THE NEOPLASTIC POTENTIALITIES OF MOUSE EMBRYO TISSUES

III. The Tumors Elicited from Gastric Epithelium

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In previous papers (1, 2) the fact has been reported that benign and malignant epithelial tumors rapidly and regularly arise from the epidermis .of mouse embryos after this has been transplanted, together with methylcholanthrene, to adults of the same strain (C strain). Some of the growths have metastasized, in animals let live long enough, and many successful transplantations have attested to their neoplastic character. The method lends itself to experimentation with other tissues of the embryo, and these have yielded a wide variety of tumors (3). The present paper is concerned with epithelial growths of gastric origin—which have been induced as readily as those of the epidermis. The findings provide a means for the experimental study of gastric carcinogenesis and yield information about the neoplastic potentialities of cells from the embryo, in addition to that already obtained.

Methods and Materials

In the work with transplanted embryo skin, advantage was taken of its tendency to form cysts lined with epidermis. Fragments of the tissue were injected into the thigh muscles of adult mice together with olive oil containing methylcholanthrene, with or without Scharlach R, a substance known to stimulate and attract epidermal cells (4). The globules of oil were soon surrounded by a proliferating layer of epidermis which in consequence became directly exposed to the carcinogenic action of the methylcholanthrene, and tumors speedily arose. The implantation of bits of embryo stomach under the same conditions has resulted in a like course of events.

In some experiments the oil introduced with the tissue was saturated with Scharlach R (Grübler) at 100°C., and then 1 per cent of 20-methylcholanthrene (Eastman) was dissolved in it. This preparation will be referred to as *OSSM*. In other tests only 1 per cent of Scharlach R was present with the carcinogen, and this preparation will be called *OSM*. The larger amount of dye, 2 per cent, was used for its presumptive stimulating effect on the gastric epithelium, the smaller mainly to show at autopsy where the oil was. Olive oil containing 1 per cent methylcholanthrene but no Scharlach R will be referred to as *OM*. The various preparations were distributed in test tubes in 1 and 2 cc. amounts, sterilized in the autoclave, and stored at 4°C. until required. When used, they were put in a boiling water bath for an

hour to dissolve such crystals as had formed in the cold, and then were shaken in a mechanical device for another hour with one-third their bulk of Locke's solution, to make an emulsion—which was injected forthwith. The shaking broke up the oil in many small globules, thus facilitating contact of it with the tissue.

C strain mice were mostly employed, a breed so homogeneous that transplanted epidermal tissue does well in every host (1). The duration of pregnancy in the animals furnishing the embryos was determined in most instances by the vaginal plug method, and fetuses 15 to 18 days old (12 to 20 mm. long) were utilized. They were decapitated, the skin of the belly was shaved, swabbed with 60 per cent alcohol, and with a cautery the abdominal cavity was opened, the uterus removed, and the embryos freed individually. Each was washed in Tyrode's solution, pinned out on a sterile board, and the body wall was cut through along each side with the cautery.[1] It was then raised by means of a forceps thrust transversely through from cut to cut, and the cuts were extended until they met. The apron-shaped flap thus freed was reflected over the chest and pinned down. The pylorus was next grasped with a fresh pair of forceps, severed with scissors on the side toward the cardia, the body of the stomach was grasped, and the organ was cut free from the esophagus by means of a V-shaped incision extending well into the gastric tissue. All esophageal tissue was excluded in this way from the material used. Different instruments were employed at each stage of the operation.

After the stomachs of all the litter had been assembled in Tyrode's solution each was clipped open along the lesser curvature, under a dissecting microscope, and emptied of its mucoid contents. In some experiments the gastric tissue was then pooled in a dish containing a few drops of the salt solution, and hashed with knives. In others the squamous and glandular portions of the organ (Fig. 1) were separated for implantation. They could be readily distinguished, the former having a smooth inner surface, with prominent longitudinal furrows, whereas the latter appeared rugose and was devoid of these. Transverse cuts were made with a sharp-pointed knife to either side of their well defined junction, and the strip in which this lay was discarded or transplanted separately. Each piece of tissue as cut free was washed in a large volume of Tyrode's solution, and when all those of the same sort had been gathered together in a little of the fluid they were cut fine. In some experiments, Locke's solution was used throughout; it served equally well.

The mice implanted were young adult males. The calf of the leg was swabbed with alcohol, the skin slit, the incision wiped with 1-1000 mercury bichloride to kill any epithelial cells which might be carried in on the injecting needle, and this was thrust into the calf muscles and pushed up into those of the posterior thigh. For each implantation 0.025 cc. of *OSM* or *OSSM* or *OM* was drawn up into a quarter cc. Luer syringe, followed by an equal amount of tissue suspension, and injection was done into the thigh muscles,—forcibly so as to scatter the materials. The layering of these in the syringe barrel prevented the tissue fragments from becoming coated with oil, which would have interfered with their survival. Sometimes, for the better dispersion of the material, 0.075 cc. of Locke's solution was drawn up into the syringe between oil and tissue suspension. Muscular contraction prevented backflow after the injection. In many instances one thigh of the mouse was utilized for a control implantation of the tissue suspension with 0.025 cc. of Locke's or Tyrode's solution.

After 2 to 3 weeks the thighs were palpated and outline drawings to size were made of any nodules, with weekly or biweekly drawings from then on. At autopsy notes were made of the findings, with sketches in most instances, the specimens were fixed in acid Zenker's fluid, and sections were stained with eosin and methylene blue. They were cut serially in many cases. Tumors for transplantation were removed aseptically and pieces of them were thrust into the

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[1] This instrument was used to insure that no other viable cells than those of the stomach would be included in the material under test.

thigh muscles of new hosts through trocars, after the skin had been slit and swabbed with bichloride.

GENERAL FINDINGS

According to a large literature the gastric epithelium of the embryo is amongst those tissues which best survive transfer to adults. There are no records of tumors having arisen from it, and no growths even remotely resembling them have developed from the control implants of the present experiments. Instead these have given rise to small clusters of discrete, rounded, thin-walled cysts with a smooth lining (Figs. 2 and 3), full of concentric keratin when this lining has consisted of squamous epithelium, or containing a colorless, thin or mucoid fluid when it was glandular in character. The enlargement of the keratinizing cysts was very slow in most instances, but those holding fluid sometimes became a centimeter or more across in the course of a few months.

Very different were the results when the tissue had been implanted with methylcholanthrene. At first a cluster of cysts formed, but soon most of them coalesced with result in one or several relatively large multilocular cysts of irregular shape (Fig. 5). Their ruddy contents, when Scharlach R was present, showed that the carcinogenic material had been included within them. Very shortly they became bigger than the controls and after some weeks they often underwent a sudden enlargement. Examination then showed papillary or fungating masses, obviously of neoplastic tissue, projecting into their interior, or else discoid or diffuse thickenings in their walls, with extension frequently into the neighboring muscle. Microscopically the projecting growths appeared to be benign or malignant papillomas (Figs. 4, 6, 7, and 20) or carcinomas, and the thickenings had the latter character (Figs. 16 and 17). Multiple growths, often of widely differing sorts, formed as time went on, and in animals let live for some months the nodules became very big, often surrounding the femur, occupying the whole upper leg, and eventually rupturing outwards and proving fatal. All contained cysts, in some instances large, with a pultaceous content and fleshy walls studded with many projecting papillomas, in other cases small and lying amidst masses of tissue, formed through the coalescence of several malignant growths. Five were transplanted while the nodules containing them were still small, and all grew rapidly in the new hosts (Figs. 9, 10, and 15), killing these when not interfered with. One of them was carried further, through three successive groups of mice (Fig. 18), and then let lapse. These findings substantiated the diagnosis that the growths were carcinomas. Hence this term will be applied to all those of similar character which are dealt with in the succeeding pages.

Since the prime aim of the work was to learn how regularly tumors occurred and to study them individually while still discrete, most of the animals were killed early. Metastases were not encountered, nor were animals kept with

a view to obtaining them, since transplantation sufficiently demonstrated the neoplastic character of the growths. The epidermal carcinomas derived from embryo skin implanted under similar conditions in the muscles seldom metastasized and then very late (2).

Experiment 1 was done with material from a litter of 5 C strain embryos 18 days old. The stomachs were 3 to 4 mm. in length. A hash of the squamous portion of the organs was implanted in both thighs of 5 hosts, the glandular tissue was put into another 5, and bits of the junction strips, consisting of both squamous and glandular tissue, into 2 more. Each implant consisted of 0.025 cc. tissue suspension followed by 0.025 cc. *OSM*. The results are summarized in Table I.

One of the mice that had received squamous tissue was killed first, on the 62nd day. It had an oblong nodule 10 mm. long in its right leg. This proved to be a cyst with a pedunculated cauliflower mass projecting into it, and a 2 mm. thickening elsewhere in its wall. The cyst contents consisted of pultaceous material stained pink with Scharlach R. Sections showed in the thickened region an epithelial growth of papillomatous character, obviously malignant and highly invasive (Figs. 7 and 8). The cauliflower mass was a benign papilloma (Fig. 6) with a singular core (Fig. 21) of which more will be said further on. Between the growths the wall was thin and had a lining of stratified squamous epithelium. Pieces from its thickened region were transplanted into both thighs of 10 new hosts, and within 12 days growths measuring up to 13 mm. had resulted at every site. They continued to enlarge rapidly, and when the animals were killed, 44 days after implantation, were found to be thick-walled cysts filled with pultaceous matter and dead, friable tissue lying amidst fluid. Sections showed the walls to be made up of carcinomatous tissue (Fig. 9) with cells much more anaplastic than those of the primary growth and undergoing hydropic degeneration instead of differentiating. More than one carcinoma may have been present in the material originally transferred.

The other leg contained a nodule 6 mm. in diameter, a thick-walled cyst filled with pultaceous, pink-stained material. Three distinct growths had arisen from its wall, as serial sections showed. Two protruded into the cyst cavity and had the morphology of benign papillomas, while the third was a downgrowing, invasive growth nearly resembling the carcinoma in the opposite thigh.

A second animal of the group was killed on the 65th day. Cystic nodules filled with pink, pultaceous material were present in both legs, that in the right 13 mm. in diameter, in the left 14 mm. The lining of the wall of the growth on the right was studded with discrete cauliflower protrusions 1 to 2 mm. high, with hemorrhagic tops and constricted bases, and there was a fleshy plaque at one spot, 3 by 2 mm. across and raised 0.5 mm. above the surface. Between the growths the wall was smooth and thin. The cauliflowers were all benign epithelial papillomas microscopically, and the plaque was a squamous cell carcinoma. Several tiny cysts lay near by in the muscle. These were cream-colored, that is to say devoid of *OSM*, and sections showed their thin walls to be lined with ordinary stratified squamous epithelium, and their contents to be concentric layers of keratin.

The big cyst in the left thigh had recently ruptured, with release of most of its pink contents into the subcutaneous tissue overlying the muscle. A football-shaped, molluscoid mass with constricted base projected inwards from its irregularly thickened wall. Microscopically this growth was a malignant papilloma. Several small, benign papillomas existed elsewhere on the cyst wall, as also a second cancer. Pieces of the molluscoid growth were transplanted to 10 sites in 10 new hosts and grew at every site, forming thick-walled masses measuring up to 24 mm. across after 41 days, when the mice were killed. Sections of the transplanted tumors showed them all to be composed of tissue (Fig. 10) like that of the original neoplasm. All were cystic, but in some instances the cyst cavity was very small.

TABLE I

Neoplastic Effects of Methylcholanthrene on Implanted Gastric Tissue

(Experiment 1: Stomachs of 20 mm. embryos)

Mouse No.	Leg	Time between implantation and		Portion of stomach implanted	Findings at autopsy
		sudden growth of nodule	death		
		days	*days*		
1	R	54	62		10 mm. cyst with pultaceous contents: *benign pap., squam. carc.* (transplanted*)
	L	47			6 mm. " " " " *2 benign paps., squam. carc.*
2	R	40	65		13 mm. " " " " *many benign paps., squam. carc.*
	L	47		**Squamous**	14 mm. " " " " *3 benign paps., malig. pap., squam. carc.* (transplanted‡)
3	R	54	76		20 mm. " " " " *squam. carc.*
	L	76			7 mm. " " " " *benign pap.*
4	R	54	76		13 mm. " " " " *2 squam. carcs.*
	L				6 mm. " " " " *benign pap., squam. carc.*
5	R		76		Two 4 mm. cysts " " " *benign pap., squam. carc.*
	L	47			16 mm. cyst " " " *malig. pap.*
6	R		46		Tiny columnar and squam. ep. cysts: *squam. carc. from one of latter*
	L	33			6 mm. fluid-filled cysts, devoid of *OSM: columnar ep. cysts*
7	R		65		Implant not found
	L	40			20 mm. cyst with pultaceous contents: *squam. carc., adenoacanthoma* (transplanted§)
8	R	33	76	**Glandular**	3 fluid-filled cysts, together 7 mm., devoid of *OSM: columnar ep. cysts, one tiny squam. cyst*
	L	33			Similar group, devoid of *OSM: columnar and squam. ep. only*
9	R	33	76		9 mm. cyst with pultaceous contents: *squam. carc., sarcoma*
	L	33			3 fluid-filled cysts, each 4 mm., devoid of *OSM: columnar ep. cysts, sarcoma*
10	R	54	76		13 mm. cyst with pultaceous contents: *2 squam. carcs.*
	L				Sarcoma
11	R		44		7 mm. group of cysts: *squam. carc.*
	L			**Glandular and squamous**	4 mm. " " " : *squam. ep. cysts*
12	R		63		10 mm. cyst with pultaceous contents: *squam. carc.*
	L				4 mm. fluid-filled cyst, 1 mm. cyst: *columnar ep. cyst, squam. cyst with carc.*

carc. = carcinoma   pap. = papilloma
ep. = epithelial   squam. = squamous
malig. = malignant

* Tumor grew progressively at all 20 sites in 10 new hosts.
‡ "   "   "   " " 10 " " 10 " "
§ "   "   "   " " 15 " " 10 " "

The remaining mice which had received squamous tissue and *OSM* were killed on the 76th day. Each of the 3 had in both hind legs a cystic nodule with ruddy contents, and there were tumors on the wall in every case (Table I). The growths were benign (Fig. 4) or malignant papillomas or frank squamous cell carcinomas more or less like those which had been transplanted.

The 5 mice implanted with glandular tissue yielded growths at 9 out of 10 sites. One animal died of intercurrent infection after 46 days. Its left leg contained several small cysts lined mostly with tall columnar epithelium but in a few places with tubular glands. The mucoid fluid in the cysts was colorless, giving no sign of the presence of *OSM*. In the right leg there were several cysts also, some lined by columnar, others by squamous epithelium, and on the wall of one of the latter was a growth only a millimeter across, but with the microscopic aspect of a squamous cell carcinoma.

The second mouse, killed after 65 days, had no growth in one leg, all the embryo fragments having died, but in the other the femur had been completely surrounded by a mass 20 mm. in diameter, which was cystic and had "pointed" and ruptured into the subcutaneous tissue of the groin, with release of much dye-stained, pultaceous matter. Most of the wall of the cyst was thin and smooth on the inner side, but there was a rugose, fleshy thickening in one region, with invasion of the muscle. Sections showed the thickening to consist of an actively invasive carcinoma, with many mitoses. Much of it had the morphology of an adenoacanthoma, in Stewart's terminology (5), but this graded into ordinary squamous cell carcinomatosis. A near-by tiny separate cyst, also with ruddy contents and lined with stratified squamous epithelium, had on its wall a papillary growth, another squamous cell carcinoma. Elsewhere in the muscle were some small cysts lined with columnar epithelium which had formed gastric glands. They appeared devoid of *OSM*.

Pieces of the adenoacanthoma were transplanted to both legs of 5 mice and to one leg of 5 others. Growths arose at all of the new sites, becoming as much as 28 mm. across within 23 days. They were cysts full of friable or pultaceous matter, with thick walls composed of adenoacanthomatous tissue like the primary tumor. Photographs of this growth have already been published (reference 2, Figs. 53 and 54).

The remaining 3 mice were killed 76 days after implantation. Small cysts, tense with clear, colorless fluid and lined with a single layer of columnar epithelium, were present in a leg of one animal. Their color gave no indication that they contained *OSM*, which lay in a separate globule. Most were lined with glandular epithelium, but one with stratified squamous. In the other leg were cysts having similar characters.

A second animal, killed after 76 days, had in one leg a cyst lined with stratified squamous epithelium, which showed a squamous cell carcinoma at one spot. Elsewhere in the cyst wall there was a spindle cell sarcoma, and nearby was a small cyst lined with columnar epithelium. The implant in the other leg yielded small cysts lined for the most part by columnar epithelium, which had formed gastric glands in some places; a sarcoma was also present in the neighboring tissue.

In one leg of a third mouse, killed after 76 days, a spindle cell sarcoma was present. In the other, a 13 mm. nodule had ruptured during the final palpation and much pink pultaceous matter was found lying free in the subcutaneous tissue. The partly collapsed cyst had thick firm walls, owing to the presence of two cancers as the microscope showed. Both were squamous cell carcinomas, differing somewhat in detail.

Of the 2 mice implanted with fragments of stomach containing both squamous and glandular epithelium, one was found dead on the 44th day. It had in one leg a group of small squamous cysts lined with stratified squamous epithelium and lying at a distance from the droplet of *OSM*. In the other leg also a group of cysts was present, and some *OSM* had been included in one of them as shown by its pink contents. From this one a squamous cell carcinoma had arisen (reference 2, Fig. 52). A similar tumor was found in one leg of the last mouse of the

group, which died of an intercurrent infection 63 days after implantation. Its other leg contained a mucous cyst lined with columnar epithelium and a squamous cyst from which a squamous cell carcinoma had derived.

It will be seen that growths with the morphology of carcinomas or of benign or malignant epithelial papillomas arose at every site where pieces of squamous stomach lining had been implanted together with *OSM*. This latter had regularly become encysted. Two of the cancers were transplanted and grew rapidly in the new hosts (Figs. 9 and 10). Where bits of the glandular portion of the stomach had been put, on the other hand, the carcinogenic material often lay at a distance from the epithelial cysts, and in such instances these were lined merely with glandular tissue or columnar epithelium. But whenever the *OSM* had been enclosed, tumors regularly arose; one of them, an adenoacanthoma, was transplanted and grew fast in the new hosts (reference 2, Figs. 52–54). At spots where tumors had not yet appeared on the cyst wall, it was often lined with a stratified squamous layer, not with glandular epithelium such as had been implanted.

Experiment 2 was carried out with gastric tissue from a litter of 7 embryos 18 mm. long. The stomachs were cut in two in the glandular region near the line of demarcation from the squamous tissue, and the portions thus separated were hashed separately. It follows that some glandular tissue was implanted with the squamous material. Seven mice served as hosts, 4 of them receiving in one leg bits of gastric tissue with Locke's solution, and in the other leg tissue with *OSSM*.

At the 6 sites where material had been put consisting mostly of squamous tissue with a little that was glandular, nodules 3 to 4 mm. in diameter had become palpable by the 12th day, irrespective of whether *OSSM* was present. In an instance of the latter sort the nodule suddenly enlarged before the 40th day, and for this reason the animal was killed. The nodule was 10 mm. long, made up of coalesced and coalescing cysts with ruddy contents. Sections showed at one spot an invasive squamous cell carcinoma (Fig. 11) containing many spaces lined with stratified epithelium, which in some places was only 1 or 2 cells deep. Elsewhere on the cyst wall was another invasive squamous cell carcinoma of somewhat different type (Fig. 12) while a third seemed only very recently to have arisen (Fig. 13). In the opposite leg, where the tissue had been introduced with Locke's solution, there was a cluster of tiny, discrete cysts, mere thin-walled sacs lined mostly with ordinary squamous epithelium 2 or 3 cells deep, but at an occasional spot with a glandular layer (Fig. 2).

A second mouse receiving the same material was found dead on the 72nd day. Only a few tiny, creamy cysts were present where the tissue had been introduced with Locke's solution, but in the leg implanted with tissue plus *OSSM* a cystic mass 20 mm. across had arisen, with thick ragged walls and pink pultaceous contents. The tissue was obviously malignant and had extended into the muscle. Sections were not made because of the advanced autolysis.

Mice 3 and 4 of the same group were killed after 92 days. They had been implanted in only one thigh, with tissue and *OSSM*, and they yielded dye-containing cysts 10 and 13 mm. across respectively. Sections showed in one case a squamous cell carcinoma, and in the other three distinct, well established cancers of this sort, with elsewhere on the cyst wall a benign and a malignant papilloma.

Mice 5, 6, and 7 had been implanted with fragments of glandular stomach. Nodules ap-

peared more slowly in their case, some not becoming palpable until 20 to 28 days had passed. No. 5 was killed after 42 days. Two translucent, cystic nodules, 5 and 7 mm. across, were then present in the leg where the tissue had been put with Locke's solution. Sections of these showed the usual lining with cylindrical epithelium and gastric glands (Fig. 3). There was no squamous epithelium anywhere. In the other leg where *OSSM* had been introduced with the tissue a multilocular, 9 mm. cyst was found (Fig. 5). Most of its lining consisted of a single layer of high cylindrical epithelium, grading at many places into stratified squamous epithelium, with a narrow belt of transitional epithelium between (Fig. 14). At one spot where this last was present a carcinoma of transitional type had arisen and extended into the adjacent tissue (Figs. 5 and 16). Nowhere did the lining consist of a thick, well-differentiated, glandular layer like that existing in the control cyst, though shallow abortive glands had formed at a few spots.

Nos. 6 and 7 were killed on the 92nd day. In No. 6 no trace could be found of the fragments implanted with *OSSM*, but a small group of thin-walled, fluid-filled cysts was present in the opposite leg where the material had been implanted with Locke's solution.

No. 7 had been implanted in one thigh only, with tissue and *OSSM*. The animal was killed on the 70th day because the resulting nodule had suddenly enlarged. It consisted of 3 cysts, in the aggregate 15 mm. across, none of them containing *OSSM*. The enlargement had been due to an accumulation of clear colorless fluid.

Experiment 3 was done with material from 5 embryos 15 days old (12 to 13 mm. in length). The stomachs, only 1.5 mm. long, were hashed *in toto* and implantations made with *OSM* in one leg of 5 hosts. Nodules appeared in all within 13 days, and in 4 instances they suddenly increased in size between the 20th and 55th days, enlarging progressively thereafter. On the 84th day, when the animals were killed, the nodules ranged from 7 to 15 mm. in diameter, and they proved to be cysts filled with pink-stained, pultaceous matter, thin-walled in most regions, but thickened and fleshy, or with cauliflower growths, at some spots. The microscope showed tumors in every instance, benign and malignant papillomas (Fig. 20) and carcinomas, as in the preceding experiments. In some of the nodules there were several of these last, all of squamous cell type and one of them markedly anaplastic.

Experiment 4. A litter of 6 embryos 18 days old (18 to 19 mm. long) was utilized. The squamous and glandular portions of the stomachs—which averaged 3 mm. in length—were cut apart and implanted separately, with *OSM*. Squamous tissue was put in both posterior thighs of 5 mice, and glandular fragments in 4 more. Nodules arose at every site. As in the previous experiments they formed soon, and some underwent sudden enlargement after a time. The findings are summarized in Table II.

Of the 5 mice that received squamous stomach 3 were killed 43 days after implantation and 1 after 44 days. At one site the single cyst was tiny and the red oil lay at a distance: it was not examined microscopically. In every other instance the nodules proved to be cysts containing keratin or mushy pultaceous material, stained red. They had irregularly thickened walls, nodulated or with mounds, hassocks, or cauliflower excrescences projecting into them. Microscopically the growths were papillomas and carcinomas like those induced in the preceding tests with the squamous portion of the stomach. Sometimes they were multiple.

The fifth mouse was killed 76 days after implantation. Much larger cysts with pink pultaceous contents were present in both legs. They had squamous cell carcinomas thickening their walls.

Of the 4 mice receiving glandular tissue, 1 died of intercurrent causes 44 days after implantation. In its right thigh was a cyst 15 mm. across, full of mucoid fluid containing droplets of *OSM* and a little blood. The cyst was lined at a few spots with abortive tubular glands,

but for the most part with a single layer of columnar cells or with stratified epithelium which in some regions was keratinizing. In the left leg there was a sausage-shaped mass 5 mm.

TABLE II

Neoplastic Effects of Methylcholanthrene on Implanted Gastric Tissue

(Experiment 4: Stomachs of 18 to 19 mm. embryos)

Mouse No.	Leg	Time between implantation and		Portion of stomach implanted	Findings at autopsy
		sudden growth of nodule	death		
		days	*days*		
1	R	33	43		4 mm. keratinized cyst: *beginning cancer?*
	L				6 " cysts, 1.5 cm. in aggregate: *benign pap.*
2	R		43		4 mm. cyst with pultaceous contents: *2 squam. carcs.*
	L	33			Many keratinized cysts, 5 mm. in aggregate: *2 squam. carcs., benign pap., malig. pap.*
3	R	33	43	Squamous	3 " " 11 " " " *2 squam. carcs., malig. pap.*
	L	"			8 mm. cyst with pultaceous contents: *squam. carc.*
4	R	44	44		5 " keratinized cyst: *squam. carc.*
	L				Tiny " " (no sections made)
5	R	54	76		12 mm. cyst with pultaceous contents: *2 squam. carcs.*
	L	40			3 cysts " " " 16 mm. in aggregate: *squam. carc.*
6	R	26	44		15 mm. fluid-filled cyst: *columnar ep. cyst*
	L	"			5 " group of creamy cysts: *squam. carc.*
7	R	"	45		10 " fluid-filled cyst, 2 mm. keratinized cyst: *squam. carc. from the latter*
	L	33			10 " " " " tiny " " *squam. carc. from the latter*
8	R	26	76	Glandular	19 mm. mass of cysts, some filled with fluid, others with pultaceous matter: *squam. carcs. from the latter*
	L	"			12 mm. mass of cysts, some filled with fluid, others with pultaceous matter: *2 squam. carcs. from the latter*
9	R	40	76		7 mm. mass of cysts, some filled with fluid, others with pultaceous matter: *squam. carc. from the latter, sarcoma*
	L	"			13 mm. mass of cysts, some filled with fluid, others with pultaceous matter: *5 squam. carcs., and anaplastic carc.*

long, composed of several ruddy cysts lined with squamous epithelium, from one of which a carcinoma had invaded the near-by tissue.

A second mouse was killed on the 45th day. Each of its thighs contained a 10 mm. cyst filled with colorless fluid, and there were some smaller cysts as well which contained keratin. In each leg a squamous cell carcinoma had derived from one of the keratinizing cysts.

Another animal was killed on the 76th day.   It had in one leg a 19 mm. mass composed in part of thin-walled cysts, lined with a single layer of columnar epithelium and containing colorless, watery fluid.   There were other cysts lined with stratified squamous epithelium,— from one of which a carcinoma of this type had arisen,—and still others, much the largest, which were lined with cylindrical and stratified squamous epithelium, grading into each other through the intermediate stage of a transitional layer.   They were full of pink, pultaceous material, and a squamous cell carcinoma was found in the wall of one.   In the other leg was a 12 mm. mass of cysts, some of them lined with squamous epithelium—from which 2 squamous cell carcinomas had arisen—and some with transitional epithelium, columnar epithelium, or a mucous membrane containing perfect gastric glands, equipped with parietal and chief cells. The cysts of this last sort contained colorless fluid, that is to say were devoid of OSM.

The remaining mouse, killed after 76 days, had a group of cysts 7 mm. across in the right leg, with the diverse characters of those just mentioned.   One had a pink, pultaceous content, with a squamous cell carcinoma in its wall and a spindle cell sarcoma as well.   In the opposite leg a 13 mm. cyst had formed which ruptured into the subcutaneous tissue at the final palpation, liberating much semisolid, necrotic material pink with OSM.   The cyst wall was thickened almost everywhere by anaplastic carcinomatous tissue of stratified squamous derivation.   Near-by was a smaller cyst, thin-walled, containing colorless fluid and lined with tall columnar cells and many tubular glands.   In this leg there were present as well 2 smaller cysts containing OSM and lined with squamous epithelium.   From one of them 5 distinct squamous cell carcinomas had arisen.

Several more experiments of similar sort were carried out which need not be detailed.   In one of them tiny individual pieces of stomach were implanted, with very small globules of $OSSM$.   Tumors appeared late.   One of them which had taken origin from glandular tissue was transplanted and flourished in the new hosts (Figs. 15, 17, and 19).   In these experiments tumors arose in every instance in which the methylcholanthrene was encysted by the gastric epithelium and this tissue survived.

The control implants of glandular stomach with Locke's solution yielded clusters of discrete cysts full of colorless, more or less mucoid fluid, as has already been stated.   At many spots the cyst lining consisted of a thick layer of well differentiated tubules with characteristic chief and parietal cells, while at others the wall was covered with a single layer of tall columnar cells (Fig. 3) showing large secretory globules.   Nowhere was there any transitional or stratified squamous epithelium.   Sometimes the cysts suddenly enlarged after a while, a sign of the presence of a tumor in the case of epidermal cysts or those derived from the squamous stomach, but owing merely to a rapid secretion of fluid by the lining epithelium in the present instance.

The findings were very different where oil containing Scharlach R and methylcholanthrene had been encysted by proliferating tissue from the glandular part of the stomach.   Fully differentiated glands developed only occasionally then; as a rule any forming were abortive and shallow (Fig. 5).   The cyst lining consisted mostly of tall cylindrical cells, but at many spots these were undergoing, or had undergone, metaplastic change, first to a transitional

epithelium—which, to judge from its small amount, represented a passing phase —and then to stratified squamous epithelium that formed keratin actively (Fig. 14). Cysts in which this metaplasia had been everywhere completed contained ruddy concentric layers of keratin when only small tumors existed on their walls, but pultaceous or necrotic tissue when the growths were large. With a single exception, in which a carcinoma of transitional type had arisen, the tumors arising from implants of the glandular portion of the stomach were all of stratified squamous type and resembled morphologically those derived from the squamous portion of the stomach. They were frequently multiple, were invasive and obviously malignant. No papillomas of any sort occurred, such as frequently arose from implants of squamous stomach tissue.

The implants of tissue from the squamous stomach, together with methylcholanthrene, yielded benign and malignant papillomas as well as squamous cell carcinomas. These last resembled morphologically those derived from the metaplastic glandular elements.

The carcinogenic preparations employed in every experiment described thus far contained Scharlach R in addition to methylcholanthrene. A test was therefore made to ascertain what rôle the dye played. This test had the more importance because Scharlach R has been shown to cause hyperplasia of the squamous portion of the stomach of adult rats (6) comparable to that which it induces in the skin (4, 6), and sometimes mimicking cancer.

Experiment 5. The stomachs of 7 embryos 20 mm. long (18 days old) were pooled and hashed as such. Eight mice were implanted, in one hind leg with gastric tissue together with olive oil saturated with Scharlach R (*OSS*), and in the other with this tissue plus the same oil containing 1 per cent of methylcholanthrene as well as the dye (*OSSM*).

The first mouse was killed after 17 days. Groups of small cysts with red contents had formed in both legs. They were similar in the gross but the microscope disclosed marked differences. Where *OSS* was present the cysts were like those resulting from implantations with Locke's solution, thin-walled structures lined with a shallow layer of flattened squamous epithelium, but in some spots with tubular glands or cylindrical epithelium. The cysts containing *OSSM*, on the other hand, had a lining of extremely hyperplastic, stratified epithelium up to 14 cells thick, from which strands of cells extended toward neighboring small globules of *OSSM*. Some of these latter had already been surrounded by the epithelium while others were in process of conveyance into the interior of the cyst as differentiation and keratinization took place of the epithelial cells round about, just as happens when the cyst lining consists of embryo epidermis (reference 1, Figs. 18–20).

Similar changes were encountered in 2 mice killed on the 21st day, but in another killed on the 28th day extension to the globules of *OSSM* had ceased. The squamous cysts at the *OSS* sites in these animals were thin-walled, and the microscope showed their lining to be moderately hyperplastic but orderly.

The fifth mouse was killed 35 days after implantation. In the leg receiving *OSS* clusters of tiny, keratinized cysts were found, and a few larger, thin-walled cysts filled with clear, watery fluid and lined partly with tubular glands, partly with columnar epithelium, next to a large reservoir of *OSS*. The other leg, where the tissue had been put with *OSSM*, contained a firm ovoid mass 7 mm. across. Red oil ran out of this when nicked, and it proved to be a

solitary cyst lined with hyperplastic, stratified squamous epithelium, irregular along the base. At one spot on this lining there had arisen a pedunculated, cauliflower papilloma covered with a thick layer of stratified squamous epithelium which looked benign. The growth filled most of the cyst cavity. A near-by cyst was lined in part with a single layer of columnar epithelium from which glands had derived in one area. But as the columnar epithelium was followed around the cyst wall, it was found to heap up into several layers, like the transitional epithelium of Fig. 14, and then to become stratified and squamous. No such metaplasia was found in any of the cysts containing *OSS* and lined with glands and columnar epithelium, though the specimens were sectioned serially and searched.

In 2 mice killed on the 86th day tumors were also found where *OSSM* had been introduced with the gastric tissue. The growth in one was a large, almost solid, squamous cell carcinoma 15 mm. across, in the other a 15 mm. cyst walled with carcinomatous tissue of the same type. Only innocuous thin-walled cysts were found at the *OSS* sites. Some of the cysts containing *OSSM* were lined everywhere with stratified squamous epithelium and full of keratin, while others showed partial metaplasia of the glandular epithelium as in the case just described. When this had been completed in some regions but not in others the cyst contained both keratinized tissue and fluid.

The last mouse was let live until the 129th day after implantation. A large cyst into which a malignant papilloma projected, practically filling it, was found in the leg where the tissue had been put with *OSSM*. In the opposite leg, where *OSS* was present, there was a cluster of tiny, thin-walled cysts, some lined with squamous tissue, others with columnar epithelium or glands. All had a pink color, attesting to enclosure of the *OSS*, pools of which lay next them. Despite this intimate exposure to Scharlach R for an extended period, not the least suggestion of neoplastic change was come upon. As in the mice killed after 86 days, the squamous epithelium exposed merely to Scharlach R was not hyperplastic, but had formed a lining 2 or 3 cells deep with a regular border against the muscle. The glandular epithelium had fully differentiated.

In this test the Scharlach R in olive oil did not induce neoplastic changes in the implanted gastric tissue of embryos (Table III). The epithelium grew around and encysted the oil globules, and the walls of the resulting cysts became slightly thicker than those deriving from control implants with Locke's solution, but the epithelial activities were transitory, not seen in specimens examined after 35 days or more. In these latter the cysts were thin-walled and resembled in other ways those due to implants with salt solution. The transitory hyperplasia induced by Scharlach R corresponds to its effect upon implanted embryo epidermis (1).

The findings were very different when *OSSM* was present. It stimulated the epithelium of the squamous portion of the stomach to active proliferation, tongues of cells extended to surround outlying globules of oil, and these eventually became incorporated in the cysts—which had a hyperplastic, stratified squamous lining. The glandular epithelium showed no such activity, and failed to encyst most of the oil. This last finding will account for the relative frequency of sarcomatous change throughout the experiments in which glandular tissue was implanted together with methylcholanthrene. Metaplasia was a common occurrence, whereas it never took place in response to Scharlach R alone.

Several other tests yielded the same general results. In one of them oil

was employed containing methylcholanthrene without Scharlach R (*OM* instead of *OSSM*). It was found to have as much stimulating and attracting effect on the epithelial cells of the squamous portion of the stomach as *OSSM*. A squamous cell cancer induced by *OM* was transplanted to 10 sites in 5 new hosts, and formed large, progressively growing tumors at each site. The growth was carried successfully through 3 subsequent generations of new

TABLE III

Comparison of the Effects of Scharlach R and Methylcholanthrene (OSSM) with Those of Scharlach R Only (OSS)

(Experiment 5: Stomachs of 20 mm. embryos)

Mouse No.	Time to death	Implants with OSS	Implants with OSSM
	days		
1	17	4 mm. group of cysts: *thin squam. lining*	5 mm. group of cysts: *cyst lined with columnar ep.; others with hyperplastic squam. ep.*
2	21	3 " " " " *hyperplastic squam. lining; columnar ep. cyst, some gland formation*	4 " " " " *same general findings*
3	21	7 " " " " " "	5 " " " " " " "
4	28	6 " " " " " "	10 " " " " " " " ; *glands at some spots*
5	35	14 " " " " *Large fluid-filled cysts lined by glands; a few tiny squam. cysts*	7 " solitary cyst *benign pap.*
6	86	6 " " " " *some cysts lined with squam. ep., others with columnar ep. and glands*	15 " " " *squam. carc.*
7	86	8 tiny cysts: " "	15 " " " almost filled with solid tumor: *squam. carc.*
8	129	8 mm. group of cysts: " "	25 mm. solid mass: *malig. pap., sarcoma*

hosts (Fig. 18), and then transplantation of it was discontinued. The glandular portion of the stomach also reacted as if to *OSSM*, becoming metaplastic and undergoing neoplastic change.

Functional Activities of the Glandular Lining of the Cysts

The tubular glands present in the control implants of glandular tissue and Locke's solution had characteristic chief and parietal cells, and the tall columnar epithelium lining the cysts in other regions contained large secretory globules.

Not infrequently one of the cysts became a centimeter or more across as time went on, and several were tapped and the colorless fluid they yielded was tested for digestive enzymes and hydrochloric acid. The fluid quickly reformed. Tests were made also of the contents of cysts containing *OSSM*. In the latter instances the mice were killed immediately after the tapping, the state of the cysts and the presence or absence of tumors was duly noted, and representative specimens were fixed and sectioned.

The pH of the aspirated materials was determined colorimetrically. Töpfer's indicator was used in tests for free hydrochloric acid, and the persistence of the yellow color was taken to mean that it was absent. In a few instances total acidity was determined by titration to phenolphthalein with N/100 NaOH. Peptic activity was tested by adding 0.1 cc. cyst fluid to 0.2 cc. of 0.4 per cent HCl, to which had been added a few fragments of fibrin got by whipping rabbit blood. The same batch of fibrin was used throughout, preserved frozen. Digestion of the fibrin was considered a positive finding (7). The presence of rennin was sought by adding 0.1 cc. cyst fluid to 1 cc. skimmed milk with 1 cc. acetate buffer (pH 5); a positive test was indicated by clotting of the milk (7). In both the pepsin and rennin tests, the tubes were held in a water bath at 40°C., together with controls containing salt solution instead of cyst fluid, and they were read at intervals up to 5 hours. The cyst fluids were somewhat syrupy, and the globules in the tall columnar cells stained red with carmine showing that they contained mucin. Neither in a control cyst tapped on the 42nd day, nor in cysts containing *OSSM* (6 specimens tapped after 50 to 92 days) was any free hydrochloric acid found. The total acidity of the fluids from 2 cysts containing *OSSM* (as evidenced by ruddy droplets), which were aspirated after 50 and 54 days respectively, was equal to 0.04 and 0.05 cc. N/10 HCl, on titration with phenolphthalein. Pepsin and rennin were present in the fluids from all of the control cysts and also in those of the cysts containing *OSSM*. One of these last had a squamous cell carcinoma on its wall.

The failure to find free hydrochloric acid led to the carrying out of a further experiment.

Fluid was obtained from the cysts of 3 mice implanted 57 days previously with a suspension in Locke's solution of bits of glandular gastric tissue procured from embryos 17 to 18 mm. long. To stimulate the secretion of hydrochloric acid 2 of the mice had been fasted for 21 hours, and given bread soaked in meat extract 4½ hours prior to the tapping. The third mouse was fasted 17 hours, then given meat extract and tapped 2½ hours later. The procedure described is known to stimulate the secretion of acid into completely denervated stomach pouches of dogs (8). No free acid was found in the present instances, but the pH of the cyst fluids was lower than in previous tests—6.5, 7, 7.2, as compared with 7.7 to 8.8. Tests for pepsin and rennin were positive.

In view of the fact that peptic activity is sometimes exhibited by autolyzed tissue not derived from the stomach, tests were made of the viscous, brownish fluid present in the cystic centers of 3 large, squamous cell carcinomas obtained by implanting fragments of mouse embryo lungs together with *OSSM* (3). The tissue lining the cysts was necrotic. No peptic action of the fluids was demonstrable, though one of them gave a faint test for rennin. The pultaceous contents of a carcinomatous cyst which arose from an implant of squamous stomach lining also gave a faint test for rennin, but was devoid of peptic action.

These experiments made plain that the cells lining the cysts formed after implantation of the glandular portion of the embryo stomach produce digestive enzymes, and that they may do so even in the presence of sufficient methyl-

cholanthrene to bring about neoplastic changes at some spots on their walls. None of the cysts contained free hydrochloric acid though sections showed parietal cells to be present, often in abundance. Mucus, which had accumulated in the closed cysts, is known to be capable of buffering the acid. The gastric pouches of dogs, into which acid was secreted, drained to the outside.

Special Histological Features

The changes occurring in the cysts formed after implantation of bits of the squamous part of the stomach, together with methylcholanthrene, differed in several respects from those resulting from the proliferation of transplanted embryo epidermis. In both instances the stratified squamous epithelium lining the cysts was soon rendered hyperplastic by the carcinogen, but the gastric epithelium sometimes desquamated while still only partially keratinized, instead of differentiating into a cornified layer as in cysts lined with epidermis and those resulting from the implantation of the gastric tissue together with Locke's solution. There was no marked accumulation of small round cells in the connective tissue immediately next the hyperplastic epithelium, such as took place in the wall of epidermal cysts containing methylcholanthrene, though a few scattered elements of the sort could be seen. Occasionally keratinizing carcinomas arose which could not be told from those of epidermal origin, but the tendency to early desquamation was accentuated in most of the cancers and the living layer of malignant cells was shallow in consequence (Figs. 10 and 12), indeed sometimes only one cell thick. Hydropic swelling was not infrequent in the period just prior to desquamation (Fig. 9). Most of the carcinomas were primary, but some derived from papillomas.

The early effects of OSSM on the glandular epithelium were singular. It caused metaplasia as already described, bringing it about so swiftly that it had been nearly or quite concluded, with result in a stratified squamous layer, before the first neoplastic change took place. The failure to obtain frank adenocarcinomas would seem attributable to this circumstance, not to any lack of neoplastic potentialities on the part of the glandular epithelium; for Stewart and Lorenz (5) succeeded in getting adenocarcinomas, as well as many adenoacanthomas, by the injection of methylcholanthrene into the stomach wall of mice. In the present work only one adenoacanthoma was procured—which proved readily transplantable (reference 2, Figs. 53 and 54) —and one carcinoma (Fig. 16) composed of transitional epithelium such as was briefly present during the course of the metaplasia. It may be that adenocarcinomas would have been got had the cells been exposed to the carcinogen in amounts which brought about metaplasia more slowly.

The technique of separating the glandular tissue for implantation was devised with the special aim of excluding any cells derived from the squamous portion of the stomach, and its validity was proven by the absence of squamous and transitional epithelium from the walls of the cysts due to the control

implants of glandular tissue. The stratified squamous epithelium which was the end result of the metaplasia frequently elaborated layers of keratin and in other ways resembled morphologically that of the squamous portion of the stomach, yet papillomas never arose from it. The difference cannot be attributed to peculiarities of the submucosa, since the cyst walls were nearly everywhere devoid of this tissue, owing to its failure to proliferate and accompany the epithelium as this extended laterally and formed the cysts.

The fleshy, plump core of one of the benign papillomas arising from a cyst due to the implantation of bits of stomach together with *OSM* (Fig. 6) consisted of large proliferating cells, apparently neoplastic, which had the general aspect of ganglion cells (Fig. 21). Such cells are notably abundant in human teratomas (9). Those encountered in the present instance were probably derived from the intrinsic autonomic plexus of the stomach.

Experiments with Mice of the I Strain

In mice of the I strain an extraordinary hyperplasia of the glandular portion of the stomach, sometimes resulting in obstruction, is known to develop in a very high percentage of adults (10). It seemed possible that adenocarcinomas might be produced in this breed by the procedure employed in the present work, so a colony was raised from a group of young I animals generously provided by Dr. L. C. Strong. In the mice thus obtained the glandular hypertrophy was far less pronounced than that described by Stewart and Andervont (10). Only in very old individuals did it become noteworthy, and it never caused the gastric lining to more than double in thickness, and this only near the line of demarcation from the squamous stomach. Nevertheless, several experiments were carried out in which bits of the glandular gastric tissue of I embryos were implanted, together with *OSSM*. No hyperplasia resulted of the glandular epithelium as such nor did any adenocarcinomas arise. A squamous cell carcinoma due to the implantation of bits of the squamous portion of the stomach together with methylcholanthrene was transplanted to 10 adults of I strain and grew progressively in them all.

DISCUSSION

The gross and microscopic characters of the growths originating from the gastric tissue, together with the successful transplantations, justify the conclusion that true neoplasms resulted from the action of the methylcholanthrene. None of the tumors had embryonic features, a fact scarcely surprising since enough time elapsed before the first neoplastic change became noticeable for the cells to have matured. In the control implants they did so during this time.

The tumors were procured far more rapidly than when adult mice are fed carcinogenic hydrocarbons or injected with them. Many months usually

elapse before any growths are noted in such experiments, even when methyl-cholanthrene has been introduced into the gastric wall (11). But in most of the reported instances the stomach has been examined by palpation through the abdominal wall, and only large growths can be perceived in this way. Furthermore, the conditions prevailing in the cysts due to implantation of gastric tissue from the embryo were highly favorable to the action of the carcinogen. Papillomas or carcinomas, often both, were found in 36 out of 39 instances in which pieces of the squamous portion of the stomach of C strain mice had been implanted together with methylcholanthrene. In the 3 instances in which tumors failed to arise, the oil was separated from the cysts by muscle. No papillomas developed where implants of the glandular portion of the stomach had been put, but carcinomas were found at 17 out of 32 sites. The glandular epithelium was not attracted toward the oil containing methyl-cholanthrene as was the squamous, and often failed to encyst the carcinogen. This fact will sufficiently account for the smaller incidence of tumors. It is possible that the cells of the embryo stomach are more susceptible to neoplastic change than those of the adult, but the present work provides no evidence on the point. It does away, however, with the idea, based on the findings in adult animals, that the epithelium of the glandular portion of the mouse stomach is refractory to the influence of the chemical carcinogens. Carcinomas speedily took origin from this tissue.

The experiments had two main objects, to learn whether distinctive morphological changes precede gastric cancer—in view of the prevailing uncertainty on this point—and to gain data bearing on current theories of tumor causation. The precancerous happenings in the squamous portion of the stomach have only a special interest since no comparable structure exists in man; and the glandular epithelium underwent metaplasia very early, as already stated, and no adenocarcinomas occurred. Metaplasia is frequently encountered in cancerous human stomachs, but it only occasionally goes so far that stratified squamous epithelium is formed (12, 13) and that cancers of this type arise, as was the common finding in mice. In man the chief and parietal cells not infrequently disappear from the glands, and the tubules become shorter, changes which have been termed "intestinalization;" but these alterations are not peculiar to the precancerous state, taking place under various other pathological conditions.

The mouse tumors were all consequent upon local, discontinuous, neoplastic changes, occurring abruptly here or there in a hyperplastic epithelial layer (Fig. 13). The most careful scrutiny of this layer disclosed no forerunning alterations to indicate the spots at which such changes would take place. The only "precancerous state" perceptible was a general epithelial hyperplasia. This finding is the more remarkable because local "precancerous" changes—epithelial disorder, marked variations in cell and nuclear size, pronounced baso-

philia and round-cell accumulation—are ordinarily to be seen in the lining of the epidermal cysts that result from the implantation of embryo skin and methylcholanthrene (1).

The fact that in the mice no benign papillomas arose from the stratified squamous epithelium consequent on glandular metaplasia, whereas such growths often took origin from the epithelium of the squamous portion of the stomach, shows how different the potentialities can be of tissues which appear alike morphologically. In work soon to be reported on the results of implanting embryo lung tissue together with methylcholanthrene in adult mice, it has been found that the pulmonary carcinomas arising from bronchial epithelium which has undergone metaplasia to a stratified squamous layer often differ strikingly from any growths thus far observed as deriving from the morphologically similar layers of epidermal or gastric origin. It may be remarked in this general relation that none of the gastric carcinomas formed of metaplastic glandular elements showed any signs of secondary change to the adenomatous form, such as Kirschbaum has reported as occurring in the squamous cell carcinomas which arise from adult mammary tissue rendered metaplastic by methylcholanthrene (14).

No effort has been made to learn how soon the first neoplastic changes take place in the gastric epithelium; but well established papillomas and carcinomas have been encountered after 35 and 40 days respectively, that is to say, as soon as in the case of embryo epidermis. This finding makes it probable that neoplastic changes may occur within less than 4 weeks after the implantation, as happens in the case of the epidermis (1).[2] The rapidity and regularity of the occurrence of such changes, when the right conditions are provided (Tables I and II), and the diverse character of the resulting growths put large obstacles in the way of supposing that they can be due to transmitted viruses with the specialized character of those now known to cause neoplasia. The matter has been discussed at some length in connection with the epidermal growths of embryonic derivation (2).

SUMMARY

Epithelial tumors have been readily obtained by the implantation of embryo stomach tissue together with olive oil containing methylcholanthrene (with or without Scharlach R) in adult mice of homologous strain. The implanted tissue from the squamous portion of the stomach rapidly encysted the oil, and benign and malignant papillomas and squamous cell carcinomas soon arose from the stratified squamous lining of the cysts. Bits of the glandular

[2] During recent work, as yet unpublished, a squamous cell carcinoma 2 mm. across was found in the wall of an epidermal cyst due to the implantation 23 days previously of embryo skin with methylcholanthrene. The experimental study of other growths of similar origin and morphology (1, 2) and of control specimens leaves no doubt that it was a true neoplasm.

portion of the stomach also formed cysts, but the gland cells underwent metaplasia in response to the carcinogen, altering first to transitional epithelium and then to a stratified squamous layer. So swiftly did these changes take place that nearly all of the tumors took origin from epithelium that had already become stratified and squamous, and the growths themselves were of this type. A single transitional cell carcinoma and an adenoacanthoma were procured, but no adenocarcinomas; nor did any benign papillomas develop, though they often resulted from the action of methylcholanthrene on the squamous portion of the embryo stomach. Search failed to disclose any distinctive precancerous changes in the gastric tissue. Five of the cancers were transplanted and they grew in every host.

No tumors arose from any of the numerous control implants. Those consisting of glandular tissue formed cysts lined partly with columnar epithelium secreting mucus and partly with tubular glands equipped with chief and parietal cells in good condition. Pepsin and rennin were found in the fluid contained in these cysts, but no free hydrochloric acid. The enzymes were present also when the cysts contained methylcholanthrene and the glands had not yet been wholly replaced by metaplastic epithelium.

The tumors appeared months sooner than when methylcholanthrene is injected into the stomach of adult animals or given by mouth; some of them were well established after 5 or 6 weeks. They arose regularly when the requisite experimental conditions were provided. The utilization of transplanted embryo tissue provides a means whereby gastric tumors free from bacterial infection can be procured swiftly and easily.

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EXPLANATION OF PLATES

All the sections were stained with eosin and methylene blue. Mr. Joseph B. Haulenbeek made the photographs.
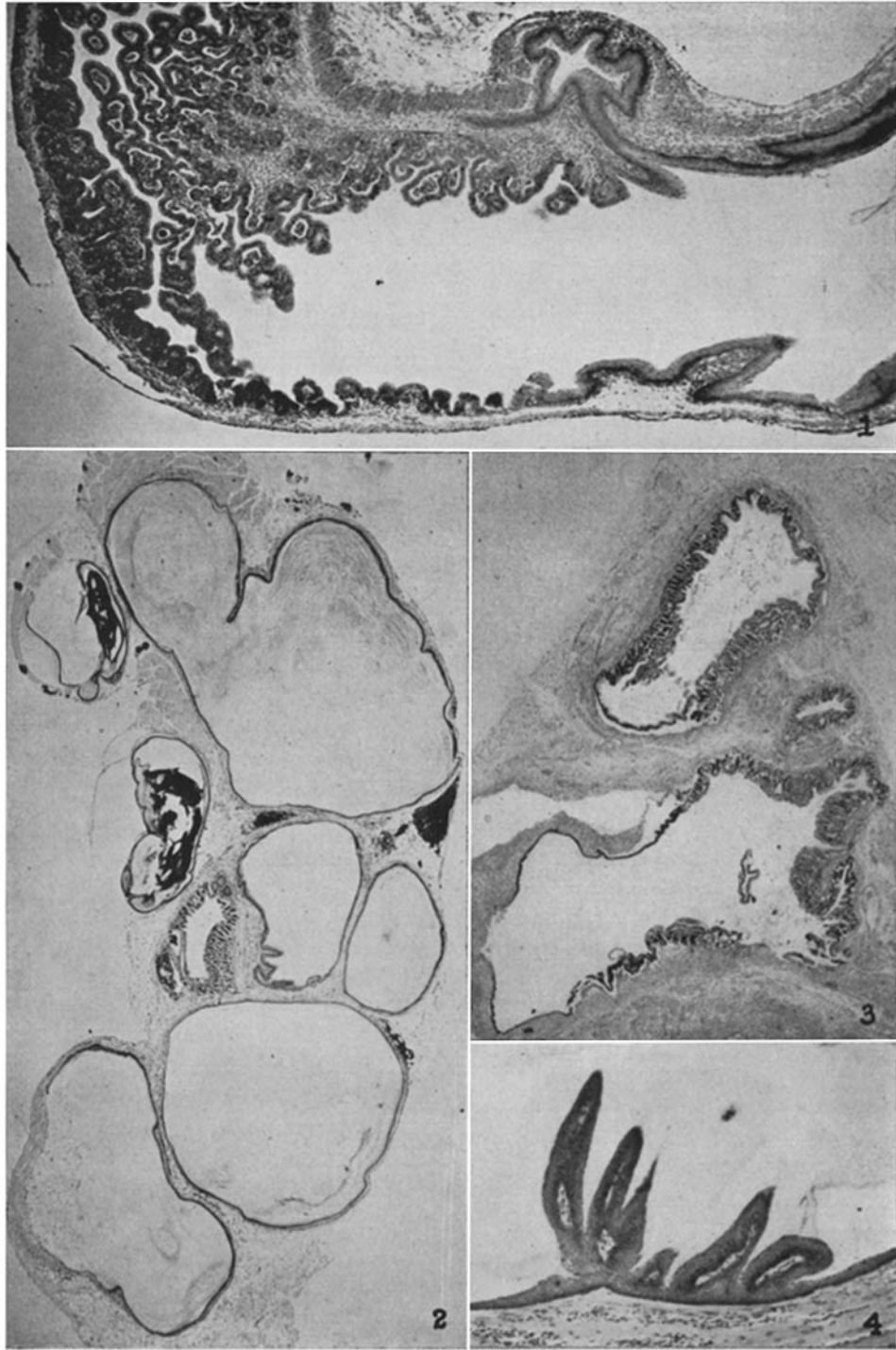
PLATE 25

FIG. 1. Longitudinal section through the stomach of a 20 mm. mouse embryo at the junction of the squamous and glandular epithelium. The glands are as yet only partially differentiated. The esophagus can be seen cut across, near the top of the photograph.   × 55.

FIG. 2. Sagittal section of a nodule due to the implantation 40 days previously of bits of the hashed stomachs of 18 mm. embryos, together with Locke's solution. The multiple cysts have remained separate. Most of them have a thin lining of stratified squamous epithelium and are full of concentric layers of keratin (scarcely visible in some cases, at others dark with methylene blue); but one is lined with a thick, glandular layer.   × 22.

FIG. 3. Cysts resulting from the implantation, with Locke's solution, 42 days previously of fragments of the glandular portion of the stomachs of 18 mm. embryos. The cysts are lined mostly with tubular glands, but in some places with a single layer of tall, cylindrical epithelium, as higher magnification showed. This did not disclose any squamous epithelium.   × 30.

FIG. 4. Benign papilloma on the wall of a cyst which had resulted from the implantation 76 days previously of fragments of the squamous part of the stomachs of 18 day (20 mm.) embryos together with OSM.   × 65.
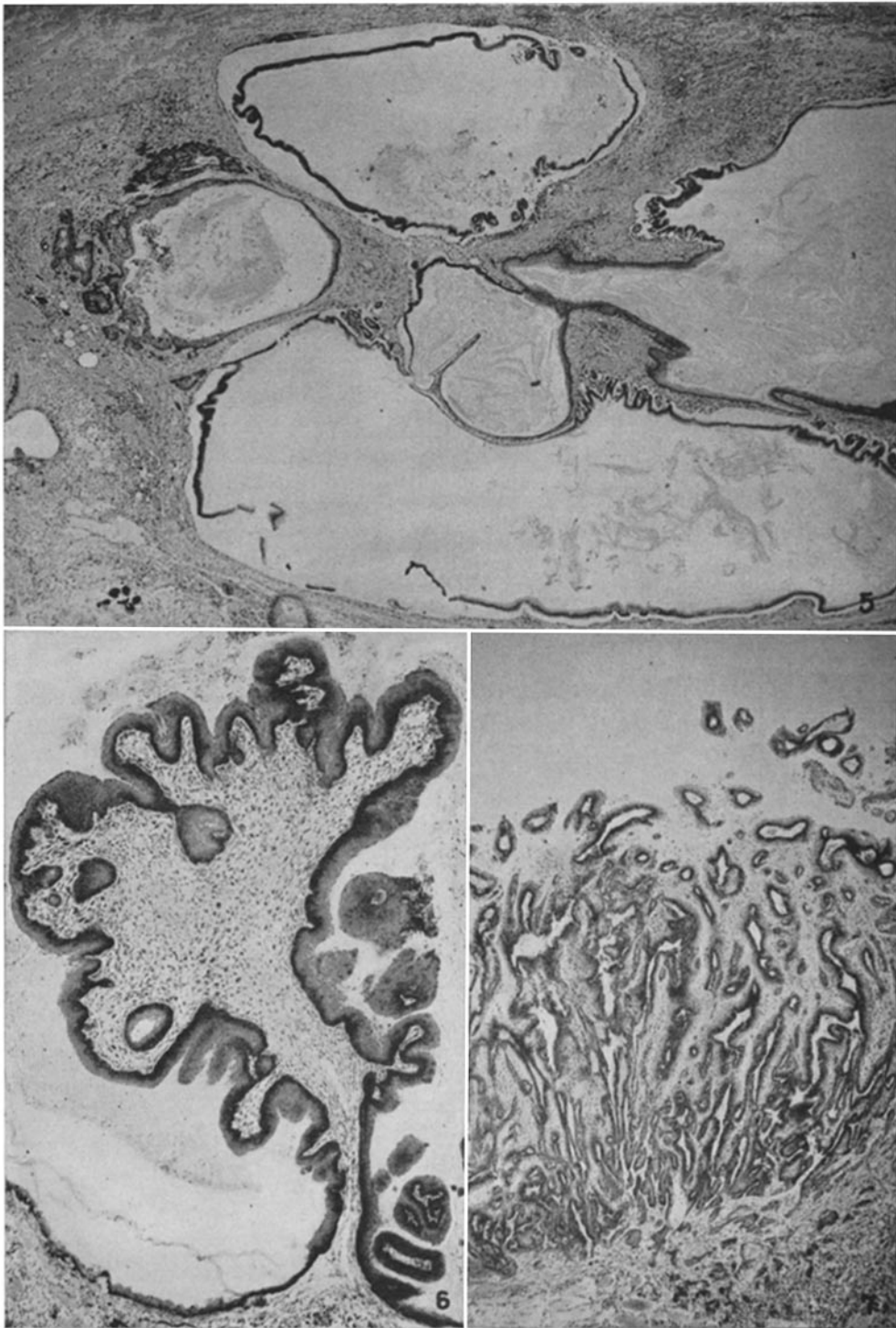
PLATE 26

FIG. 5. Nodule resulting from implantation of glandular stomach tissue with *OSSM*. The growth is from the opposite thigh of the animal furnishing Fig. 3. The cysts opened into one another, as serial section showed. They were lined at a few spots with ill-developed glands, but elsewhere with cylindrical epithelium that graded into a stratified squamous layer (see Fig. 14). Toward the left of the photograph an invading carcinoma has arisen. $\times$ 55.

FIG. 6. Benign papilloma due to an implantation of tissue from the squamous part of the stomach with *OSM*. The mouse was killed after 62 days. The growth, like that of Fig. 4, is from an animal of Table I. It connects by a narrow pedicle with the wall of the cyst in which it is contained. The large cells visible in the core of the tumor looked like ganglion cells that had become neoplastic; they were in mitotic division here and there (see Fig. 21). Elsewhere on the cyst wall a carcinoma was present (Fig. 7). $\times$ 75.

FIG. 7. Malignant papilloma invading along the base; from the cyst wall furnishing the growth of Fig. 6. The tumor proved readily transplantable (Fig. 9). $\times$ 40.

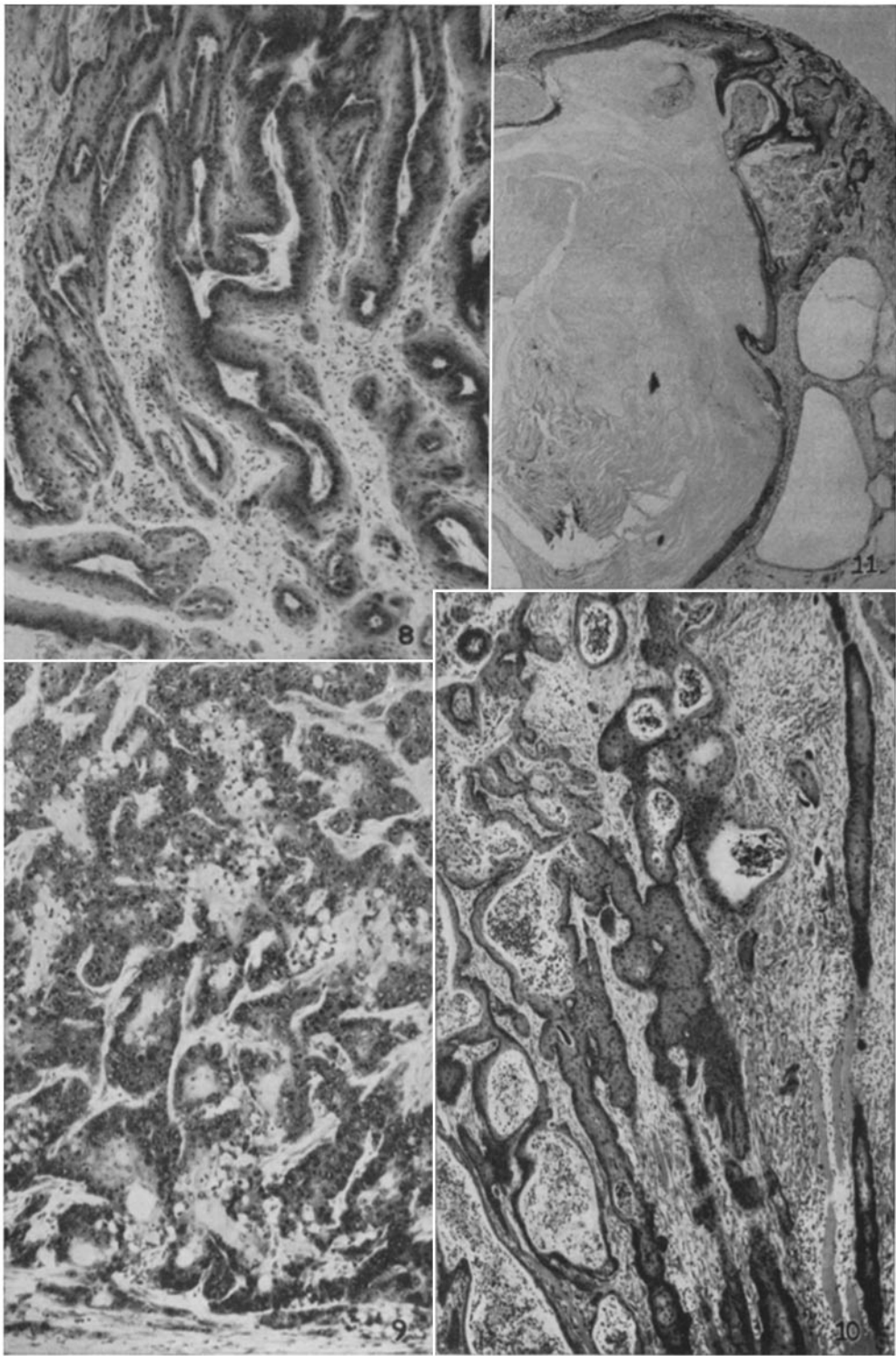(Smith: Neoplastic potentialities of mouse embryo tissues.   III)

PLATE 27

FIG. 8. Higher magnification of part of the growth shown in Fig. 7. Here it is a squamous cell carcinoma desquamating before keratinization has been completed. × 100.

FIG. 9. Part of a rapidly growing tumor which resulted from the transfer to new hosts of pieces of the growth pictured in Figs. 7 and 8. The morphology of the neoplastic cells is very different. Many have undergone hydropic degeneration. × 125.

FIG. 10. Replacement of muscle fibers by a squamous cell carcinoma derived from the squamous portion of the stomach. The tumor was transferred to new hosts 65 days after the original implantation of embryo tissue, together with *OSM*, and the picture is from one of the resulting growths removed after only 11 days. × 58.

FIG. 11. Squamous cell carcinoma extending out from the wall of a cyst that had resulted from the implantation 40 days previously of the hashed gastric tissue of embryos (see *Experiment 2*). Most of the tissue had come from the squamous portion of the stomachs but a little of it was glandular; hence the precise epithelial derivation of the tumor is uncertain. The empty spaces just outside the cyst wall had contained *OSSM*. There were two other minute carcinomas elsewhere on the wall (Figs. 12 and 13). Nothing remotely resembling any of them was present in the cluster of cysts resulting from a control implantation in the opposite thigh of the gastric tissue with Locke's solution (see Fig. 2). × 33.

(Smith: Neoplastic potentialities of mouse embryo tissues.   III)
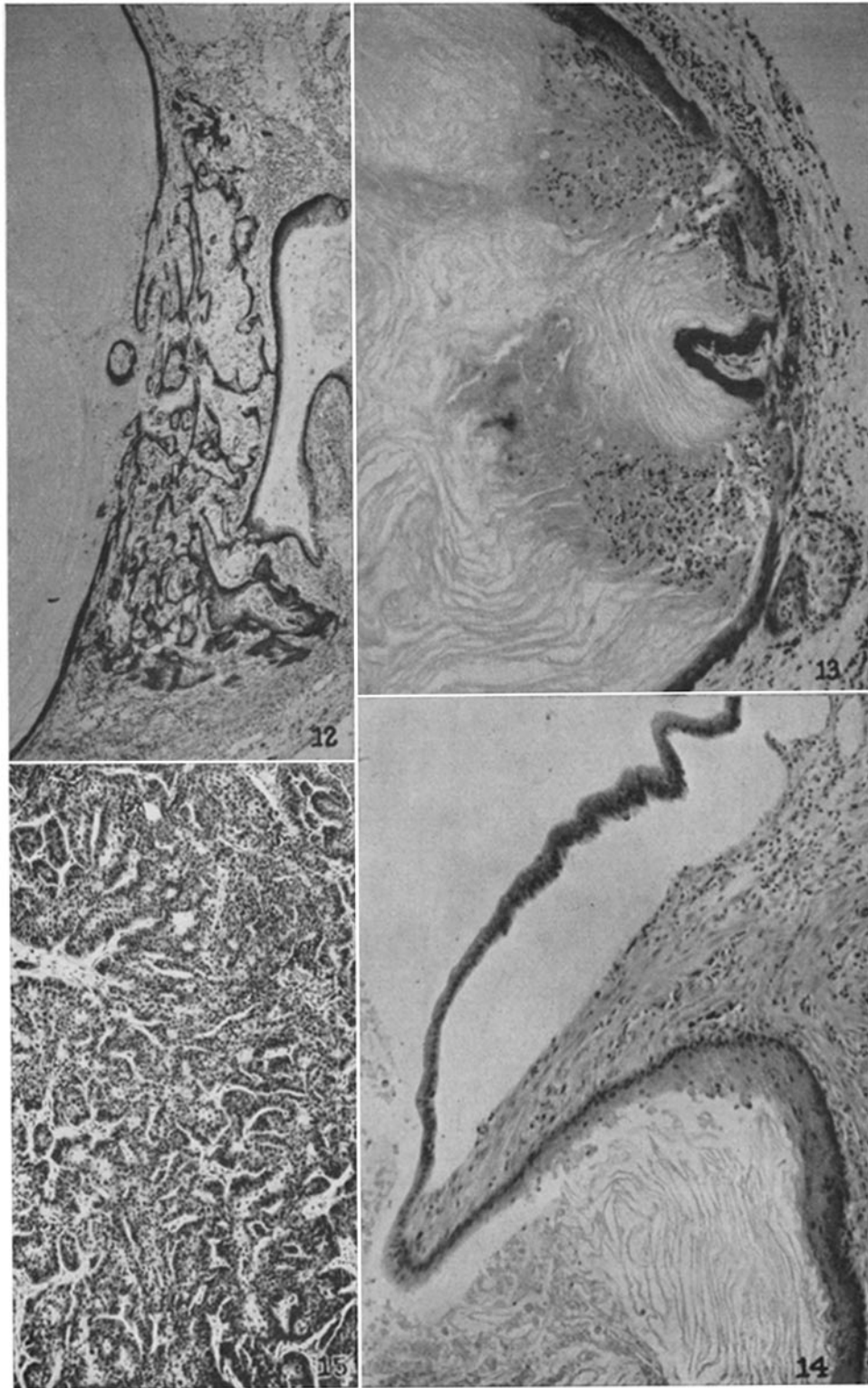
PLATE 28

FIG. 12. One of the other carcinomas just referred to as extending out from the wall of the same cyst. In most places the cancer cells are no longer keratinizing but are dying early and desquamating. × 45.

FIG. 13. The third carcinoma. It has only begun its extension from the diffusely hyperplastic cyst wall, but had been present some time as evidenced by the cellular debris that has come away into the cyst contents. Where the epithelium is not neoplastic stratified layers of keratin overlie it. Tongues of the neoplastic tissue have extended under a projecting fold in the cyst wall. × 95.

FIG. 14. Metaplasia of glandular epithelium to a stratified squamous layer, under the influence of OSSM (Experiment 2); from the multilocular cyst pictured in Fig. 5. A few patches of abortive glands were present in the latter, but most of the cyst lining consisted of cylindrical epithelium. At the spot now pictured this epithelium goes over first into a transitional layer, and then takes on the stratified squamous character. These changes find expression in the cyst contents. Keratinized layers lie immediately over the stratified squamous layer, but there are desquamated cells above them as if such differentiation had not previously taken place. A few cells have been cast off by the transitional epithelium now existing. The space over the columnar epithelium appears empty because only fluid was here. The knife has pulled the columnar layer away from the supporting connective tissue. × 130.

FIG. 15. Growth resulting from transplantation to a new host of a squamous cell carcinoma that originated from implantation of a tiny piece of the glandular portion of the stomach of an 18 day embryo together with OSSM. × 60.
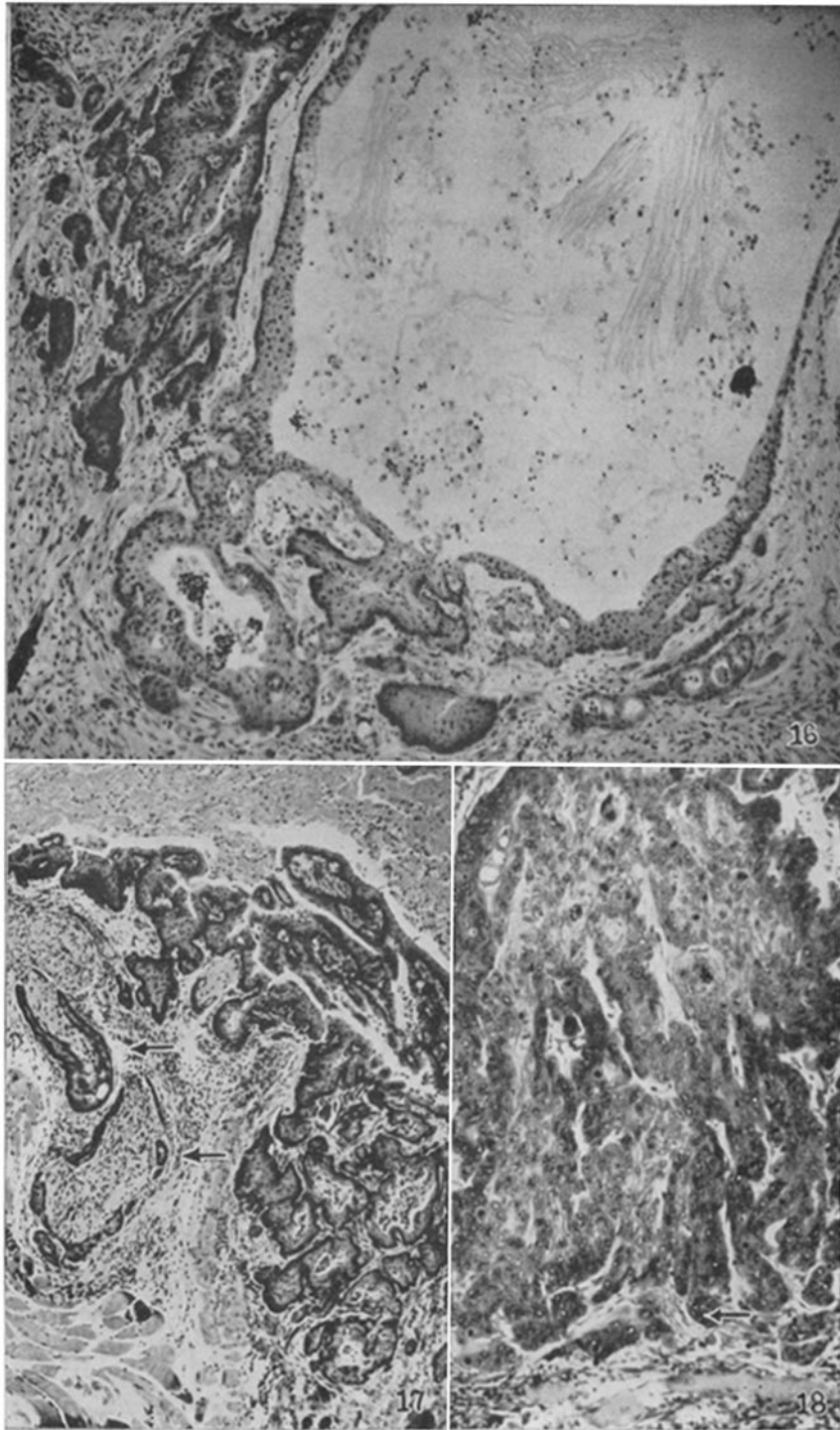
(Smith: Neoplastic potentialities of mouse embryo tissues.   III)

FIG. 16. Carcinoma consisting of transitional epithelium; the same growth pictured in Fig. 5. × 85.

FIG. 17. Squamous cell carcinoma resulting from the implantation 176 days previously of a tiny piece of the glandular portion of the stomach of an 18 day embryo together with *OSSM*. The cancer has extended from the cyst wall amidst the muscle fibers and grown around two large nerves (arrows). A similar growth from the opposite thigh of the same animal was transplanted (Fig. 15). × 58.

FIG. 18. Margin of a carcinoma growing in the third successive mouse to which it had been transplanted. The tumor originally sprang from the epithelium of the squamous portion of the stomach, and the first transfer of it was made 78 days after implantation of the embryo tissue, together with *OM*. The cancer is invading and replacing muscle fibers (arrow). × 130.
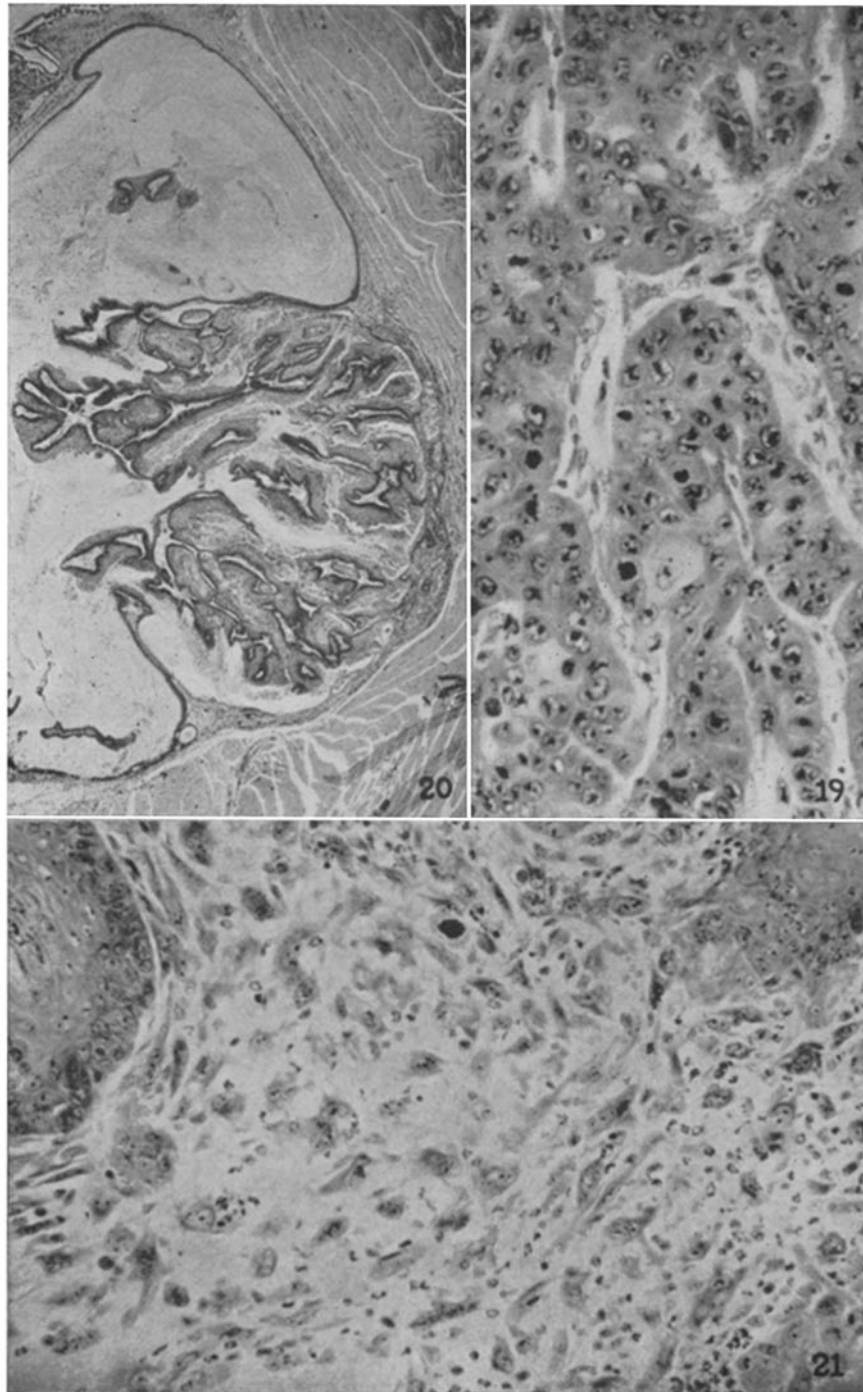
(Smith: Neoplastic potentialities of mouse embryo tissues.   III)

PLATE 30

FIG. 19. The transplanted growth of Fig. 15 at a higher magnification—to show the very numerous mitoses.   × 325.

FIG. 20. Malignant papilloma projecting into a cyst which had resulted from the implantation of hashed embryo stomachs, together with *OSM* 84 days before.   The growth was anaplastic along its base, as higher magnification showed, and was invading the muscles.   × 20.

FIG. 21. Core of the papilloma of Fig. 6, to show what appear to be ganglion cells that have undergone neoplastic change.   One of them is in mitosis.   × 265.

(Smith: Neoplastic potentialities of mouse embryo tissues.   III)