protein prior to the administration of the drug.

Summary. Ethionine given to young male rats of the Wistar strain on a complete diet caused only inconsistent and moderate pancreatic damage. Rats that had been on a protein depletion diet for a period of at least 6 days showed extensive destruction of the pancreas following the administration of equal amounts of the drug.

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Successful Subcutaneous Growth and Transplantation of Human Tumors in X-Irradiated Laboratory Animals.* (18854)

HELENE WALLACE TOOLAN. (Introduced by C. P. Rhoads.)

From Sloan-Kettering Institute for Cancer Research, New York City.

Neoplasms fail to grow in heterologous hosts unless one or both of two conditions are met. Either the tumor is one which has been transplanted for many generations or the implantation is made in sites at which the host offers minimal resistance to foreign tissue. Examples of these sites are the anterior chamber of the eye, brain, testes and immature tissues such as avian embryos and newborn animals. Spontaneous neoplasms, such as those of man, have been propagated heterologously only when implanted in the anterior chamber of the eye(1). The percentage of failures has been high, even under these circumstances, the rate of growth of the tumors slow, and the transplantability poor(2). To achieve an improved method for growing human neoplasms in animals, the work here reported was undertaken

Murphy showed(3) that rats which had been X-irradiated would support the growth of subcutaneously implanted mouse tumors for the duration of the lymphopenia produced by the radiation. A modification of this procedure has been employed in our experiments.

Methods and materials. Young adult female albino rats (Carworth Farms) and mice (Banks strain of Swiss) were employed. The rats received 3 doses each of 200 r, total body X-irradiation, on alternate days and the mice 4 doses of 150 r on successive days. Two 180 kv. tubes were employed (one above, one below the animal).

Previously, it had been established that this procedure would reduce the total white count in the particular strains of animals used, to approximately 400-800 mm^3. Several extra animals were included in each group irradiated to check the degree of lymphopenia produced. Counts were *not* made on the animals to receive implants since too great a variable is introduced by the cardiac puncture required to obtain a true picture of the total white count of rodents; tail blood is not usable because of its hemoconcentration.

The divided irradiation doses caused no symptoms of toxicity in the animals whereas a single dose large enough to produce the same reduction of white cells often caused diarrhea and even an occasional death. It must be em-

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TABLE I. Results of Subcutaneous Implantation of 100 Human Tumors into X-irradiated Rats and Mice.

No. of tumors	Pathological diagnosis	Mitosis for more than one generation	Mitosis at end of 1st generation (8 days) only	Failed
I – Malignant				
48	Epidermoid Ca.	21	15	12 (6 infected)
10	Ca. breast	1 (poor)	3 (poor)	6 (1 ")
10	Ca. colon, sigmoid or rectum	1	2 "	7 (4 ")
4	Melanoma	1 "	2 "	1 (")
3	Ca. ovary	1 "	2	—
3	Osteogenic sarcoma	2	—	1
2	Leiomyosarcoma	1	1	—
2	Fibrosarcoma	1	1	—
2	Synovioma	1	1	—
2	Rhabdomyosarcoma	1	—	1
2	Ca. lung	—	1 "	1
1	Ca. prostate	1	—	—
1	Malignant mixed tumor parotid	1	—	—
1	Fascial sarcoma	—	1 (not transferred)	—
1	Ca. adrenal cortex	—	1 (poor)	—
1	Ca. kidney ("clear cell")	—	1	—
1	Angiosarcoma	—	—	1
1	Wilms	—	—	1 (")
1	Ca. uterus	—	—	1
96		33*	31	32
II – Benign				
2	Mixed tumor parotid	—	2	—
1	Hemangioma	—	1	—
1	Leiomyoma	—	—	1
4		0	3	1
Total: 100		33	34	33

* Of 33 tumors in which mitotic cells were present for more than one generation, 18 showed active mitosis at end of the 2nd generation, 9 at end of the third, and 6 had actively growing cells at end of the 4th generation (approximately a month after the original tumors were obtained); one epidermoid carcinoma was transferred successfully for 9 generations.

phasized that different strains of the same species of animal may vary greatly in their susceptibility to radiation. This factor must be determined carefully for each strain used.

Samples of human neoplastic tissue were implanted 1-8 hours after removal in X-irradiated animals which had received their 3rd, and final treatment 1-3 days previously.

Of the 100 tumors implanted, the first 47 were pressed through a 40 mesh monel metal sieve into a sterile Ringer's solution buffered to a pH of 7.35 (3 mg of glucose and 200 units of penicillin added per cc). Sieving was employed to insure suspensions of cells with relatively few clumps for implantation. As a result, any large masses of tumor subsequently found at the implantation sites could be ascribed to multiplication of cells rather than survival of a graft *in toto*. Each of the next 6 tumors was divided into two parts. One was sieved as usual, the other minced fine enough with scalpels so that the suspension would pass through a No. 20 needle. The remaining 47 tumors were minced only. All the tissue transplanted beyond one generation was minced.

With few exceptions each tumor suspension was implanted subcutaneously in both X-irradiated mice and rats, usually in groups of 6 to 10 each. Mice were given 0.5 cc of the concentrated suspension in each flank. The rats were either implanted in similar manner or in 4 sites, both groins and both axillae. On the 8th day after implantation (occasion-

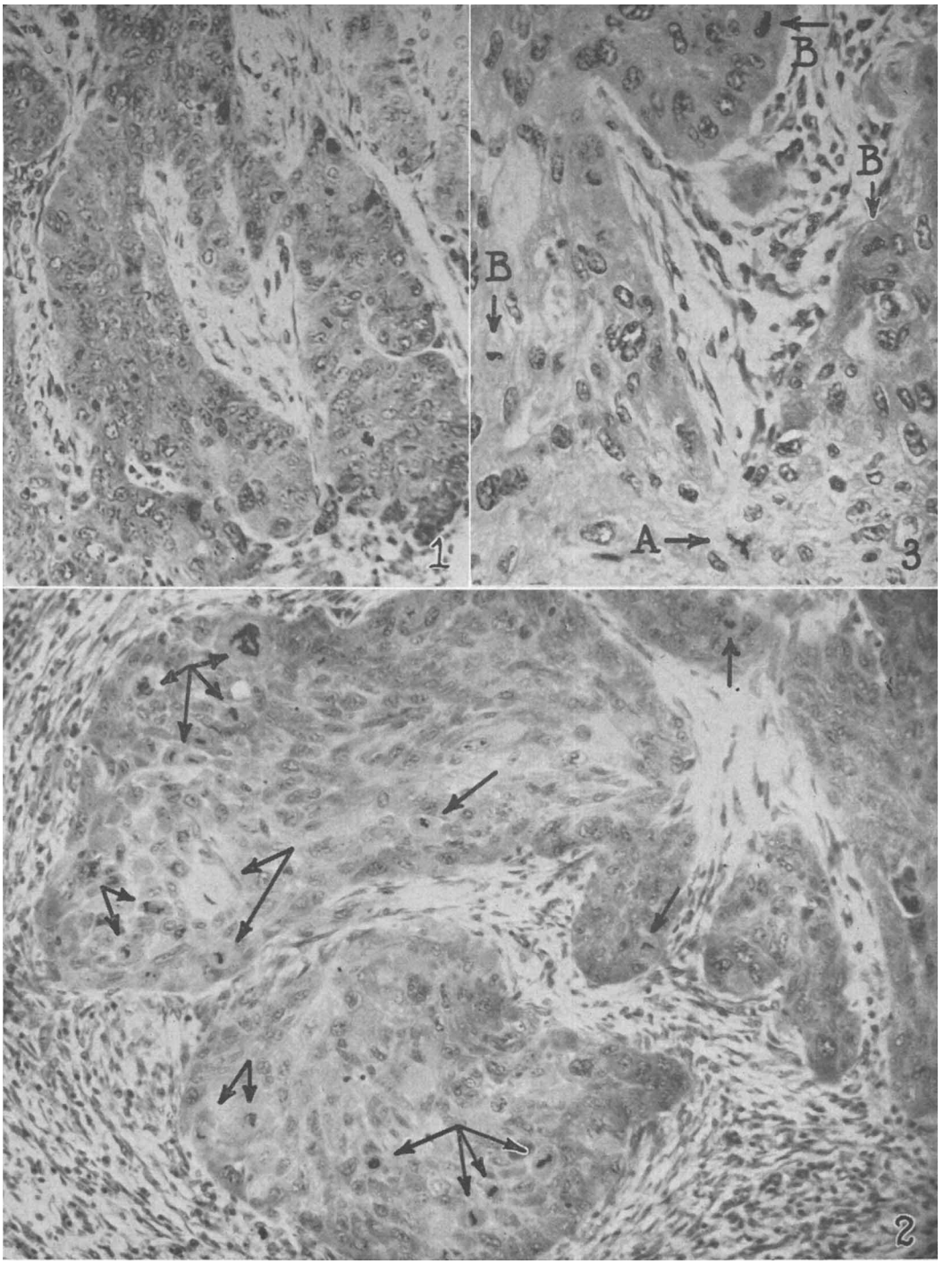

FIG. 1. Epidermoid ca. of larynx; original tumor. × 220.

FIG. 2. Same tumor as in Fig. 1 after 3 generations (25 days) in X-irradiated rats; note the many mitotic figures (arrows). Original implantation a sieved suspension of cells. × 200.

FIG. 3. Same tumor as in Fig. 1 after 3 generations (25 days) in X-irradiated mice; note tripolar mitosis (arrow A) and other mitotic figures (arrows B). Original implantation a sieved suspension of cells. × 350.

ally on the 7th or 9th) the animals were killed and the nodules at the sites of implantation were removed, samples taken for histological examination, and the remainder pooled, minced and reimplanted in a new group of irradiated animals. After 8 days the procedure was repeated—the number of subsequent transfers varying with the tumor.

Choice of the 8th day as an optimum time for transplantation was due to the finding that the ratio of implanted tumor cells in good condition to the amount of host reactive tissue was most favorable to the tumor at this time. Study of implantation sites in animals allowed to survive 10-14 days before sacrifice showed that the tumor cells had disappeared in a mass of reactive tissue.

About every fifth tumor was implanted in normal as well as in irradiated animals. No tumor cells were found after 8 days in the untreated hosts.

Results. On the basis of microscopic examination of the samples taken at times of transplantation, the results of implanting 100 human cancers in X-irradiated animals fall into 3 equal groups (Table I): (1) Failures: In these none of the implanted cells were in active mitosis after 8 days in the heterologous hosts even though surviving cancer cells might be present. (2) Single generation success: Here proliferating cells were present at the time of the first transplantation attempt but none at the second. (3) Multiple generation success: Vigorous mitosis was present for two or more generations.

With very few exceptions the findings were the same for a particular tumor whether implanted in mice or rats, and, in general, they were similar for all the animals of a group. The size of the nodule at an injection site was no indication of the success or failure of the implantation since it might be composed entirely or in large part of reactive tissue.

Epidermoid carcinomas grew most successfully and a number were transferred for several generations. This type of tumor was particularly satisfactory, furthermore, because it is easily distinguished from the subcutaneous, or reactive, tissues of the hosts (Fig. 1-6). Other types of neoplasms sometimes grew equally well (Fig. 7-9). The 10 infiltrating duct carcinomas of the breast, on the contrary, did poorly even though female hosts were used. This might have been due to the large amount of stroma and comparatively few cancer cells characteristic of this tumor.

The growths obtained from the sieved cell suspensions were as good as those from the minced tissue implantations though more cells survived when the minced material was used. Fig. 2 and 3 show a 3rd generation transplant 25 days after removal from the patient, and Fig. 7 is also a 3rd generation, 22 days after removal. The large masses of cancer cells, such as those in Fig. 2, necessarily indicate multiplication since the implanted sieved suspensions were composed of individual or at most, small clumps of cells.

It is of interest that almost all the tumors which survived for 2 generations or more in the heterologous host showed considerably more mitotic activity than did the original implants (Fig. 1-6), and none had less. It may be that some selective adaptation of cells occurred. Implants in successive generations were well vascularized (Fig. 6, 9), furthermore, whereas those that only survived for only one generation often were not. As shown in Table I, 6 tumors were transferred successfully for 4 generations and one was transplanted for 9. In many instances no attempt was made to transfer the material beyond the third generation since it was found that the tumors eventually died out. The reason appears to lie in the fact that only unusual mitotic activity during the short 8-day period before recovery of the animal from irradiation could make up for the considerable mechanical loss of cells at the time of transfer. The small groups of tumor cells still left in the later generations showed no diminution of vitality, however, for often fully a fifth of them were in mitosis.

Two factors were correlated with failure of the original implantation to survive: (1) Infection. Of 33 tumors in the group of failures, 13 were contaminated as compared with none in the more successful groups. (2) Delay in implantation. Time between removal of the growth from the patient and implantation in

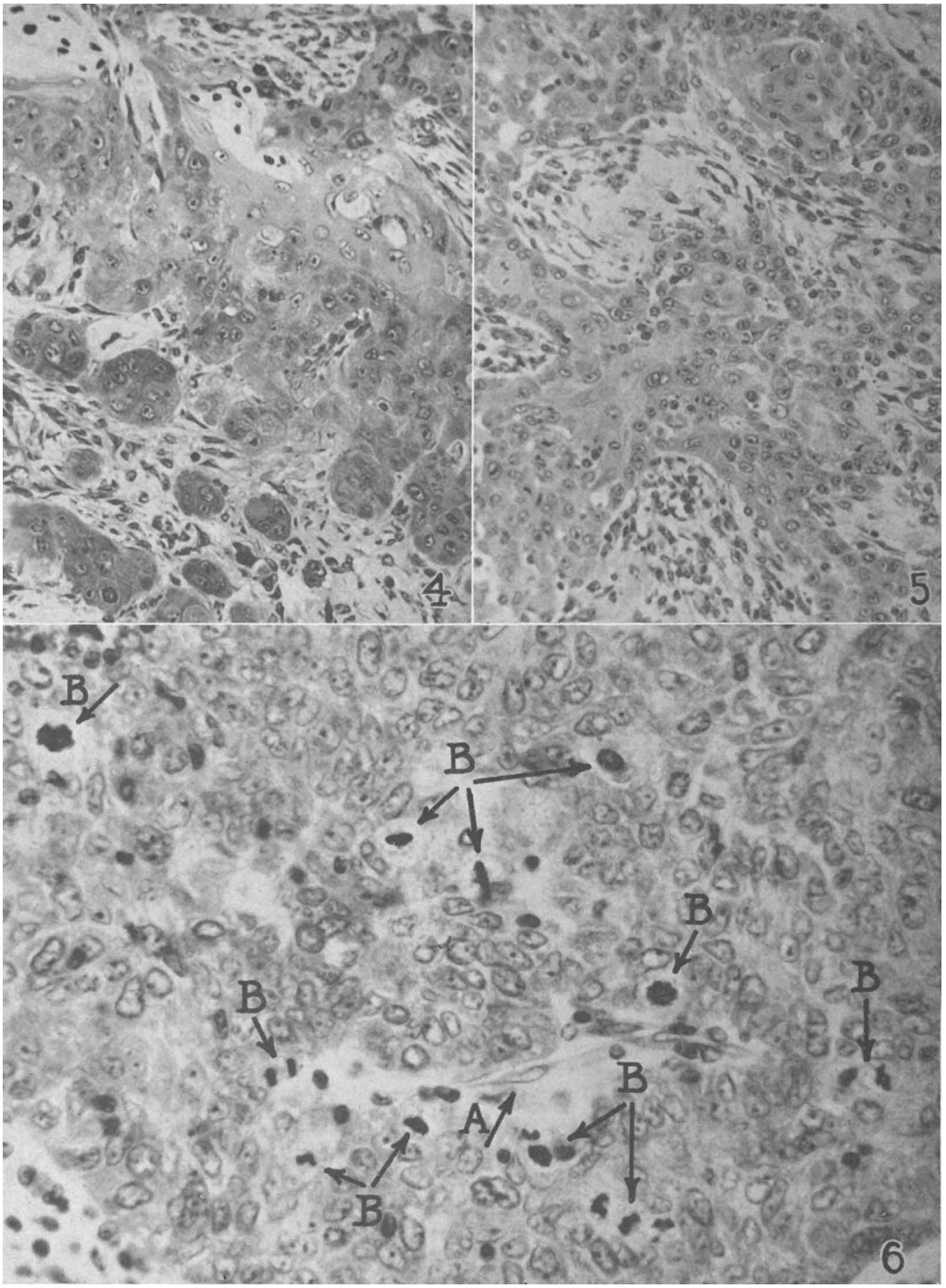

FIG. 4. Transplant of a recurrent squamous ca. after 2 generations (16 days) in X-irradiated rats. × 200.

FIG. 5. Transplant of a metastatic squamous ca. from radical neck operation after 2 generations (16 days) in X-irradiated rats; tumor well vascularized and similar to original specimen. × 200.

FIG. 6. Transplant from epidermoid ca. of tongue after 2 generations (17 days) in X-irradiated rats; note blood vessel (arrow A) and numerous mitoses (arrows B). × 600.

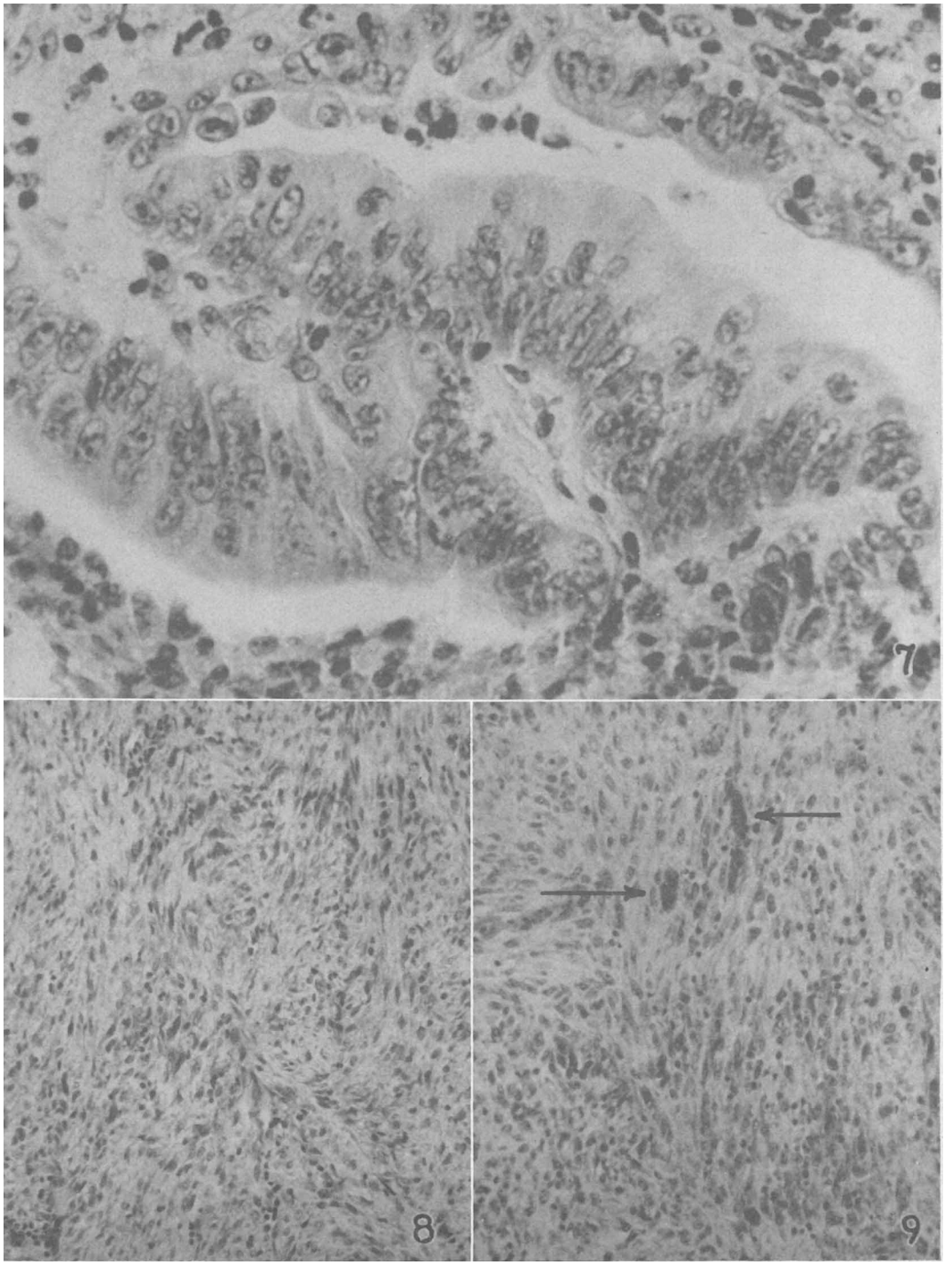

FIG. 7. Transplant of an adenocarcinoma of the rectum after 3 generations (22 days) in X-irradiated mice. The resemblance to the tissue of origin is striking. Original implantation a sieved suspension of cells. × 800.

FIG. 8. Fibrosarcoma of the leg, original specimen. × 180.

FIG. 9. Transplant of fibrosarcoma (Fig. 8) after 2 generations in X-irradiated rats. Growth very similar to original and well vascularized (arrows point to small blood vessels). × 180.

the experimental animals was found to be most important. As this exceeded one hour, less favorable results were obtained.

The ideal demonstration of viability would have been reimplantation of the material grown in heterologous hosts back into the original human donor. This was not feasible, and so tissue culture was employed to provide confirmatory data. Pieces of 3 original epidermoid carcinomas were cultured in roller tubes and the sheets of epidermoid cells which grew out were compared with those obtained in tissue culture from the subcutaneous nodules of the first, second, and, in one case, third animal transplantations of the same growths; they were quite similar. Some mouse or rat fibroblasts were present but the typical epithelial cells predominated. One of these epidermoid carcinomas was highly anaplastic with many multinucleated cells. This same type of cell grew out in the tissue cultures from the original tumor and from the second transplant nodule. Unfortunately, no effort was made to culture material from the first transplant generation.

Summary. (1) Of 100 human neoplasms implanted subcutaneously in X-irradiated rats and mice as sieved or minced cell suspensions, 33 had no mitotic cells at the end of the first generation (8 days), 34 showed cellular proliferation at the end of the first generation but could not be transferred successfully to a second, and 33 were transplanted for 2 generations or more with evidence of active growth and vascularization. (2) Epidermoid carcinomas were most successfully propagated. (3) Failures were in part due to infection and to delay between surgical removal of the tumor and its implantation in the heterologous hosts.

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A Microbiological Method for the Determination of L- and D-Aspartic Acids.* (18855)

MERRILL N. CAMIEN.† (Introduced by M. S. Dunn.)

From the Laboratoire de Biochemie, Institut Léon Fredericq, Université de Liège, Liège, Belgium.

It was shown previously(1) that D-aspartic acid supported only limited growth of *Leuconostoc mesenteroides* P-60 with relatively short incubation times in asparagine-free media. When low concentrations of L-asparagine were present, however, the growth response to D-aspartic acid was nearly the same as, or better than, that to L-aspartic acid. This suggested that *Leuconostoc mesenteroides* might prove useful for the determination of both aspartic acid enantiomorphs in hydrolysates of biological materials. The method which was subsequently developed for this purpose is described.

Experimental. The stock standard solution contained 4.00 mg of L-aspartic acid,‡ 1.00 mg of D-aspartic acid,‡ and 0.05 ml of 12 *N* hydrochloric acid per ml. It was stored at room temperature and diluted as required just before using. The diluted standard solutions were added in amounts of from 0.2 ml to 1.0 ml per tube to give a range of total aspartic acid concentrations of from 0 to 70 γ per tube in steps of 5 γ per tube.§ Each level was tested in triplicate. The standard was set

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† U. S. Fulbright Scholar. Present address: Department of Chemistry, University of California, Los Angeles.

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‡ These products, the same as those described previously(1), were kindly supplied by Dr. Max S. Dunn.

§ A 3 *N* NaCl solution was added, taking into account amount of NaCl formed in neutralizing HCl acid present.