CONDITIONS DETERMINING THE TRANSPLANTABILITY OF TISSUES IN THE BRAIN.

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Plates 13 to 16.

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The study of the transplantability of animal tumors is largely responsible for the view that it is impossible among warm blooded animals to graft tissues from one species to another, and that even grafting within a species has definite limitations. Leo Loeb,[1] among others, has brought forward evidence indicating that the conditions governing the transplantability of tumors are applicable essentially to normal tissues, and Rous[2] has shown that the methods of inducing resistance to tumor transplantation apply also to normal tissue grafts. In his well known zig-zag experiments, Ehrlich[3] showed that the limit of growth of a tumor graft in a foreign species (rat tumors in mice, or *vice versa*) is from 7 to 9 days, but in no instance has such a graft been successfully implanted into a second individual of the foreign species without being first returned to the original or homologous species. One of us demonstrated that this restriction of growth does not apply to the embryo, since rat tissues are capable of growing over long periods and through successive transplantation in chicken embryos.[4]

The explanation of the strict specificity of tissue transplantability has been sought in various ways and led to not a few hypothetical views. A certain number of indubitable facts have been discovered, but whether or not they are to be regarded as the essential or primary

[1] Loeb, Leo, *Proc. Am. Phil. Soc.*, 1908, xlvii, 3; *J. Cancer Research*, 1917, ii, 135; *J. Med. Research*, 1920–21, xlii, 137.

[2] Rous, P., *J. Exp. Med.*, 1910, xii, 344.

[3] Ehrlich, P., *Arb. k. Inst. exp. Therap. Frankf.*, 1906, No. 1, 77.

[4] Murphy, Jas. B., *J. Exp. Med.*, 1913, xvii, 482.

cause of the phenomenon or merely the secondary or concomitant and attendant results has not yet been determined.

Thus, it has been noted that a short time after the implantation of a heteroplastic graft, the latter becomes surrounded by a zone of cells in which the lymphoid variety of cell is dominant; the intensity of the cellular reaction is roughly proportionate to the degree of relationship existing between the two species concerned in the experiment.

On the other hand, it has been found that with the rat tumor graft in chicken embryos, referred to above, the cellular reaction is much delayed and does not become evident until the 19th day of incubation and coincides with the appearance of degenerative changes in the graft, which now suffers rapid and ultimately complete absorption.[5] It is significant that the cellular reaction about, and subsequent disintegration of the graft are hastened by the implantation into the embryo of a fragment of spleen from an adult chicken.[6]

These findings indicate unmistakably that the lymphoid cell reactions are associated with the resistance mechanism, and the importance of this association is emphasized by the fact that adult animals deprived of their lymphoid tissue by repeated exposures to suitable small doses of x-rays, like the embryo, fail to destroy foreign tissue grafts, which not only continue to grow actively, but may be transferred repeatedly to other individuals which have been prepared for their reception by the x-ray treatment.[7] One would expect that an animal with its resistance so reduced that even inoculated foreign tissue survives and grows actively would prove to be non-resistant to transplants from homologous spontaneous tumors, but this is not the case. Grafts from a spontaneous tumor undoubtedly survived for a longer period in x-rayed than in untreated animals, but we did not succeed in transplanting a tumor, under these conditions, which failed to grow in untreated or normal animals. One instance has been reported of transplantation of a rat tumor in x-rayed animals which failed in untreated rats,[8] but this method falls far short of what might be expected.

[5] Murphy, Jas. B., *J. Exp. Med.*, 1914, xix, 181.
[6] Murphy, Jas. B., *J. Exp. Med.*, 1914, xix, 513.
[7] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459.
[8] Chambers, H., Scott, G., and Russ, S., *J. Path. and Bact.*, 1920, xxiii, 384.

The site of tissue transplanation has received but little attention, and as a rule the experimental studies have been made on grafts in the subcutaneous tissue or muscle. New points for consideration have been introduced by recent experiments reported by Shirai,[9] who has employed the brain as the locus of heteroplastic tumor transplants, in which situation, according to him, grafted tissue grows as readily in an alien as in an homologous host. The important bearing of this observation on the question of the resistance mechanism has led us to undertake an investigation of the subject.

Heterologous Tumor Grafts in the Brain.

Since Shirai has not described adequately the procedure he employed, we had first to work out a practical method.

Method.—After etherization of the animal, the head is shaved and a midline incision made, enabling the skin and fascia to be retracted. A small hole of sufficient size to admit a No. 18 gauge trocar is made in the skull. The trocar is provided with a shoulder of metal about 3 mm. from the point, in order to limit the depth of penetration. The point is beveled slightly so as not to cause unnecessary damage to the brain tissue. The material for inoculation is loaded into the trocar and pushed into the brain by means of a plunger, after which the trocar is withdrawn slowly so as not to remove the graft. Bleeding may be stopped by pulling the fascia over the opening or by applying a small piece of muscle.

Mouse Tumors in the Brains of Rats.—The cerebellum was chosen as the location for the first inoculations, but for reasons not altogether clear, this proved to be unsatisfactory. Occasionally excellent growths resulted, but in many animals the graft remnant was found imbedded in a mass of reaction tissue similar to that which appears about an heteroplastic graft in the subcutaneous tissue. Implantations into the posterior lateral part of the cerebrum also failed to give uniform results, because as was found, a graft lying in the ventricle or even coming into contact with the ventricle leads to a similar reaction. On the other hand, when the graft lay entirely in the brain substance, it grew almost invariably with great rapidity.

This observation led us to select the anterior part of the frontal lobe on account of the thickness of the cortex and the shallowness of the ventricle at this point. Grafting in this region gave 80 to 90 per cent

[9] Shirai, Y., *Japan Med. World*, 1921, i, 14, 15.

of tumors in the animals inoculated. In practically every instance in which the implant failed to grow, examination showed that it had come into contact with the ventricle.

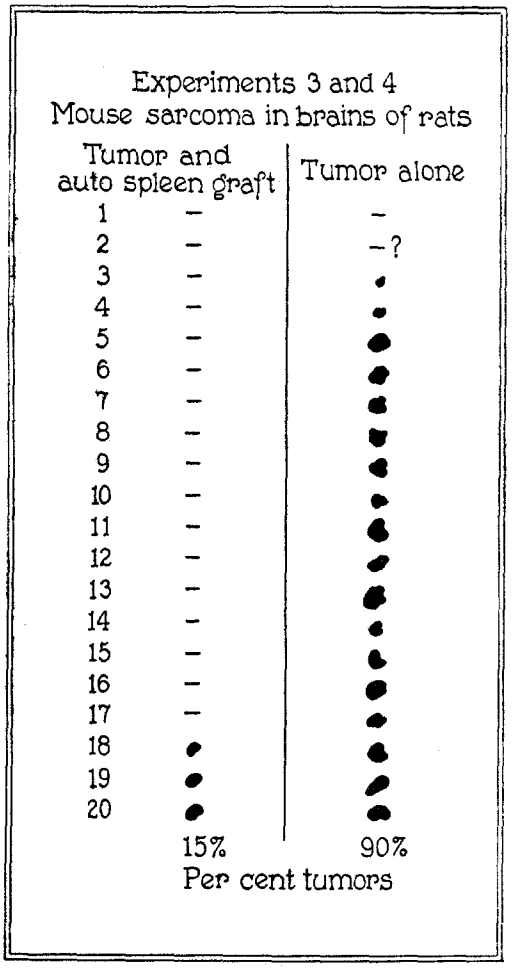
The growth resulting from inoculation of the rat brain with mouse sarcoma is generally a discrete, rounded nodule, pushing the brain substance away, rather than invading it directly. The tumors are copiously supplied with blood vessels and rarely show considerable areas of necrosis, which is in contrast with nodules developing in the subcutaneous tissue of the native host, in which the entire center of the growth is often necrotic. The number of mitotic figures is remarkable (Fig. 1). No cellular reaction is present about the edges of the typical growth lying entirely in the brain substance, but nearby vessels often show a pronounced collar of round cells (Fig. 2), and the lumina of small vessels are often blocked with lymphocytes. Other than exceptions to be mentioned, the cellular accumulations remain confined, and do not invade the brain tissue. If, in the course of growth, the tumor reaches the ventricle, the choroid plexus becomes enormously swollen and engorged with lymphocytes (Figs. 3 and 4). Under these circumstances the cells frequently invade the tumor, and necrosis of the portion of the tumor lying near the ventricle takes place (Figs. 5 and 6).

The mouse sarcoma used in these experiments grows with great rapidity in the brains of rats,—pin-head sized grafts producing tumors in 7 to 9 days which replaced the entire frontal lobe. This mouse tumor has also been grown in the brains of guinea pigs and pigeons. Moreover, a mouse carcinoma has been successfully implanted in the brains of rats, but the kind of growth obtained is somewhat different in that the tumors show a tendency to flatten on the surface of the brain and to extend apparently by invasion, sending finger-like processes into the brain (Fig. 7). In addition, necrosis is observed more frequently in the carcinoma than in the sarcoma, although less in extent than occurs in the subcutaneus tumor of the native host.

Effect of Autografts of the Spleen on the Growth of Foreign Tissue in the Brain.

As previously noted, an organism which is non-resistant to heteroplastic tissue, like the chick embryo, may be rendered resistant by a

graft of adult spleen. This, with the other evidence associating the lymphoid cell with the resistance mechanism, leads one to consider the possible importance of the observation that no cellular reaction



TEXT-FIG. 1.

occurs about a foreign tissue in the brain, and the following experiments were therefore undertaken to throw some light on this question.

Method.—The tip of the spleen of a rat was removed under ether anesthesia, and a small bit of the tissue, together with a graft of a mouse sarcoma, was im-

planted into the brain of the same rat. As a control, a graft of the tumor alone was introduced into the brain of another rat.

Of 50 rats receiving a graft of mouse sarcoma and an autograft of spleen, only eight developed tumors, and practically without exception these were small, often only nests of cells. On the other hand, 40 of the 48 controls developed tumors, and the growths generally replaced the entire frontal lobe of the brain (Table I and Text-fig. 1).

TABLE I.

Experiment No.	Tumor and auto spleen graft.		Tumor alone.	
	Takes.	No. of rats.	Takes.	No. of rats.
	per cent		*per cent*	
1	11.1	9	66.6	9
2	33.3	9	80.0	10
3	10.0	10	80.0	10
4	20.0	10	100.0	10
5	8.5	12	88.8	9
Average or total.....	16.0	50	83.3	48

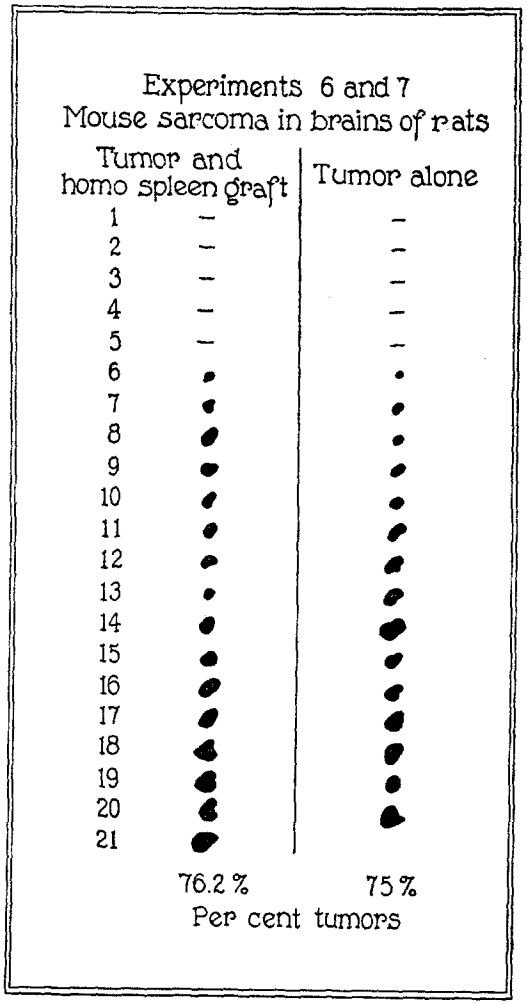
In two additional experiments, homografts of spleen instead of autografts, were introduced along with a graft of mouse sarcoma into the brains of rats, but absolutely no inhibitory action on the tumor was effected. Sixteen out of the twenty-one rats receiving tumor and homo spleen grafts developed sizable tumors, while in the controls with sarcoma alone, fifteen out of twenty had tumors (Table II and Text-fig. 2).

TABLE II.

Experiment No.	Tumor and homo spleen graft.		Tumor alone.	
	Takes.	No. of rats.	Takes.	No. of rats.
	per cent		*per cent*	
6	72.7	11	80.0	10
7	80.0	10	70.0	10
Average or total.....	76.2	21	75.0	20

This complete lack of activity on the part of the homo spleen graft is in strong contrast to the almost complete inhibitory action of the

auto spleen grafts. As a further control it was shown that autoplastic testicular grafts exert no inhibitory action, nor does autologous blood injected with the tumor affect its development.[10] In the experiment with auto spleen graft reported above, it was noted that



TEXT-FIG. 2.

the lymphoid cells disappear from the fragment very soon after the death of the tumor cells, and it has also been noted that when an auto spleen graft is inoculated alone into the brain, the lymphoid elements

[10] Maisin, J., and Sturm, E., *Compt. rend. Soc. biol.*, 1923, lxxxviii, 1216.

disappear still more rapidly. In fact it is rare to find any surviving cells of this type after 48 hours, while the cells of a similar graft in the subcutaneous tissue survive for a longer time.

Homoplastic Tumor Grafts in the Brain.

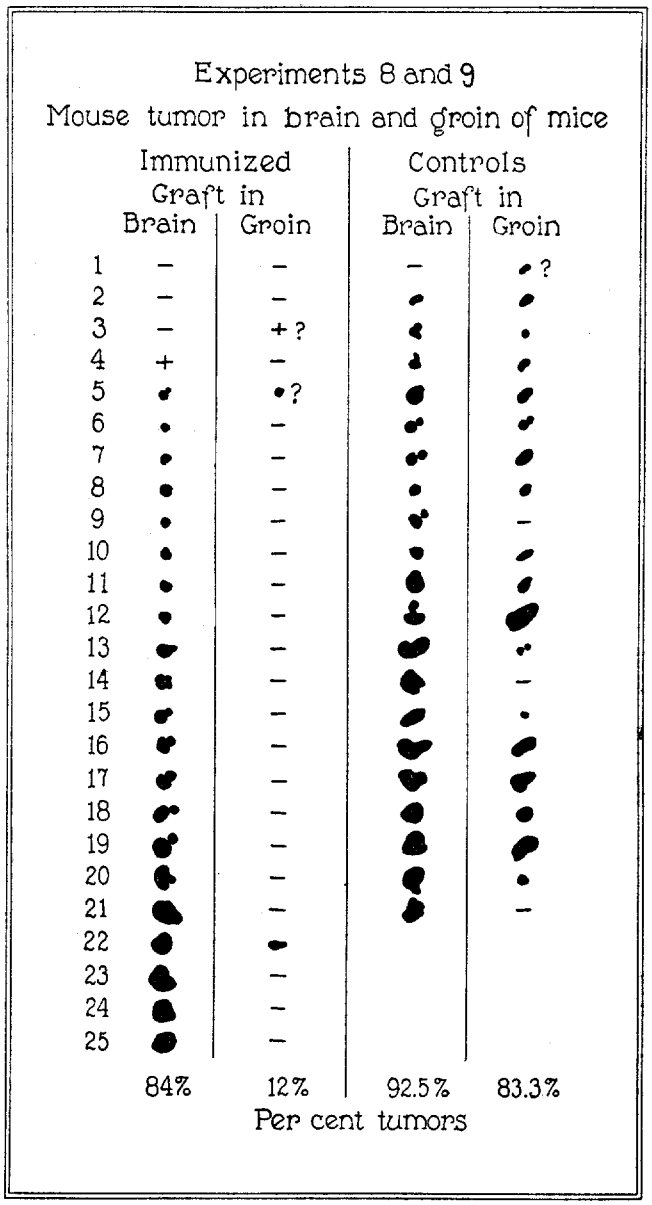
All the factors studied indicate that the mechanism involved in the destruction of the heteroplastic tissue graft is also responsible for the natural resistance possessed by a proportion of animals to homoplastic tumor grafts. Even induced resistance to tumor transplants shows itself locally by the same series of events as those which take place in natural resistance to both homo- and heteroplastic grafts. If this similarity is more than a superficial manifestation, we should expect, from the preceding observations, that animals highly resistant to homoplastic tumors, transplanted in the usual locations, would be susceptible to inoculation in the brain.

Experiment.—A number of mice were immunized against tumors by the injection of 0.2 cc. of defibrinated mouse blood, and 10 days later were inoculated with a transplantable mouse adenocarcinoma, both in the brain and in the subcutaneous tissue of the groin. As controls, normal mice were given similar inoculations with the same tumor. The results of six experiments carried out on 127 mice are given in Table III. Text-fig. 3 shows the results in two of the experiments.

TABLE III.

Experiment No.	Immunized mice.			Control mice.		
	Tumor in brain.	Tumor in groin.	No. of mice.	Tumor in brain.	Tumor in groin.	No. of mice.
	per cent	*per cent*		*per cent*	*per cent*	
8	80.0	10.0	10	100.0	77.7	9
9	86.6	13.3	15	91.6	83.3	12
10	91.6	25.0	12	72.7	81.8	11
11	88.8	12.0	9	100.0	90.0	10
12	100.0	22.0	9	100.0	80.0	10
13	90.0	30.0	10	90.0	80.0	10
Average or total.....	89.2	21.5	65	91.9	82.2	62

It may be concluded from the results of these experiments that while the immunized mice show the usual high rate of immunity against subcutaneous grafts, the same animals have almost a complete


Experiments 8 and 9
Mouse tumor in brain and groin of mice
Immunized
Graft in
Brain
Groin
Controls
Graft in
Brain
Groin
1 – – – –?
2 – – – –
3 – +? – –
4 + – – –
5 –? – –
6 – – –
7 – – –
8 – – –
9 – – –
10 – – –
11 – – –
12 – – –
13 – – –
14 – – –
15 – – –
16 – – –
17 – – –
18 – – –
19 – – –
20 – – –
21 – – –
22
23 –
24 –
25 –
84%
12%
92.5%
83.3%
Per cent tumors

TEXT-FIG. 3.

lack of resistance to similar grafts in the brain. Inoculations in the latter locality resulted in tumors in 89.2 per cent of the animals, which is practically the same rate as that shown in the brain of non-immunized controls and a higher rate than resulted from an inoculation in the groin of even the control mice.

The histological study of specimens from the preceding groups of animals brought out an interesting fact relating to the heteroplastic tissue experiment in that when a graft came in contact with the ventricle in a resistant mouse, a marked cellular reaction occurred, resulting in the complete or partial destruction of the tumor. On the other hand, grafts in susceptible mice frequently grew into the ventricle and invaded the choroid plexus without inducing a reaction greater than that seen about a subcutaneous graft in a susceptible animal.

In order to test the effect of auto and homo spleen grafts on the growth of tissue in the brains of immunized and non-immunized mice, a further investigation with homoplastic tissue was undertaken.

Experiment.—55 mice were immunized by the injection of 0.2 cc. of defibrinated mouse blood, and 10 days later, twenty-seven of these were inoculated in the brain with a mouse carcinoma and a bit of analogous spleen tissue. The remaining twenty-eight mice were inoculated in the brain with tumor alone. In addition, all the animals of the two groups were given a graft of tumor in the groin. As a control, normal mice were inoculated in the groin and in the brain with tumor alone.

In another experiment, normal mice were inoculated in the brain with autologous spleen grafts and tumors, and in a second series with homologous spleen and tumor. All animals received in addition a graft of the tumor alone in the groin, and control animals were also inoculated with the same tumor in the brain and in the groin. For comparison, the results of the two experiments are given in Table IV.

TABLE IV.

Experiments 14, 15, and 16.

Immunized mice.				Control mice.	
27 mice inoculated with		28 mice inoculated with		30 mice inoculated with	
Auto spleen graft and tumor in brain.	Tumor alone in groin.	Tumor alone in brain.	Tumor alone in groin.	Tumor alone in brain.	Tumor alone in groin.
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
29.6	25.9	89.3	31.4	96.6	83.3

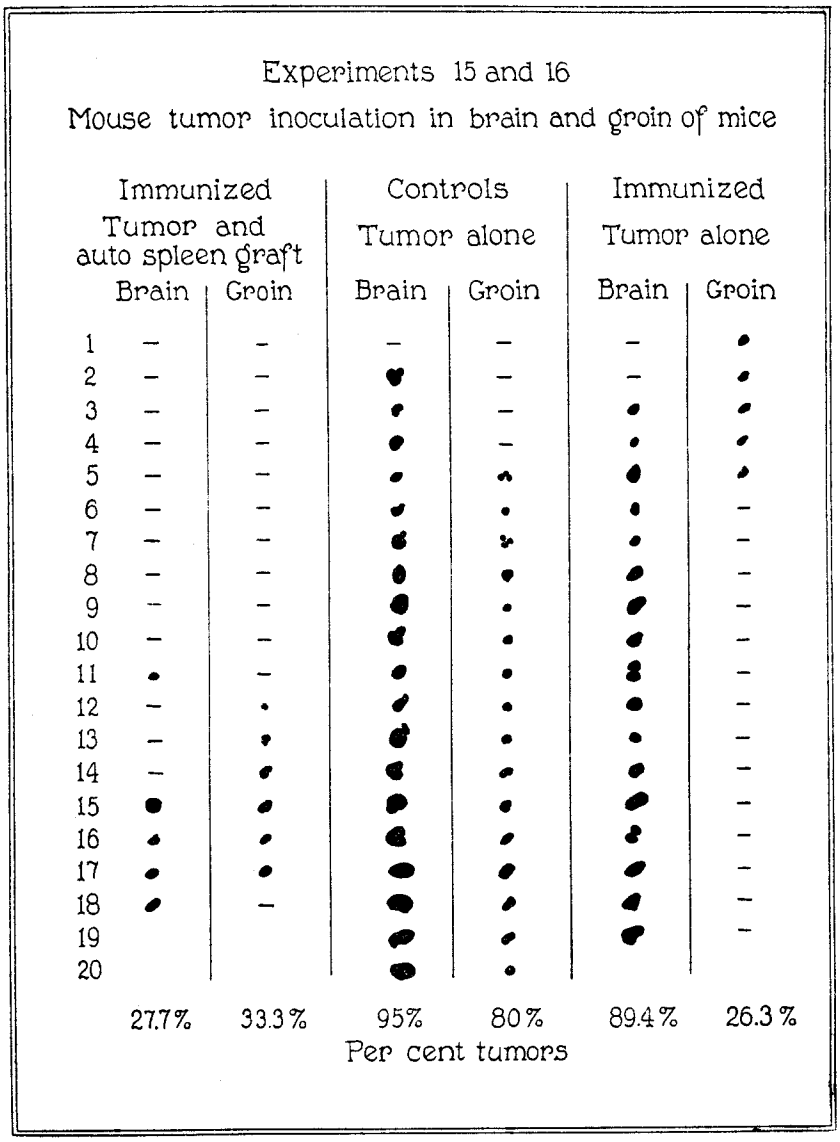
TABLE IV—*Concluded.*

Experiments 17 and 18.

Non-immunized mice.				Control mice.	
20 mice inoculated with		19 mice inoculated with		20 mice inoculated with	
Auto spleen graft and tumor in brain.	Tumor alone in groin.	Homo spleen graft and tumor in brain.	Tumor alone in groin.	Tumor alone in brain.	Tumor alone in groin.
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
55.0	75.0	84.2	84.2	95.0	70.0

It may be deduced from the above table that immunized mice inoculated into the brain with homologous spleen and tumor grafts have considerable resistance, while immunized mice inoculated with tumor alone are almost completely lacking in a defensive reaction to tumor growth (Text-fig. 4). Even non-immunized mice, inoculated with auto spleen and tumor grafts show some resistance, but much less than that exhibited by the immunized animals. In contrast to the effectiveness of the auto spleen graft in its inhibitory action is the almost complete inactivity of homo spleen grafts. In this respect we have another agreement in the behavior of hetero- and homoplastic grafts in the brain.[11]

[11] Some time ago we carried out an experiment to test the effect of auto- and homosplenic tissue on the fate of tumor grafts inoculated in the subcutaneous tissue, the splenic tissue in this experiment being mixed with the tumor cells before inoculation. The results based on the inoculation of 114 animals were 54.6 per cent only of takes with auto- and 80 per cent of takes with homoplastic spleen, while the controls gave 94 per cent of takes. This harmonizes with the above result.


Experiments 15 and 16
Mouse tumor inoculation in brain and groin of mice
Immunized
Tumor and auto spleen graft
Brain
Groin
Controls
Tumor alone
Brain
Groin
Immunized
Tumor alone
Brain
Groin
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
27.7%
33.3%
95%
80%
89.4%
26.3%
Per cent tumors

TEXT-FIG. 4.

Failure of Grafts of Spontaneous Tumors in the Brain.

With the usual methods the spontaneous tumors of animals have proved to be rarely transplantable even to animals of the same species, and in some species no successes have been recorded. As already noted, resistance to such transplants may be somewhat mitigated by exposing the animals to x-rays, a procedure which has made it possible to transplant at least one tumor which otherwise failed, but even this method was too capricious to be of real value. The seeming complete lack of resistance of the brain naturally led to a consideration of this locality as a site for the inoculation of spontaneous tumor, with the expectation that perhaps a larger proportion might be secured for study.

Some sixty rats have been inoculated into the brain with nine different spontaneous tumors of the mouse without a single success. Over a hundred mice have been similarly inoculated with seventeen different spontaneous mouse tumors and in only ten animals could any surviving tumor cells be found at the end of 10 days or 2 weeks. In only one or two instances was there anything like a tumor formation (Fig. 8), while in the others small islands only of tumor cells imbedded in reaction tissue remained.

DISCUSSION.

It is not clear why there should be an absence of a cellular reaction about a heteroplastic graft in the brain, but two possible explanations suggest themselves; first, that for mechanical reasons the cells are unable to migrate beyond the perivascular spaces; second, that the lymphoid cells find the brain tissue an uncongenial environment. In support of the first idea are the occurrence of extensive perivascular reactions near the implant, and also the observation that the small vessels in and near the tumors are frequently crowded with, and sometimes blocked by cells of the lymphoid type. In support of the second possibility is the fact that lymphoid cells implanted alone in the brain disappear more quickly than from the subcutaneous tissue. These points are suggestive only, for we have no real knowledge of what is responsible for the absence in the brain of the usual cellular reaction about a foreign tissue graft.

There seems little doubt that the absence of a reaction of this type is the principal reason for the failure of the brain to destroy heteroplastic tissues which are so readily taken care of in other locations in the body. This notion is strengthened by the observation that a foreign tissue graft in contact with the ventricle induces the typical cellular reactions and is as promptly combated as would be a similar graft in the subcutaneous tissue. Furthermore, the necessary resistance factor may be supplied to the otherwise passive brain by the inoculation of a bit of spleen tissue along with the foreign tissue graft, this being sufficient to suppress the growth of the latter,—a condition similar to that observed in the chick embryo. On the whole, it may be said that the results described confirm and extend our earlier views on the association of cellular reaction with the resistance mechanism in respect to heteroplastic grafting.

From our previous observations it was to be expected that in the absence of cellular reactions immunity to homoplastic tumors of the transplantable type would be lacking in the brain tissues, but the explanation for the failure of spontaneous cancer grafts to grow readily even in homologous animals is not clear and must await further experimentation. It is possible, of course, that this failure may be determined as much by some inherent quality of the cancer cells as by the resistance on the part of the new host.

SUMMARY.

In confirmation of Shirai's observation, we find that transplantable mouse tumors grow actively when inoculated into the brains of rats, guinea pigs, and pigeons, whereas subcutaneous or intramuscular grafts in the same animals fail. This growth of foreign tissue in the brain, however, takes place only when the grafted material lies entirely in the brain tissue; if it comes in contact with the ventricle a cellular reaction takes place with resultant destruction of the graft.

The growth of foreign tissue in the brain may be completely inhibited by simultaneous inoculations of a small bit of autologous but not by a bit of homologous spleen tissue.

Mice highly immune to subcutaneous transplants of mouse cancer show no resistance to such tumors when the inoculation is made into the brain.

Although the brain is without obvious power of resistance to implants of transplantable heteroplastic mouse tumors, yet grafts of spontaneous tumors fail to grow there even, as a rule, when tumor implanted and animal host are of the same species.

EXPLANATION OF PLATES.

PLATE 13.

FIG. 1. A mouse sarcoma growing in the brain of a rat. *M*, mitotic figures.

FIG. 2. Perivascular infiltration in the brain of a rat, which occurs near a foreign tumor graft.

PLATE 14.

FIG. 3. Choroid plexus engorgement with round cells resulting from the encroachment of a foreign tumor.

FIG. 4. The same as Fig. 3, but showing the normal plexus at a lower part of the ventricle.

PLATE 15.

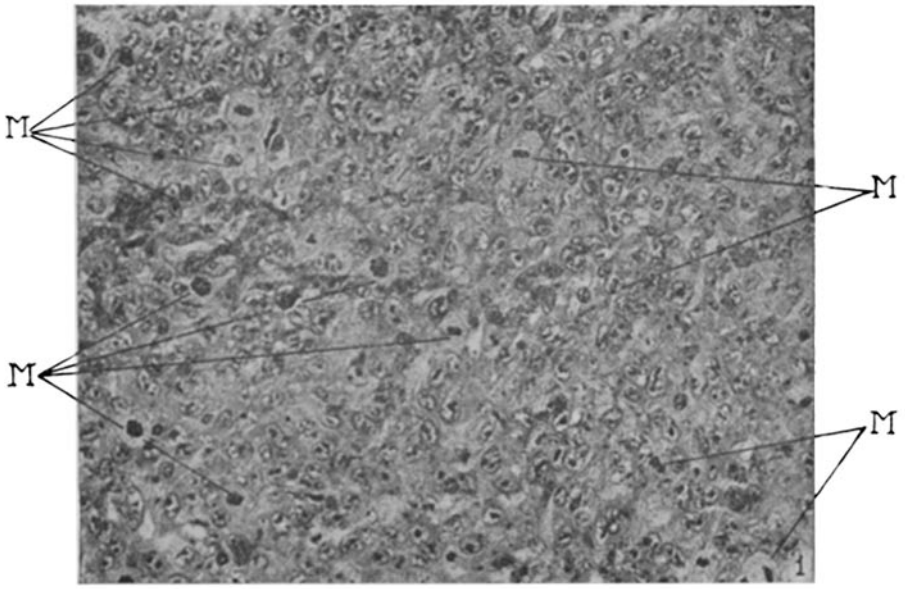
FIG. 5. Result of encroachment of a foreign tumor on the ventricle.

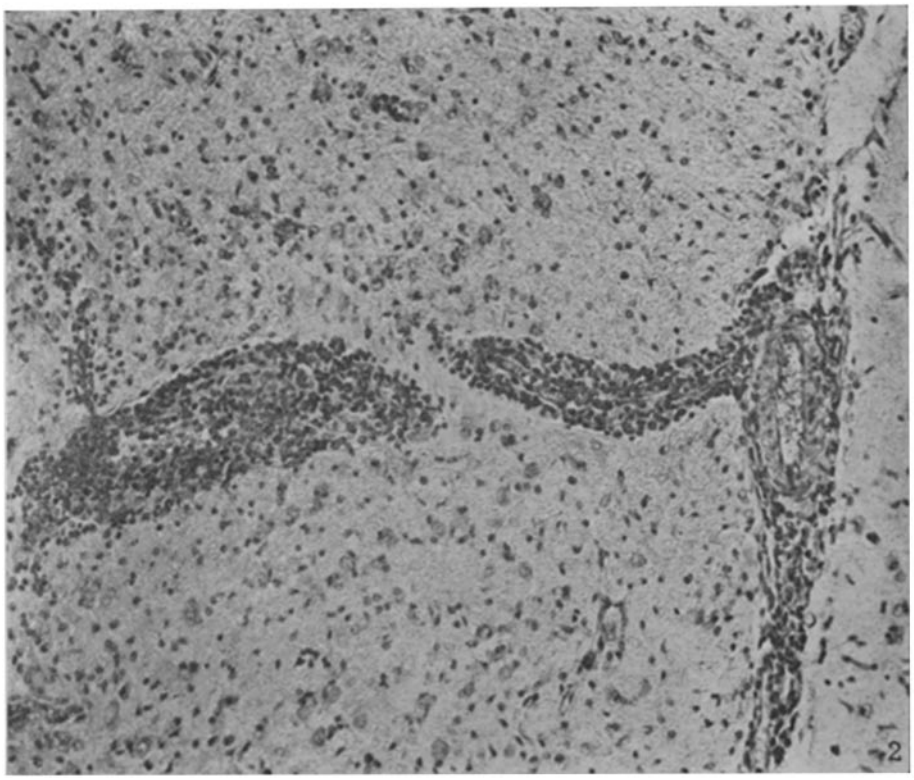
FIG. 6. The same as Fig. 5. The engorged choroid plexus is seen in the lower part of the section.

PLATE 16.

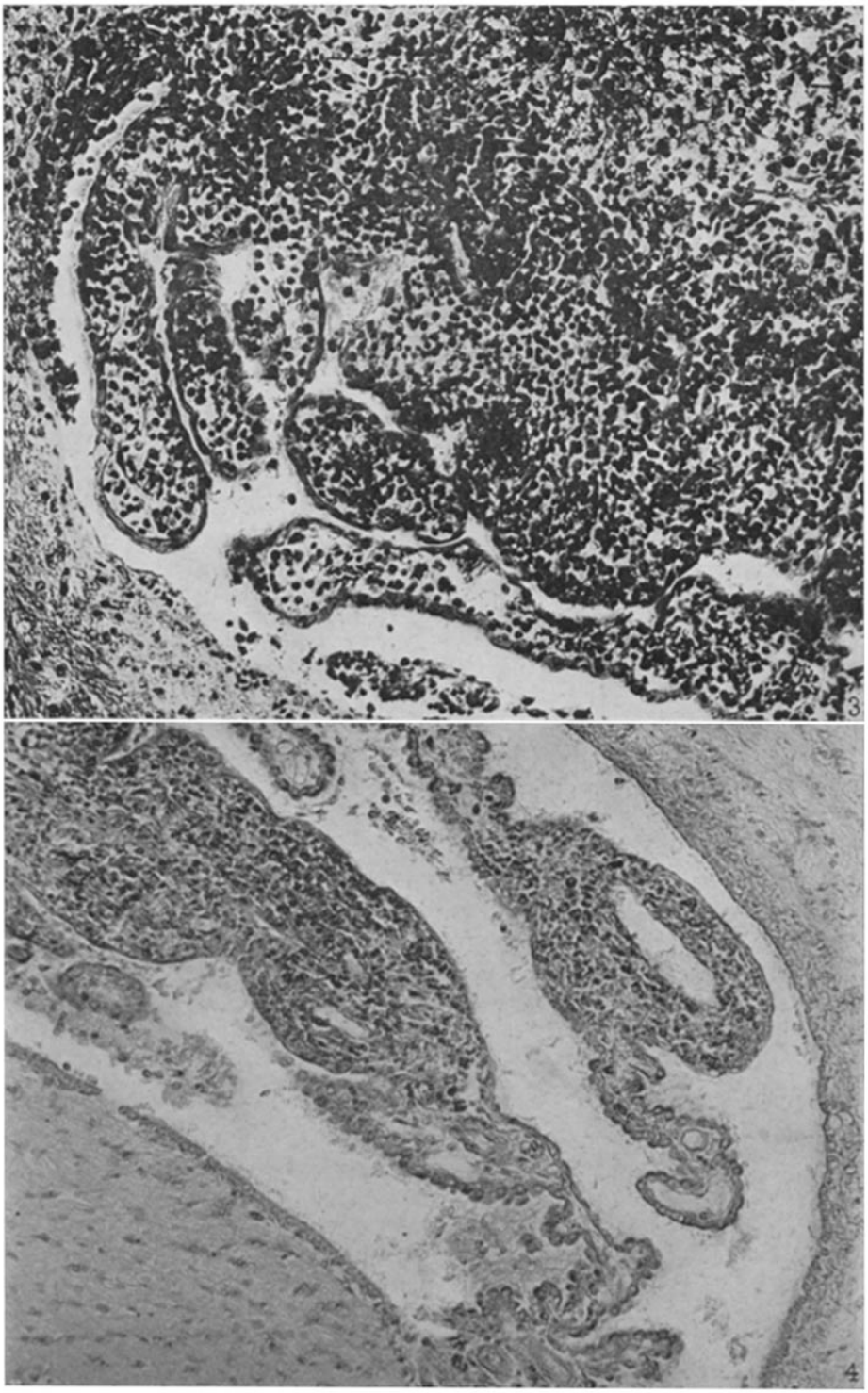
FIG. 7. The growing edge of a mouse carcinoma in the brain of a rat.

FIG. 8. Spontaneous tumor of a mouse growing in the brain of another mouse.





(Murphy and Sturm: Transplantability of tissues in the brain.)



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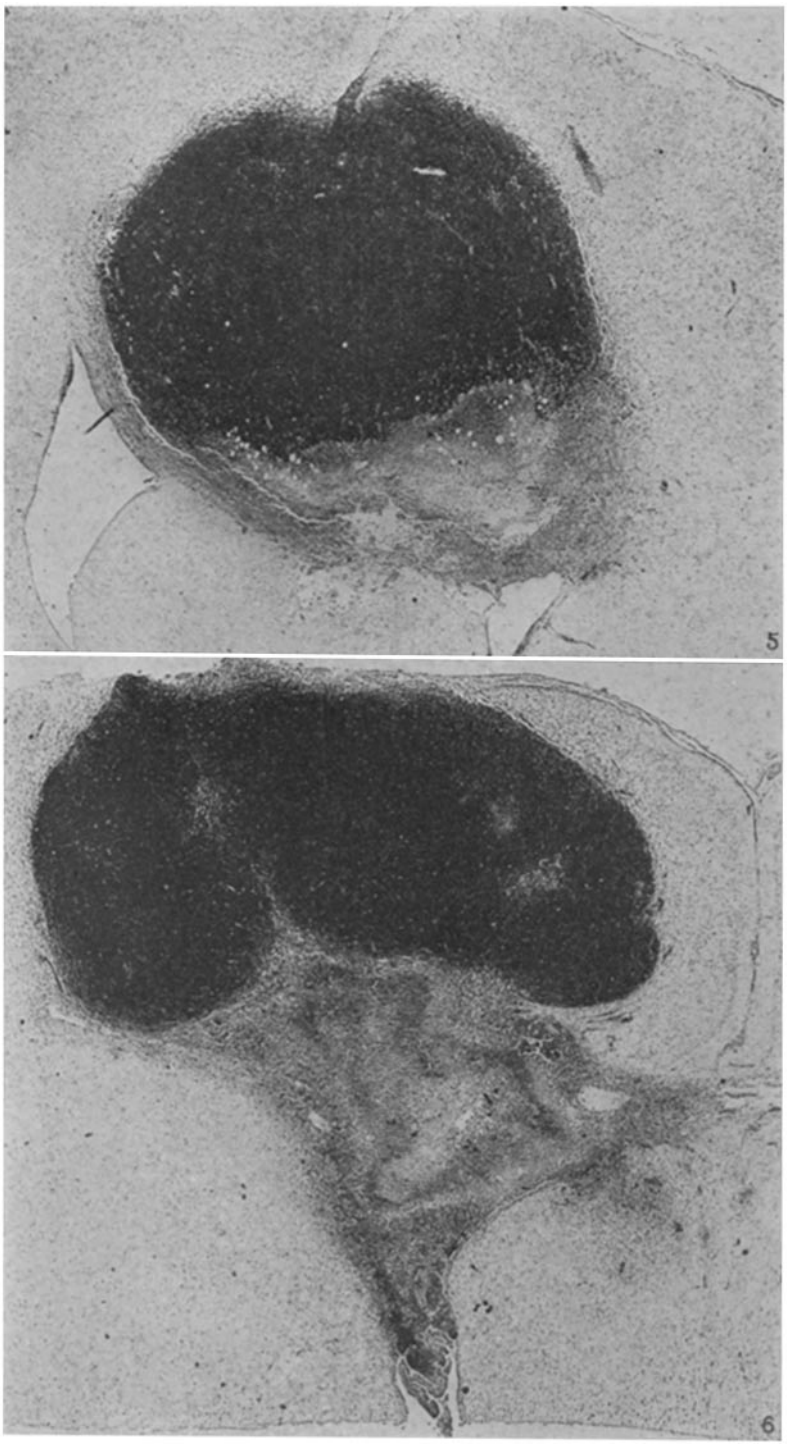

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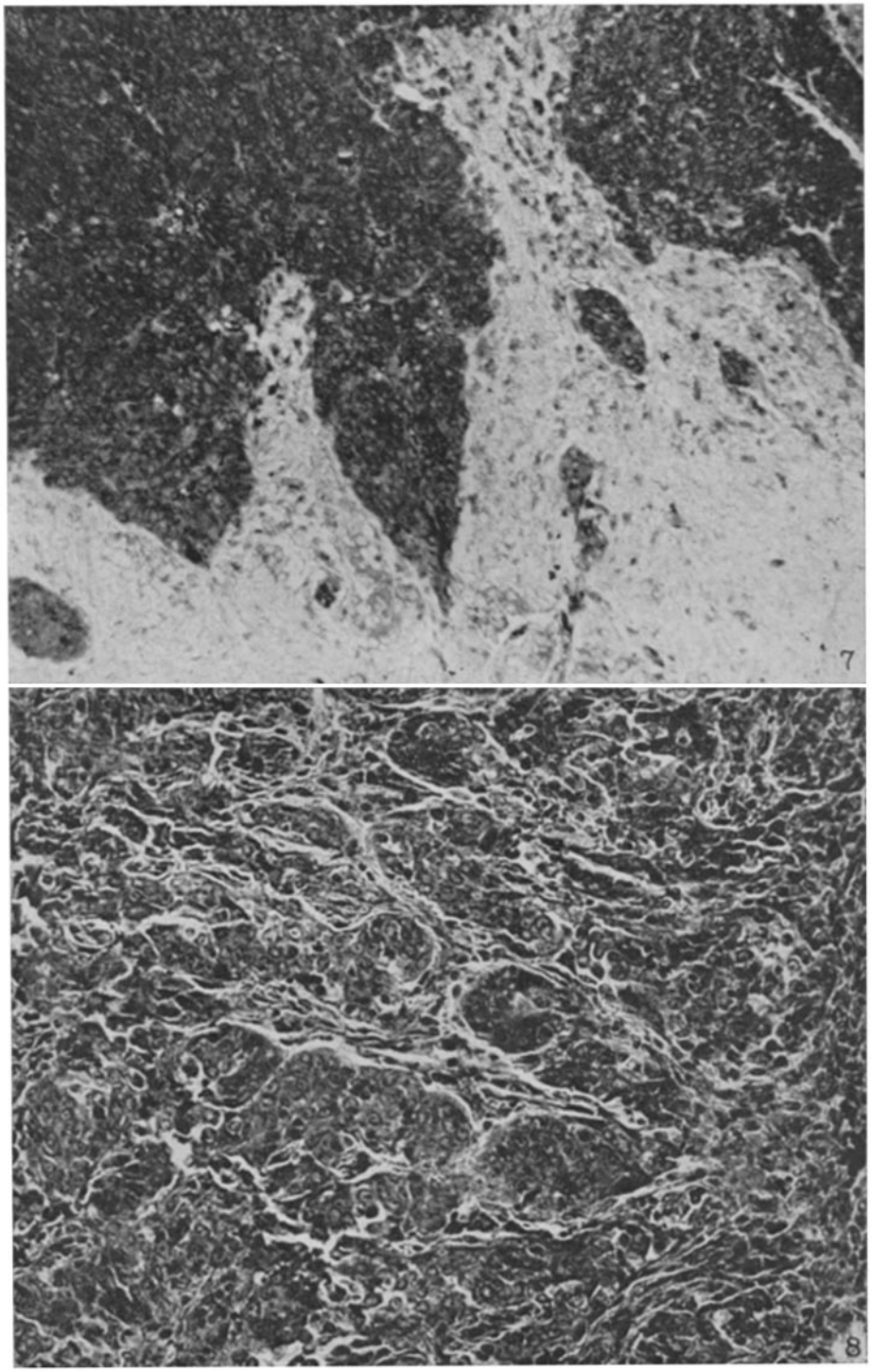

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