomata, of which the carcinoma and two sarcomata were transplanted.

EXPERIMENTAL STUDIES

Four doses of 0.2-0.3 c.c. of undiluted thorotrast were injected into the base of the most caudal nipple on the right side in each of 20 young female guinea-pigs. The injections were made on June 28, July 31, Aug. 27, and Oct. 16, 1935. The first growth was noticed on Feb. 20, 1938, but it was ulcerated and evidently had been overlooked during preceding weeks. At this time 8 other guinea-pigs were alive; 3 subsequently developed tumours, while 2 died at the end of 160 weeks without tumours, and there are none in the remaining 3 animals which are now (Dec. 21, 1938) alive.

Thorotrast Tumour No. 1 (T. 1)

(Transplantable Carcinoma)

The tumour was removed, as completely as possible, on Feb. 22, 1938, 138 weeks after the first injection of thorotrast. It was ulcerated, had destroyed the nipple, and had invaded the deeper tissues of the groin. Auto- and homo-transplantations were attempted. Six weeks later the autoplasts were large; fragments were removed for transplantation but complete excision was impracticable. The guinea-pig died five days later, April 10, 1938, having large ulcerated autoplasts, recurrence at the site of the primary tumour, and metastatic deposits in liver, spleen, and lungs.

Tumours grew in 2 out of 3 guinea-pigs inoculated with 0.05 c.c. of minced tissue removed at the first operation, but not in 3 others in which small fragments were implanted by means of a transplanting needle. In all subsequent transplantations of this and of the later thorotrast tumours, 0.05 c.c. of minced tumour was inoculated subcutaneously in the flank by means of a transplanting syringe. The tumour is now in the 15th transplanted generation. In the first ten generations 47 tumours resulted from 66 inoculations (approximately 73 per cent); one tumour disappeared, following ulceration, but there were no
other regressions. Tumour was disseminated in the abdominal cavity in five guinea-pigs, but there were no metastatic deposits in the organs. Transplanted tumours grew rapidly and required transference after about three weeks, when they usually measured $7 \times 2.5 \times 2.5$ cm. They were soft, white, and homogeneous during the first two or three weeks, but thereafter they were prone to ulceration and extensive central haemorrhage and necrosis.

The primary tumour was composed of polyhedral cells arranged in alveoli which were limited by thin-walled blood vessels and sparse fibrous tissue (Fig. 1). Mitotic figures were conspicuous by reason of their abundance and their abnormalities (Figs. 1 and 2); there were tripolar and tetrapolar divisions and cells in which the chromosomes were in complete disorder owing to the absence of a spindle. There was a corresponding diversity among the
resting nuclei. Commonly these were large and spherical, with a large nucleolus, but there were small cells less than the average and, at the other extreme, giant cells which were multinucleated or had a single giant nucleus, usually irregular in shape. Thorotrast was present in the tumour, especially at its periphery, but also in the central portions, where it was within macrophages.

The structure of the transplanted tumours (Figs. 3 and 4) closely resembled that of the original tumour. In some tumours the alveolar arrangement was especially distinct, while in others the cells were less orderly, arranged in large masses. Mitotic figures were numerous and often irregular.
No trace of differentiation into acini or ducts or into squamous epithelium was found in the primary or transplanted tumours. It is concluded that T. 1 is an alveolar carcinoma which originated from the mammary gland.

*Thorotrast Tumour No. 2 (T. 2)*

*(Transplantable Sarcoma)*

An ulcerated growth, $3.5 \times 2.5 \times 1.5$ cm., at the site of injection of thorotrast, was removed by operation on May 21, 1938, 151 weeks after the preparation was first administered. Autoplasts and implants in two normal
guinea-pigs did not grow. The animal died fourteen weeks later, Aug. 29, 1938, having two small nodules at the operation site and growths in the liver and lungs. Tumours grew in each of 4 guinea-pigs inoculated with tissue from the subcutaneous tumour and in 1 of the 4 inoculated with the internal growths. The subcutaneous transplanted tumours were small, but there was extensive intra-abdominal growth, especially in the omentum and mesentery; in one animal there was a solitary tumour in the liver and in the left lung. One of the tumours was again transplanted, after 58 days, into 4 guinea-pigs. Three tumours developed and one was recently used, when 49 days old, for the third passage.

FIG. 7. SARCOMA T. 2: TRANSPLANTED TUMOUR OF FIRST GENERATION. X 225

The primary growth (Figs. 5 and 6) was a sarcoma with considerable pleomorphism. Most of the cells had abundant cytoplasm and were rounded, polygonal, or fusiform, with little collagen between them. There were many multinucleated cells and numerous mitotic figures, many of them atypical. In some areas the cells were thinner and elongated and collagen was more plentiful. Thorotrast was not seen in the two portions of the tumour which were sectioned. The recurrent tumour and the two transplanted tumours which have been examined histologically were almost entirely composed of thin spindle cells in bundles (Fig. 7). There was a moderate amount of collagen uniformly distributed throughout the growths. Mitotic figures were frequent. Pleomorphism, comparable with that in the original growth, was seen only in the secondary deposits present in the liver and lung of one animal bearing a transplanted tumour. Its occurrence, though rare, discounts the possibility that the "recurrent" tumour was an independent new growth.
Thorotrast Tumour No. 3 (T. 3)
(Transplantable Sarcoma)

A small swelling was felt at the site of injection of thorotrast several weeks before progressive growth was apparent. One hundred and sixty-two weeks after the first injection, when the tumour measured $2.5 \times 1.5 \times 1.5$ cm., it was removed by operation, Aug. 5, 1938. The animal died three weeks later, but no autopsy was done. Tissue removed at operation was implanted in 6 guinea-pigs and 4 developed tumours. Transplantation of one of these after 33 days was successful in each of 4 guinea-pigs. The first attempt to make a third passage was unsuccessful, but a second transplantation has resulted, after 27 days, in the growth of 4 tumours in 6 guinea-pigs.
One animal of the first passage, dead on the 84th day, had an irregular mass, $11 \times 4 \times 4$ cm., in the omentum and another mass, $9 \times 3 \times 3$ cm., in the mesentery. Another animal of this series, killed after 33 days, had smaller growths in the omentum, while an animal of the second passage, which died after 45 days, had a small subcutaneous tumour, other tumours on the dia-
phragm, and a large mass, $6 \times 3 \times 3$ cm., in the omentum. The intra-ab-
dominal spread had precisely the same characteristics in T. 3 as in T. 1 and T. 2, while similar dissemination of a Daels and Biltris sarcoma was figured by Watson (19). This type of extension seemingly occurs when a tumour penetrates the abdominal wall, usually as a result of deep inoculation, and is not a characteristic of any particular type of neoplasm.

The primary tumour (Figs. 8 and 9) was a sarcoma composed principally of spindle cells closely packed together with their long axes parallel; collagen was scanty. There was considerable variation in the size of the cells. Mitotic figures were numerous and often atypical, but they were less conspicuous than in T. 1. Thorotrast, within macrophages, was scattered irregularly through the tumour, and there were large accumulations at its periphery. The structure of the transplanted tumours (Fig. 10) was similar, though in some the cells were shorter and less regularly arranged, while in others small cells or multinucleated cells were more numerous. Collagen was scanty in all of the tumours.

**Thorotrast Tumour No. 4 (T. 4)**

*(Fibrosarcoma)*

A small swelling was felt at the site of injection of thorotrast about 164 weeks afterwards; its growth was scarcely perceptible from week to week until
about the 172nd week. Having reached a size of $2 \times 2 \times 1.5$ cm., it was re-
moved by operation on Nov. 11, 1938. Two autoplasts were inserted and 3 
normal guinea-pigs were inoculated, but none of the implants, including the 
autoplasts, has grown after 40 days.

The tumour was a fibrosarcoma with abundant collagen (Fig. 11). Mitotic 
figures were present but were not numerous. Thorotrast, in variable amount, 
was scattered through the tumour and there were large accumulations at the 
periphery. There was a calcified nodule, 3 mm. in diameter, in the midst of 
an accumulation of thorotrast at the edge of the tumour, and single calcified 
cells were present among the cells containing thorotrast in other parts of the 
tumour. Calcification was not observed in the deposits of thorotrast in or 
around the other primary tumours.

![Fig. 11. Fibrosarcoma T. 4: Primary Tumour. × 225](image)

**DISCUSSION**

There are few records of the experimental production of tumours in guinea-
pigs. Neither tar nor a carcinogenic hydrocarbon has produced cancer of the 
skin. Many of the experiments were certainly terminated too soon, though 
Leitch (10) applied tar for two years without result. The skin of guinea-pigs 
is at least as resistant as the skin of rats, but other tissues are susceptible. 
Daels and Biltris (4) introduced radium by various methods into several posi-
tions and obtained two sarcomata of the meninges, two sarcomata of the kid-
neys, and three bile-duct carcinomata, one of which was accompanied by a 
glandular carcinoma of the stomach. The incidence of tumours was low 
(about 3 per cent), but many animals died early and the technique was not 
standardized. Biltris (1), who used the most satisfactory of the methods 
previously tested, recorded that in one experiment where radium was implanted 
in the liver, 4 out of 13 guinea-pigs which survived for more than a year 
developed bile-duct carcinomata; metastatic deposits of carcinoma were pres-
ent in the lungs of one of these guinea-pigs. In other experiments Biltris obtained two bile-duct carcinomata, three spindle-cell sarcomata, and two malignant tumours of the spleen. One animal having a tumour of the spleen died after eight months; with this exception the tumour-bearing animals in Daels and Biltris' experiments survived from fifteen to thirty-three months, on the average about twenty-four months.

There are two reports of the production of sarcoma in guinea-pigs by 3:4-benzpyrene. Haagensen and Krehbiel (7) made repeated subcutaneous injections of a solution of 3:4-benzpyrene in lard, and found sarcomata in 5 guinea-pigs out of 13 which survived for 342 days, the average period required for the development of a tumour. Shear (18) injected crystalline 3:4-benzpyrene subcutaneously into 23 guinea-pigs. One tumour was detected in the fourteenth month and a second in the twentieth month, while 19 animals were alive at the time of the report.

It is not certain that the full yield of thorotrast tumours has been obtained in the present experiments. So far, tumours have developed in 4 out of an "effective total" (Fieser, 6) of 9 guinea-pigs which were alive when the first tumour was observed. Fieser recommends that the rate of tumour production should be expressed as the "average induction time" but, for reasons mentioned by him, its accurate determination for the thorotrast tumours is not possible; an approximate estimate is thirty-seven months, or three times the average recorded by Haagensen and Krehbiel for 3:4-benzpyrene. The average induction time was not recorded by Daels and Biltris, but it was evidently greater than in Haagensen and Krehbiel's experiment, though the earliest tumours in the two series probably developed after similar intervals. It seems that thorotrast, in the dosage used, needs two or three times as long to produce tumours as 3:4-benzpyrene or the radioactive preparations used by Daels and Biltris. Judging from the observations on animals, sufficient time for thorotrast to produce tumours in man has not yet elapsed since its introduction as a radiological aid.

The slow-growing liposarcoma described by Murray (12) was, until recently, the only transplantable tumour of the guinea-pig available in Great Britain. Jones (8) transplanted a spontaneous mammary carcinoma through eight generations and Lubarsch (11) transplanted two sarcomata; apparently these strains were not continued. Watson (19) described a transplantable sarcoma, obtained from Daels and Biltris, which is still propagated in this laboratory. The thorotrast carcinoma is, I believe, the second transplantable carcinoma of the guinea-pig on record and the only one now in existence. The success attending transplantation of the thorotrast tumours controverts the opinion, formerly held, that tumours of guinea-pigs are rarely transplantable. Possibly induced tumours are more easily transplanted than spontaneous ones, but it is difficult to decide what proportion of spontaneous tumours was adequately tested; some failures might be attributable to unsuitable methods, for it seems that the "needle" method, commonly used for mouse tumours, often fails when used for the tumours of larger animals. The guinea-pigs used for my transplantations were bred in the laboratory; considerable in-breeding no doubt occurred, but no attempt was made to breed pure strains.

The well known carcinogenic hydrocarbons produce various types of sar-
coma, but the carcinomata, including those of internal organs, are always squamous. Dobrovolskaïa-Zavadskaià and Adamova (5) conclude that there is a fundamental difference between squamous carcinoma and sarcoma on the one hand, and glandular carcinoma on the other hand; in the former an exogenous agent is decisive while in the latter the constitutional factor is dominant. They assert that carcinogenic agents cannot produce glandular cancer in animals which are not predisposed to it.

The experimental production of tumours in guinea-pigs is of especial interest on account of the rarity of spontaneous tumours, there being only 21 on record (9). It is safe to conclude that the tissue from which thorotrast produced the T. I carcinoma was not predisposed to neoplastic disease. The carcinoma developed at the exact site of the thorotrast injection, contained thorotrast, and appeared in the same order of time as the other thorotrast tumours; it was, beyond reasonable doubt, the direct result of the action of thorotrast. Daels and Biltris described a glandular carcinoma of the stomach in a guinea-pig implanted with radium, but the nature and origin of the growth were uncertain. Their bile-duct carcinomata were undoubtedly produced by the action of radium and were, moreover, malignant growths, for one had metastasized. Boyland and Brues (2) found that a hydrocarbon, 3:4:5:6-di-benzcarbazole, produced squamous carcinoma of the skin or sarcoma of the subcutaneous tissues in mice, according to the mode of administration and, furthermore, produced hepatoma. Cook and Kennaway (3) state that the same substance produced, in two rats, lesions indistinguishable microscopically from mammary carcinoma. Other substances, notably o-amidoazotoluol produce hepatoma, but no tumours at the site of application (17). Thus, some carcinogenic agents, including radioactive substances, can produce more than one type of carcinoma as well as sarcoma. The failure of the well known carcinogenic agents to induce glandular cancers does not demonstrate that an exogenous stimulus is ordinarily unessential; probably glandular cancer fails to develop because the stimulus is inappropriate in kind.

**Summary**

Four injections of 0.2–0.3 c.c. of thorotrast, made into the base of a nipple, produced tumours in 4 out of 9 guinea-pigs which survived until the earliest growth was detected. The average induction time was about thirty-seven months. The tumours comprised one carcinoma, two sarcomata, and a fibrosarcoma; the first three were transplantable and the carcinoma has been transmitted through 15 generations. The experiment demonstrated the production of a carcinoma, other than a squamous carcinoma, by the local action of a carcinogenic agent.

**References**