THE NEOPLASTIC POTENTIALITIES OF MOUSE EMBRYO TISSUES*

I. The Findings with Skin of C Strain Embryos Transplanted to Adult Animals

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Plates 33 to 39

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The conception that tumors arise from cells of embryonal character lying latent in the organism until intercurrent factors call forth their inherent capabilities occupies a large place in the history of cancer research. The clinic is continually propounding this hypothesis. Human instances have made plain not only that the adult body now and again contains fetal remnants but that from them tumors may arise, often growths of extreme malignancy. Sometimes they are composed of cells resembling embryonal elements and very occasionally they are tridermal in composition, as if the original neoplastic change had taken place very early and affected elements possessing the potentialities of all the germ layers. Yet as against these facts, which might be taken to bespeak some special liability to tumor formation, there stands the failure of a great host of efforts to produce genuine neoplasms by the implantation of embryonic tissues in other individuals. Again and again these tissues, however stimulated experimentally, have formed nothing more than self-limited teratoid growths. True, a neoplasm has occasionally arisen (1); but the very rare carcinomas have occurred under equivocal conditions, while the sarcomas might well have derived from the stroma supplied to the implant by the host, as critics have repeatedly pointed out (2). In attempting further tests of the potentialities of embryo cells it is obviously essential to select a tissue which can be readily discriminated from the structures of the new host, which does well after transfer, and which has proved capable in the adult state of undergoing neoplastic change. The epidermis of the mouse is such a tissue. The experiments to be reported here show that when the skin of mouse embryos is implanted in the voluntary muscles of adults of the same inbred strain and continually exposed to the action of methylcholanthrene benign and malignant epidermal tumors rapidly take origin from it.

General Plan

A first need in the experiments was to bring the embryo epidermis into direct contact with the carcinogen while shielding the adjacent connective tissue from

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its sarcoma-inducing influence. Advantage was taken of the tendency of implanted skin to round up into cysts lined with epidermis, as further of the influence of Scharlach R in olive oil to attract epidermal cells and cause them to proliferate and enclose the oil (3). It seemed likely that if bits of embryo skin were implanted, together with oil containing Scharlach R and methylcholanthrene, some of the droplets would be included primarily in the forming cysts and extension would take place to others outside, with result that many of them would be incorporated too. The methylcholanthrene would thus be brought to bear on the epithelial lining of the cyst while the surrounding connective tissue would be walled off to some extent from its influence. Such was the actual course of events.

Material and Methods

The minced tissues of mouse embryos of mixed breed usually die within a few weeks when transplanted to adults (4), and hence closely inbred animals were employed. The skin fragments were implanted in the thigh muscles.

C strain mice were chosen as a notably homogeneous breed, on the advice of Dr. C. C. Little, and a big colony was raised from young individuals supplied by the Jackson Laboratory. The C strain is the product of brother and sister matings for more than fifty generations (5), and the assumption that embryo tissues would do well after transfer within the strain has been borne out by the results.

The embryos of a single female were utilized in each experiment. At first their age was inferred from the length from forehead to rump, but later the gestation period was determined by the vaginal plug method until a correlation had been made with the length when this again was relied upon.[1] The pregnant mouse was decapitated, the uterus removed with due care for asepsis, and in all save the earliest experiments it was opened with a cautery knife. After removal of an embryo the placenta separated rapidly with some hemorrhage, but by having a thick layer of absorbent gauze under the uterus any danger that the next embryo to be removed might be contaminated with maternal blood could be avoided. As soon as each of them was procured it was washed in Locke's or Tyrode's solution, in two or three changes ordinarily. Embryos 18 mm. long (17–18 days old), were taken for the bulk of the work, as providing enough material for many implantations, and the skin was peeled off inside out, like shirt and trousers, after a cut had been made across the back. That of fetuses 13–15 mm. long (16 days old) was flimsy, stripping with difficulty, and only patches could be obtained from embryos 11–12 mm. long (15 days old). No sufficient separation of it was possible with smaller ones and hence they were cut fine,—after the brain had been pressed out and the viscera removed in most cases. For this purpose a curved forceps was inserted back of the heart, after the body cavities had been opened, and their entire contents torn out in a mass. Occasionally prior to hashing embryos 7–8 mm. long (12 days old) the skin was separated from the musculature at some points so that it might the easier round up into cysts. The youngest embryos employed were approximately 10½ days old (3–3.5 mm. long). They were handled with a pipette or section lifter and eviscerated under a dissecting microscope.

[1] Embryos of identical age often differed in size, both from litter to litter and those of the same uterus. For example the length of one set of 7 embryos 15 days old ranged from 11–13 mm., whereas another set of 7 were all 15 mm. long.

The tissue was minced with knives until it could be drawn up into an injecting syringe through an 18 or 20 gauge Luer needle. The addition of a few drops of salt solution facilitated this. Enough skin could be got from a big litter of 18 mm. embryos for more than 30 implantations without any addition of saline, whereas that of embryos 10–11 mm. long had to be suspended in a relatively large amount of fluid if it was to be placed in only a few hosts. As a rule the implants consisted of 0.025 cc. of minced skin or suspension, together with an equal amount of olive oil containing Scharlach R and methylcholanthrene, or,—in the case of controls,—0.025 cc. of Tyrode solution or oil containing only the dye. In a few of the early tests the skin fragments were so thoroughly mixed with the oil that they nearly all failed to become vascularized and for this reason died. Later the materials were layered in the syringe barrel in such wise that the oil followed the tissue on injection, and this was done forcibly to scatter the materials and make a pocket in which the oil would lie without running back along the needle track. When injection was slow the oil and tissue often lay separate from each other. The best results were had when 0.075 cc. of Locke's or Tyrode's solution was interpolated between the oil and the embryo hash in the syringe. Under these circumstances such dispersal of the fragments took place at the implantation site that the oil came into contact with them only secondarily as the salt solution was absorbed.

The mammary tissue of the new hosts had to be thought of as a complicating factor since squamous cell carcinomas sometimes take origin from it (6) and it is widely distributed over the bodies of female mice. To avoid it young male mice of 19–26 gm. were nearly always selected as hosts and the implants were placed in the thigh muscles after a slit had been made to expose them. The skin was incised over the calf of the leg, the wound swabbed with 1:1000 mercuric chloride solution to remove or kill any loose epithelial cells which might else be carried in with the needle, and then it was plunged into the calf muscles and thrust up through the tissues on the outer side of the knee or deep within the popliteal space until the middle of the posterior thigh was reached. During the injection the mouse was held head down so that the oil would lodge lower than the fragments, since autopsies showed that it sometimes worked toward the hip; and prior to withdrawing the needle it was gripped between finger and thumb together with the tissues, to prevent backflow. With practice the injections could be made into almost precisely the same spot, wholly within the muscles, time after time. Usually an implant was placed in each thigh, and when any comparison was to be made of differing materials in the same individual their situation was alternated from right to left in the group of animals.

To learn whether the needle touched mammary tissue anywhere, the injection procedure was carried out on some parturient mice with functioning breasts, and they were killed and dissection done while the needle was still in place. Nowhere had it come in contact with the mammary tissue. In two experiments implants were put in the subcutaneous tissue of the axillae and groins as well as in the thigh muscles, to learn the relative fitness of these situations. The results were poor at the subcutaneous sites, the embryo skin soon ceasing to grow.

The implants were not palpated for 9–14 days to avoid disturbing the new vascularization. They had by then in most instances given rise to firm nodules, which were charted weekly afterwards in their approximate size and shape, with notes on special features,—always by the same person. After a mouse was killed the muscle overlying the nodules was clipped away to expose them, and they were described and in most instances sketched, prior to fixation in acid Zenker and the cutting of representative sections. Often the whole mass was sectioned serially. Large thin-walled cysts with pultaceous or fluid contents were sometimes nicked, emptied, pressed flat, and rolled into cylinders which were tied about with thread, fixed, and cut into segments for sectioning. The stains were eosin and methylene blue.

In some early experiments 1 per cent solution of methylcholanthrene (20-methylcholanthrene: Eastman Kodak Co.) in olive oil was found to cause cancer much sooner than 0.5 per

cent, and hence it was used thereafter. It proved close to the maximum tolerated by the embryo epidermis. A single lot of olive oil was employed throughout. The presence in it of much Scharlach R seemed desirable since the extension of epidermal cells to oil globules containing the dye is known to stop when it is absorbed; so for most of the experiments 1 per cent of methylcholanthrene was added to the oil and then it was supersaturated with Scharlach R (Grübler) by heating it with an excess of the dye in boiling water for approximately an hour. It was tubed while still hot in 1 or 2 cc. amounts, with subsequent autoclaving, and the tubes were stored at room temperature. When one was to be used it was placed in boiling water to redissolve the dye, one-third its volume of Locke's solution was added, and shaking was done with glass beads in a mechanical shaker for three-quarters of an hour. During this time many crystalline spicules of Scharlach R came out, but they remained in the oil phase (continuous phase) of the emulsion,—which did not separate until after much longer than the injections took. This constituted the standard carcinogenic material. It broke up readily when more salt solution was added and brief shaking done by hand, with result in many tiny oil droplets to which the dye crystals remained attached. For certain experiments oil was used containing methylcholanthrene only (1 per cent), but at autopsy it was difficult to find because of its pale yellow hue and hence in some later tests 1 per cent of Scharlach R was added to serve as a tell tale. No emulsification with Locke's solution was done in these instances.

The following abbreviations will be used in telling of the experiments:

OSSM—Olive oil containing 1 per cent of methylcholanthrene, supersaturated with Scharlach R and shaken with one-third its volume of Locke's solution.

OSS—A preparation generally similar but containing the Scharlach R only.

OM—" " " " " " " methylcholanthrene only.

OSM—Olive oil containing 1 per cent of methylcholanthrene and 1 per cent of Scharlach R.

Hemorrhage occasionally took place into the cysts which arose from the implants, making it uncertain whether Scharlach R was present also. The point was settled by searching for red globules of oil under the microscope, or by crushing the specimen in water whereupon they rose to the surface. Ultraviolet light was employed to find droplets devoid of dye but containing methylcholanthrene, these fluorescing a rich purple. The fluorescence is masked by Scharlach R but it emerges when acetone is added, diluting the dye. Olive oil itself does not fluoresce.

At first there was much variation from the procedures outlined. It was thought that the amount of *OSSM* introduced with the tissue might not suffice to induce neoplastic change, so attempts were made to inject more of it into the epidermal cysts after a time. In other instances half the standard quantity of *OSSM*, or even less, was injected with the tissue fragments and they were suspended in much Locke's solution: cancer rarely followed. In one test mineral oil (Soconol) was used instead of olive oil, 2.0 per cent of methylcholanthrene was dissolved in it, and it was shaken with six parts of 6 per cent gum acacia in 0.9 per cent salt solution and injected in the usual 0.025 cc. amounts together with the embryo tissue. No growths were obtained.

One large group of mice, some of them females, were injected in one thigh with *OSSM* alone in almost twice the usual amount, and in the other thigh with twice as much skin as usual (from 15 mm. embryos). This was done to learn whether the carcinogen would act from a distance on embryo skin, and the *OSSM* was not injected until 5 days after the tissue in order to rule out any possibility that it might reach the latter directly through the accidental penetration of a vessel by the injecting needle. The mice were kept for months yet no carcinomas arose anywhere, only sarcomas in some instances where the *OSSM* had been put.

Many of the tumors were transplanted. For this purpose the skin of young adult C mice, mostly males, was slit over the haunch and a piece of the neoplastic tissue about a millimeter across was deposited by means of a sterilized trocar in the posterior muscles of each thigh.

Results with the Skin of Embryos Near Term

The skin of fetuses 18 or 20 mm. long (about 17 and 18 days old) was tested first.

In *Experiment I* the minced skin of 20 mm. fetuses was implanted, in both the thigh muscles and the subcutaneous tissue of the axillae and groins of 3 of 4 new hosts. *OSSM* was put with the tissue fragments at all these sites on one side of the body, whereas for the other side a preparation was employed which was generally similar but contained only 0.5 per cent of methylcholanthrene. Oblong nodules 2–4 mm. in length formed within a fortnight at the injection sites, but they scarcely grew thereafter until the 55th to 77th days when some of those where the tissue with *OSSM* had been placed suddenly became spherical and in the course of a week enlarged to about 10 mm. across on the average.

One of the mice was killed on the 84th day. The spherical mass proved to be a cyst with a fleshy, ragged wall enclosing moist, grumous material, ruddy pink with Scharlach R. Sections of the wall showed six invasive, histologically distinct growths which had taken origin from its epidermal lining and appeared carcinomatous, one of them having oat-shaped cells. In a second animal an attempt was made on the 75th day to inject a small implantation cyst with more *OSSM* (0.1 cc.) through a skin slit. It enlarged rapidly from then on, and when the mouse was killed on the 111th day had extended into the subcutaneous tissue anterior to the groin and was 25 mm. across, nodular, and fluctuating at some spots (Fig. 1). Cross-section showed the mass to consist of three large cysts containing turbid, brownish-red fluid. In the intramuscular region the cysts were walled with fleshy tissue which had grown out amidst the muscle fibres, but where they had extended into the loose subcutaneous tissue their walls were very thin. The microscope showed the fleshy tissue to consist of several more or less confluent growths having the morphology of carcinomas, one of them wholly anaplastic (Fig. 2); and where the needle of the supplementary injection had passed there was a spindle cell sarcoma, this serving at some spots as stroma for one of the carcinomas. Transplantation of the neoplastic tissue into 10 mice (both thighs) resulted in rapidly enlarging nodules at all 20 sites (Tumor 14, Table I). Within a few weeks they had become huge, bulging into the subcutaneous tissue as roughly spherical, fluctuating growths, blue-domed as seen through the skin, owing to hemorrhage into them. A few of the host mice were killed early and the others died of the tumors. These consisted of one to several ragged-wall cysts containing pultaceous dead tissue and clear or bloody fluid, with much solid neoplastic tissue between and about them in the intramuscular region (Fig. 3). Several neoplasms had been successfully transplanted,—a malignant papilloma, the very aggressive, anaplastic, pleomorphic carcinoma of Fig. 2, another nearly resembling a spindle cell sarcoma, a typical squamous cell carcinoma, and also in some cases the real sarcoma mentioned above. All of these tumors had been present in the tissue utilized for transplantation, as sections disclosed. Some of the resulting growths contained only one or two of them.

In the original host the axillary implant with *OSSM* had been found to contain a minute growth with the morphology of a carcinoma, but no neoplastic changes were present at the other injection sites. However, a third mouse, killed on the 109th day, had what appeared to be a tiny carcinoma where the embryo tissue had been placed together with the preparation containing 0.5 per cent of methylcholanthrene.

For control purposes 4 animals had been implanted at the intramuscular and subcutaneous sites of one side with embryo skin plus an equal quantity of Locke's solution and on the other side with skin plus *OSS*. Small nodules developed which never became neoplastic.

The success of the transplantations proved that the larger growths which had the morphology of neoplasms were truly such. Wholly similar growths

too small for such test had also arisen where the embryo tissue had been exposed to methylcholanthrene, whereas nothing of the sort could be found on section of the control implants or in the controls of later experiments. Hence the conclusion seems warranted that these small growths were tumors too and others of the sort will from this point on be referred to as such.

Fragments of 20 mm. embryo skin plus *OSSM* were injected into one thigh of 13 mice, and skin plus Locke's solution or *OSS* into the other thigh. In 2 of 4 animals killed on the 42nd day the nodules containing *OSSM* showed microscopic carcinomas, while in a third mouse, killed on the 55th day, a spherical cyst had formed with ruddy pultaceous contents and a thick ragged wall from which multiple small cancers were extending into the muscle. In a fourth animal sacrificed on the 61st day no growths were encountered; but a fifth, examined on the 69th day, had a cyst 10 mm. across walled with anaplastic, carcinomatous tissue. Mice

T

The Tumor

Tumor No.	Experiment No.	Mouse No.	Embryo length	Tumor		Character of neoplasms transplanted	Hosts	Implants	Fate of imp		
				First noted	Transplanted after				Grew	Regressed	S vi
			mm.	*days*	*days*						
1	37	*6*	11–13	38	45	Benign, pedunculated pap. with anaplastic carc. at one spot in it	11	15	2		
2	19	*10*	8*	26‡	70	Benign pap.	5	10			
3	14	*12*	18	59	60	Malig. pap.	5	10	1	9	
4	15	*12*	18	68	71	" " —becoming squam. carc.	5	10	2	8	
5	"	*14*	18	89	91	" " " " pleomorphic carc.	11	22		22	
6	19	*4*	8*	90	100	Malig. pap.—becoming squam. carc.	7	14	9	5	
7	7	*16*	20	54	69	Anaplastic carc.	4	8	8		
8	14	L§ *15*	18	45	97	" "	5	10	8	2	
9	15	L§ *15*	18	68	99	Moderately anaplastic carc.	5	10		5	
10	15	R§ *15*	18	82	99	" " "	6	12			
11	18	*3*	7–8*	55	65	Composite: malig. pap., squam. carc.	5	10	8	2	
12	15	*13*	18	40	76	" " " " pleomorphic carc., anaplastic carc.	5	9	6	2	
13	14	R *15*	18	59	97	Composite: 5 distinct carcs., 1 carcinosarc.	10	20	19		
14	1	*6*	20‖	73	111	Composite: malig. pap., pleomorphic carc., squam. carc., carcinosarc.	10	20	20		
15	18	*7*	7–8*	83	92	Composite: carcinosarcoma	10	20	20		
16	2	*8*	12¶	92	105	" squam. carcs., sarcoma	9	18	9	2	
17	28	*4*	3.5¶	80	89	Spindle cell sarcomas	6	11	11		
18	8	*11*	11	94	103	" " "	5	10	10		

* Mince of embryos used from which the viscera and brains had been removed.

‡ Early enlargement probably due to secretion into cysts lined with mucous membrane of gastrointestinal tract.

§ Tumor in other thigh also transplanted. R, L,—right and left thighs respectively.

6 and 7, killed on the 75th and 82nd days, had spherical carcinomatous cysts about 6 mm. across at the sites where *OSSM* was present, while in No. 8 there was a cyst 20 mm. across by the 105th day with seven distinct, invasive carcinomas in its wall. The other 5 animals had died of intercurrent causes soon after implantation.

In the same experiment 4 additional mice received skin plus *OSSM* in one thigh, and *OSSM* alone in the other,—where it caused no growths. But one nodule resulting from skin plus *OSSM* became spherical within 20 days, grew steadily thereafter, and by the 55th day, had a diameter of 13 mm. Autopsy now disclosed a cyst full of red, pultaceous material, with a nodular wall in which were 9 distinct, well established carcinomas (Fig. 4), as serial section showed, and many others just beginning to extend from the epidermal layer in which they had originated. An adjacent tiny cyst showed a tenth cancer.

A second mouse killed on the 55th day had small cysts with a lining that was cancerous at one spot. In a third animal a nodule suddenly enlarged and became spherical between the

lantations

ed	Character of new tumors	Remarks
	Original anaplastic carc. Tiny cysts lined with benign pap. tissue and ordinary adult epithelium	Primary host 9 days old at implantation
	Like original " " " "	Cysts 4–12 mm. across prior to regression. The tumor supplying the grafts was enveloped in a horde of lymphocytes Cysts 11–20 mm. across regressed " 4–15 mm. across prior to regression
	Malig. pap., squam. carc., separately or together	In one host both grafts regressed
	Like original " " " " " "	In the animal in which the tumor survived it was found at one tiny spot The regressing tumors were in different hosts Cysts 3–14 mm. across regressed. Primary tumor appeared as malignant as that in other thigh
	Like originals " all 3 originals: also spindle cell sarc. All originals grew, severally or together All originals; also spindle cell sarcoma and a carc. nearly resembling it Mostly sarcomas; a few carcinosarcomas Benign pap., 2 carcinomas, carcinosarcoma, sarcoma	Regressions were in same mouse " " " " " Most hosts poorly nourished because of lung disease New tumors extremely vigorous. Was the 20th site implanted? Sarcomatous tissue predominated in hosts killed last The sarcoma outgrew the carcinoma " " " " carcinomas
	Like original " "	

imate length.
of whole embryos used.

47th and 54th days, and on the 69th it was football-shaped and 12 mm. long, with a wall everywhere 2–3 mm. thick, consisting of anaplastic carcinomatous tissue. Bits were implanted in both thighs of 4 young male C mice where they gave rise in every case to tumors which enlarged with enormous rapidity, becoming as much as 22 mm. across in 14 days (Tumor 7, Table I). Much of the enlargement was due to fluid accumulation, the growths proving to be more or less spherical cysts walled with carcinomatous tissue like that of the original neoplasm.

The fourth mouse of the group was thin and sick when killed. At about the 61st day the nodule where embryo tissue and *OSSM* had been put began to enlarge rapidly but later grew scarcely at all, and it was only 7 mm. across, a cyst with ruddy, keratinized contents, when the animal was killed after 75 days. The wall of the cyst was very thick, however, and showed several cancers microscopically.

No sarcomas arose in any of the animals.

The findings were consistent in these experiments and in others described further on. Where skin had been implanted together with Locke's solution or *OSS* small sausage-shaped or oblong nodules formed, consisting of cysts lined with ordinary epidermis and full of lamellated, dry keratin embedded in a matrix of connective tissue. By the end of 2 weeks they were flattened and 3–7 mm. long, thereafter growing very slowly. The nodules were only slightly larger at first where *OSSM* had been placed with the fragments, but as early as the 20th day, though more often at about the 40th day or within the next fortnight, many of them rapidly rounded into spheres and began to grow fast. Not infrequently now they suddenly became soft and much smaller when palpated between thumb and finger for charting; and immediate incision disclosed a quantity of red pultaceous matter lying free next a ruptured and collapsed cyst, often of considerable size. As the experiments were continued and the gross and microscopic findings were compared it became clear that the presence in a cyst of pultaceous material instead of dry laminated keratin bespoke the presence of a tumor or tumors, and that the sudden enlargement was due mainly to the rapid accumulation of fluid. Where a cyst broke on palpation a carcinoma had weakened the wall or else it had been thinned by interior pressure. Owing to this pressure many of the cysts "pointed" after a time in the direction of least resistance, bulging out into the loose tissue of groin or haunch, and thenceforth enlarging at a more rapid pace. The primary situation of the tumors was on the older, intramuscular portion of the cyst wall. Sometimes they were discrete, projecting inwards as verrucae or raised discs, with a smooth epithelial lining between them, while in other instances they had formed a continuous fleshy mass or layer, ragged on its inner surface owing to necrosis. They ranged in character from benign stalked papillomas to the most anaplastic of carcinomas.

The amount of fluid and pultaceous matter in the cysts became so large after a while that staining with Scharlach R was no longer perceptible in the gross and only scattered red droplets of oil could be discovered on search. Ultra-

TABLE II

Neoplastic Effects of Methylcholanthrene on Implanted Skin

(Experiment 14: skin of 18 mm. embryos)

Mouse No.	Technic	Time between implantation and: Sudden growth of nodule	Time between implantation and: Death	Findings
	Materials were:	*days*	*days*	
1	Mixed		6	Most fragments dead; cyst formation just begun
	Layered		"	One cyst formed, another forming: droplet inclusion going on
2	Mixed		"	" " " others forming
	Layered		"	" " " " "
3	Mixed		10	Completed cyst: droplet inclusion going on: *precancerous changes*
	Layered		"	" " " " " " " "
4	Mixed		"	Incomplete cysts: most fragments dead
	Layered		"	Completed cyst
5	Mixed		"	" "
	Layered		"	" "
6	Mixed		14	Incomplete cyst
	Layered		"	Actively proliferating cyst
7	Mixed		"	Tiny cyst: most fragments dead
	Layered		"	Actively proliferating cyst: *precancerous changes*
8	Mixed		"	" " " " "
	Layered		"	" " " " "
9	Mixed		18	Multilocular cyst: *precancerous changes, dubious carcinomas*
	Layered		"	Several cysts: " " *branching papilloma, squamous cell carc. (?)*
10	Mixed		24	Several cysts: *precancerous changes, branching papilloma, squamous cell carc. (?)*
	Layered		"	Several cysts: *precancerous changes, branching papilloma, squamous cell carc. (?)*
11	Mixed	24	34	Several cysts, one 12 mm. long: *precancerous changes, anaplastic carc. with giant cells*
	Layered	"	"	Several cysts: *precancerous changes*
12	Mixed	59	60	Spherical 12 mm. cyst: *malig. pap.* (transplanted)*, *squamous cell carc.*
	Layered	"	"	Sausage cyst 1 cm. long: *precancerous changes*
13	Mixed	"	68	Ovoid 11 mm. cyst full of *malig. (?) pap.*
	Layered	"	"	Cyst lining dead save where *several small carcinomas*
14	Mixed	66	76	Football 10 mm. cyst: *benign(?) pap., malig. pap., squam. carc.*
	Layered	"	"	Three smaller cysts: four small *squam. cell carc.*
15	Mixed	59	97	Ovoid 27 mm. cyst: 5 differing *carcs., carcinosarcoma* (all transplanted)‡
	Layered	45	"	" 20 " " *multiple carcs.* (one transplanted)§
16	Mixed	59	118	30 mm. mass of cysts: *benign and malig. paps.*, multiple *carcs.*, and a *sarcoma*
	Layered	"	"	Spherical 23 mm. cyst: *4 differing carcinomas*‖

* Papilloma grew fast at first, then retrogressed in all 5 new hosts (see Table I).

‡ All tumors grew fast, severally or together, in all 10 new hosts.

§ No growth of the moderately anaplastic carcinoma in 5 new hosts but it survived at one implantation site.

‖ Animal died of its tumors.

TABLE III

Neoplastic Effects of Methylcholanthrene on Implanted Skin

(Experiment 15: Skin of 18 mm. embryos)

Mouse No.	Carc. material	Time between implantation and: Sudden growth of nodule	Time between implantation and: Death	Findings
		days	*days*	
1	*OSSM*		9	Complete cyst
	OM		"	3 small complete cysts
2	*OSSM*		"	Sausage cyst 11 mm. long
	OM		"	Incomplete sausage cyst 11 mm. long: *precancerous changes*
3	*OSSM*		"	" cyst: *precancerous changes*
	OM		"	Most fragments dead: cyst formation just begun
4	*OSSM*		15	Several cysts: *precancerous changes*
	OM		"	" " " "
5	*OSSM*		"	Ramifying cyst
	OM		"	2 cysts
6	*OSSM*		15	Several cysts: *precancerous changes*
	OM		"	" " *dubious carcs.*
7	*OSSM*		19	" " *precancerous changes*
	OM		"	" " no perceptible methylcholanthrene effect
8	*OSSM*	26	26	Spherical 9 mm. cyst, ruptured where *anaplastic carc.*, elsewhere *precancerous changes*
	OM	"	"	Several cysts lined with unhealthy or dying epithelium
9	*OSSM*	29	29	Ovoid 9 mm. cyst and 2 others: *squamous cell carc.*, (bullet bodies), *dubious carc.* and *precancerous changes*
	OM	"	"	Ovoid 9 mm. cyst and 2 others: *benign(?) pap.*, *anaplastic carcs.*
10	*OSSM*	47	48	Small cyst: *precancerous changes* and *anaplastic carc.*
	OM	"	"	Spherical 11 mm. cyst, half full of *malig. pap.*: *precancerous changes*
11	*OSSM*	40	55	Football cyst 12 mm. long and 3 others: *multiple small carcs.*
	OM	47	55	Spherical 12 mm. cyst and 3 others: *multiple small carcs.*
12	*OSSM*	68	71	Ovoid 12 mm. cyst full of *pap. becoming squam. carc.* (transplanted*)
	OM	"	"	Cysts 7 and 8 mm. across: *malig. pap.* invading abdominal wall: *precancerous changes*
13	*OSSM*	60	76	Spherical 9 mm. cyst: *carc. solidum* and *squam. cell carc.*
	OM	40	"	Ovoid 15 mm. cyst: *malig. pap.*, *pleomorphic carc.*, *anaplastic carc.* (transplanted‡). Also *squam. carc.*
14	*OSSM*	89	91	Ruptured spherical 15 mm. cyst: *benign paps.*, *malig. pap.* (transplanted§) and *multiple carcs.*
	OM	68	"	Spherical 1 mm. cyst: *multiple carcs.*

TABLE III—*Concluded*

Mouse No.	Carc. material	Time between implantation and: Sudden growth of nodule	Time between implantation and: Death	Findings
		days	*days*	
15	*OSSM*	68	99‖	4 rounded cysts 10–13 mm. across: *benign and malig. paps.* Several *squam. carcs.* (one transplanted¶).
	OM	84	"	Spherical 18 mm. cyst full of *malig. pap.* (transplanted**). Also small. *carcs.*
16	*OSSM*	54	105‖	Spherical 18 mm. cyst: multiple *carcinomas*
	OM	"	"	" 24 " " *carcinosarcoma*
17	*OSSM*	"	"	Cyst 11 mm. across and several others: multiple *carcs.* and a *sarcoma*
	OM	"	"	" 23 " " full of *malig. pap.: squam. cell carc.*

OSSM = Olive oil containing 1 per cent of methylcholanthrene and supersaturated with Scharlach R.
OM = " " " " " " " " only.

* Papilloma grew fast in all 5 new hosts, forming cysts up to 20 mm. across, and then regressed. (See Table I, as for the other transplanted tumors).

‡ All 3 tumors grew in some hosts, in others regressed after early proliferation.

§ Very malignant-looking papilloma grew fast in all 11 new hosts, forming cysts up to 20 mm. across, and then regressed.

‖ Poor condition: lung disease.

¶ Moderately anaplastic squamous cell carcinoma grew progressively in all 5 new hosts.

** Implants gave irregular results in the 6 hosts: no tumors at 7 sites, early regression at 3, and later regression at remaining 2, where cystic growths 13 mm. and 14 mm. across had arisen.

violet light provided some striking pictures. The interior of the cysts containing *OM* glowed in rich purple, with a mist of the same fluorescence just outside, the muscle further away appearing dark, or dotted with brilliance where globules containing the carcinogen still lay scattered in it. A few of the cysts seemed solid when first cut into, but this was because they were full of close-packed papillae (Fig. 5) with fluid lying between them.

Incidence of the Tumors

Two extensive tests were made with the skin of 18 mm. embryos to learn how regularly and how soon tumors could be engendered.

A subsidiary aim of *Experiment 14* was to find whether mixing *OSSM* with the skin fragments prior to injection yielded conditions as favorable as having it follow the latter in. The mice received in one thigh 0.025 cc. of skin mixed with the same amount of *OSSM*, while for the other thigh the *OSSM* was first drawn up into the syringe and then the skin, suspended in 0.05 cc. of Locke's solution, the result being that it entered the thigh first. The 14 animals were killed 6 to 118 days after implantation (Table II).

In *Experiment 15* the carcinogenic effects of *OM* and *OSSM* were compared in the same mice. One or the other of these preparations was drawn up into the syringe to the amount of 0.025 cc., with 0.075 cc. of Locke's solution layered next, and then 0.025 cc. of minced skin. The 17 animals were killed 9 to 105 days after injection (Table III).

Most of the implants removed within the first 3 weeks were sectioned serially.

The experiments had nearly identical yields of neoplasms (Tables II and III). In every mouse kept more than 34 days, 5 in Experiment 14 and 8 in Experiment 15, tumors developed that were recognizable as such on palpation, and the microscope disclosed many others. In 12 of the 13 hosts they arose from both implants. These findings are the more noteworthy because the animals in which the largest nodules formed during the first days after implantation, those in which the conditions might very well have been most favorable to tumor formation, were sacrificed early to learn what was happening. Layering of the materials in the injection syringe so that the oil followed the tissue into the muscle resulted in a somewhat better formation of cysts than when preliminary mixing was done; yet there was no discernible difference in the ultimate tumor yields. *OM* gave as good results in this respect as *OSSM*.

The first gross evidence that neoplastic change had taken place was the rupture during palpation on the 26th day of a nodule that had recently become spherical (mouse 8, Table III). Immediate autopsy showed a thick-walled cyst, 9 mm. across in the collapsed state. Its pultaceous contents, lying free next it, was ruddy with dye and contained much methylcholanthrene (ultraviolet test). An anaplastic carcinoma was present where the break had occurred (Fig. 6). In a second animal killed after 29 days (mouse 9, Table III), a spherical cyst 9 mm. across had formed, and serial sections showed at widely separate spots on its wall a papillomatous ingrowth which may have been benign (Fig. 7), a very active anaplastic squamous cell carcinoma, which had invaded the adjacent muscle, replacing its fibres individually (Fig. 8), and an invasive, small-celled carcinoma (Fig. 9) differing tinctorially from the hyperplastic epidermis from which it had taken origin. In a third mouse killed after 34 days (mouse 11, Table II), an invasive growth with giant and multinucleated epithelial cells (Fig. 10) had taken origin from a cyst 12 mm. across.

The Histological Findings

The skin of fetuses 15–18 mm. long has been mainly used in studying the microscopic changes, but that of younger embryos has yielded essentially similar findings.

When 0.025 cc. of skin was implanted together with an equal quantity of Locke's or Tyrode's solution many of the tissue fragments died, but the epidermis of those which survived rounded up into tiny cysts within 5 or 6 days, and a palpable nodule resulted. This enlarged very slowly, keratin accumulating within the cysts as an opaque, creamy mass of concentric lamellae, dense, inelastic, and nearly dry. Often there were 8 or 10 cysts in the beginning, but those lying immediately next each other usually coalesced secondarily, with result in larger, flattened, multilocular cysts, or else they formed a nodular sausage, perhaps 6–8 mm. long and 2 mm. across after 2 weeks yet scarcely a millimeter deep. The flattening was in the plane of the muscle. Occasionally one of the cysts became more than a centimeter in diameter, flattened like a lima bean. The wall of even the largest was translucent and very thin, and the keratinized stuff shelled away from it leaving a smooth surface.

The skin of 15–18 mm. embryos has rudimentary hair follicles and is covered with stratified

squamous epithelium 5 to 8 cells deep (Figs. 11 and 12). It is very unlike the adult epidermis, which builds up no layer expressive of differentiation, but is only 1 or 2 cells deep, keratinizing abruptly. After implantation however the embryo skin matured very rapidly, taking on the adult form within less than 2 weeks (Fig. 13), and during this time numerous sebaceous glands and hair follicles of ordinary aspect developed. The glands and follicles were mostly situated in and near the original pieces of skin, now incorporated in the cyst wall, with few or none in that part of the latter which had formed by lateral extension of the epithelial layer. The cellular corium underwent little differentiation during the first 2 weeks (Fig. 13) but formed a compact layer later (Fig. 16). Much tissue of the adipose sort, but without recognizable fat cells as yet, had been transferred with it, and from this a thick layer of mature fat cells soon developed, with the hair follicles embedded amongst them (Fig. 16). Many of the follicles underwent an enormous hyperplasia, pushing through the fatty layer to the muscle of the host, where they were checked and in consequence became clubbed and bent (Fig. 13).

These were the findings with the skin of 18 mm. embryos. By the end of the 3rd week the corium and adipose tissue together were nearly three times as thick as in a sectioned embryo of the same litter (Fig. 11). Save as providing vascularization and perhaps some stroma, there was no evident cellular reaction of the host tissues to the implant. Even after many weeks it lay directly against the muscle without any interposed reactive tissue, though occasionally a few lymphocytes had appeared beneath the foreign epidermis, and more rarely a sparse accumulation of them had taken place at the occasional spots where it had not matured but was still hyperplastic, probably from local disturbance.

As months passed and keratin accumulated within the cysts their epidermal lining was stretched, the mouths of the follicles gaped, and they surfaced or atrophied, no longer forming hairs. The sebaceous glands also became fewer, the corium thinned, and large cysts lying near the muscle surface often "pointed" in the direction of least resistance. Here the lining epithelium sometimes died, as if from pressure; and occasionally it succumbed everywhere, leaving only a mass of keratin, surrounded and penetrated after a while by reactive tissue. The presence nearby of cysts with living epithelium proved its death to have been a fortuitous happening. Generally, the epidermis remained in good condition for long periods,—up to 140 days, the maximum time of observation. None of a large number of implants examined after 2 to 4 months showed the least sign of neoplastic change. Indeed the findings were surprisingly uniform and they never offered difficulties of interpretation.

When skin fragments and olive oil were injected together, cyst formation with differentiation took place like that just described, but the epidermis matured more slowly and was still hyperplastic after 2 weeks (Fig. 15). Occasionally a few small oil globules could be seen amidst it, which the epithelial cells, proliferating laterally, had met and grown around, but there was no extension to any of the droplets lying near by, and the developing corium and adipose tissue soon pushed these further away. Where the cells of the differentiating and hypertrophying hair follicles came into contact with the oil they seemed uninfluenced by it.

Very different were the results with oil containing Scharlach R (*OSS*) (Figs. 18–20). This so greatly stimulated and attracted the epidermal cells of the several skin fragments that they frequently extended along to droplet after droplet, and merging into a layer, formed one or several large cysts instead of more numerous small ones. The cysts had a thick lining of stratified squamous epithelium, and differentiation of the follicles was delayed, these remaining rudimentary and more cellular than ordinary for 10 days or more. Sebaceous glands appeared only slowly and no recognizable adipose tissue formed. The sides of the cysts were completed sooner than the ends, the epidermis rapidly extending along in the plane of the muscle fibres. As it did so it encountered and enclosed numerous oil droplets and grew out to a few, with result that they too became incorporated in its layer; but once this layer had formed, extension out-

wards from it seldom took place. At the incomplete ends of the cysts, though, tongues of actively proliferating epidermis frequently penetrated far amidst and around the droplets, simulating cancer, if only superficially.

The initial activities of the cells were migratory with few mitoses, as where repair of an epidermal wound has just begun (7), but proliferation and differentiation took place soon after with result in a stratified squamous layer enclosing the droplets. It had a well defined stratum granulosum (Figs. 19 and 20) and its basal cells, irregular in arrangement at first, quickly became well ordered, presenting an even front to the connective tissue. Within a few weeks, follicles and sebaceous glands had differentiated, the epidermis matured, the cysts flattened, and after 30 to 40 days they could not be told from those due to the implantation of skin and Locke's solution, save in that they were frequently solitary (Fig. 21).

In extensive comparative tests implantations were made of skin with Locke's solution or olive oil or *OSS* in one thigh and with *OSSM* in the other: the animals were killed at intervals of a few days. The *OSSM* was obviously damaging. Polymorphonuclear leucocytes sometimes collected about it during the 1st week (Fig. 27), as not about oil or *OSS*, and much of the skin that had been introduced with it promptly died. The epidermis of the surviving fragments proliferated so rapidly however that within another week or two cysts had formed much larger than those due to the controls. They tended to be solitary or few, as in the case of *OSS*, and they formed in the same general way, with inclusion in the epidermal layer of many oil droplets, these riding on into the cysts amidst the differentiating and desquamating cells about them. A very active extension of the epithelium to outlying oil droplets often took place at the forming ends of the cysts. At first it was migratory in character (Fig. 22), like that to droplets of *OSS*, but soon mitoses became numerous and a thick, crowded, stratified squamous layer formed. Its cells differed much in size and shape, and the basal elements often appeared to lie separate in stained sections, probably owing to edema, with no line of demarcation between them and the connective tissue (Fig. 23). A profuse, very cellular reactive tissue formed, separating the epidermis from the muscle (Fig. 24) and lymphocytes appeared, sometimes as early as the 6th day. Later on they became so numerous as to form a thick zone in the reactive tissue (Figs. 5, 24, and 25), and some wandered into the epidermal layer. This was undergoing pronounced changes. Its hyperplastic differentiating cells failed to produce a stratum granulosum, they became progressively more basophilic and disorderly, and came to differ widely in size. Where they had extended in tongues to outlying droplets of *OSSM* (Fig. 27) the abnormalities were especially marked, and the general aspect was not infrequently that of an active carcinoma. Sometimes the keratin formed by the proliferating elements was abnormally dense and eosinophilic.

Where the skin was hit hardest by methylcholanthrene the rudimentary hair follicles that had been present at implantation disappeared instead of developing, and the lining of the cysts consisted merely of a stratified squamous layer (Fig. 24) which often died later. Where the damage was less some of the follicles persisted (Fig. 14) and even functioned actively for a little while,—as attested afterwards by the presence of hairs amidst the keratin (Fig. 17). Sebaceous glands also formed sometimes but within 2 weeks had disappeared, and the follicles, becoming more and more distended with keratin, either atrophied or surfaced. No differentiation of adipose tissue occurred but instead the epidermis was separated from the neighboring muscle by reactive tissue in which lymphocytes accumulated.

During subsequent weeks no sebaceous glands or follicles formed but the epidermis continued hyperplastic, showing numerous mitoses and increasing disorder. Lymphocytes also became more abundant (Figs. 5 and 25). They were not found where the cysts were "pointing" and the epithelium in consequence was thin or lacking, but hordes of them collected where it was thick and actively proliferating. As a rule neoplastic changes soon followed. We will refer to the condition as the preneoplastic state.

In a mouse killed after 141 days there was still a dense zone of lymphocytes in the wall of a cyst lined with hyperplastic epidermis from which several small discrete carcinomas had taken origin; but ordinarily, there were few lymphocytes around cysts examined so late. The methylcholanthrene had generally disappeared long before, the fluorescence test failing to show it after 2 months in most specimens. Usually tumors formed within this time.

The extension to outlying oil droplets sometimes continued in diminishing degree for several weeks, and when a large globule lay immediately next a cyst the epithelium usually extended around and enclosed it.

Methylcholanthrene in olive oil (*OM*) had as pronounced an influence as *OSSM* to stimulate and attract the epidermal cells (Fig. 28), and in Experiment 15 it proved as effective in causing tumors. So too with *OSM* (oil containing methylcholanthrene and enough Scharlach R to serve as a tracer). Even when dissolved in mouse fat the carcinogen exerted a stimulating and attracting influence, though in lesser degree. These findings will be the subject of a later paper.

Results with the Skin of Younger Embryos

So little skin could be got from individual litters of embryos 15 mm. or less in length that it had to be suspended in a considerable amount of Locke's solution if many implants were to be made; and as result it often failed to come into contact with the carcinogenic material injected with it. The skin from 6 litters of embryos 11–15 mm. long (14–16 days old) was employed in as many tests, 86 animals receiving it in all. The results wholly resembled those with embryos near term. Whenever the *OSSM* had been included in the epidermal cysts, neoplastic changes took place,—unless the animal was killed early or all of the epithelial cells died because injured by the carcinogen. The younger the embryos the more liable was the skin to injury, and sometimes its epidermis succumbed entirely after forming cysts. In not a few cases though, it lived long enough for carcinomas to take origin from it and grow out beyond harm from the methylcholanthrene; but when the growths were limited to the cyst wall they died with the rest of the epithelial lining, as serial sections and the pattern of the necrotic tissue showed. Such happenings were rare in implants of the skin of 18 mm. embryos.

Results with Minced Embryos

The skin could not be got in quantity from embryos less than 10–11 mm. long, and hence as already stated these were chopped fine, usually after evisceration and removal of most of the brain. The hash was implanted as such in 0.025 cc. amounts together with the same quantity of carcinogenic material.

Findings with Embryos 12 Days Old (7–8 Mm. Long).—Three experiments were done. In *Experiment 18*, 7 mice were implanted with embryo tissue and *OSSM* but 2 were killed for the study of early changes before 2 weeks had elapsed. A third, autopsied on the 44th day, had at this time in one thigh a cyst 18 mm. across, with 3 distinct carcinomatous masses in its wall, while in the other thigh there was a small cyst with a cancer just beginning to invade. The remaining 4 mice, sacrificed after 65, 65, 70, and 92 days, all had squamous cell carcinomas in both thighs, with a carcinosarcoma as well in the animal killed last. The implant

yielding the 18 mm. cyst in the animal killed after 44 days had begun to enlarge suddenly between the 26th and 33rd days, and an implant in another mouse, which later proved cancerous, did the same between the 47th and 54th days.

Pieces of one of the tumors procured from an animal killed after 65 days were transplanted to both thighs of 5 young male C mice. Section showed a malignant papilloma and an anaplastic squamous cell carcinoma to have been present in the graft material, and both neoplasms did well in the new situations (Tumor 11, Table I). A carcinosarcoma from the mouse killed after 92 days gave growths in each of 10 new hosts (Tumor 15, Table I), some of them resembling the original tumor, some wholly sarcomatous.

Experiment 19 had as subsidiary aim a direct comparison of the early effects of *OSSM* and *OSS*. The implant with *OSSM* began to enlarge rapidly soon after the 34th day in the first animal to show cancer, and autopsy on the 57th day disclosed 6 separate carcinomas on the wall of a cyst lined for the rest with epidermis in the preneoplastic state. In another mouse the nodule with *OSSM* suddenly enlarged between the 41st and 48th days, and examination on the 70th day disclosed a solitary benign papilloma (Fig. 29) nearly filling a cyst 18 mm. across which was mostly lined with epidermis in the preneoplastic state. The cyst contents was pink with Scharlach R but did not fluoresce in ultraviolet light when the dye was diluted with acetone. On transplantation the papilloma succeeded in establishing itself at 7 of 10 new sites (5 hosts), (Fig. 30), but did not proliferate into a mass at any (Tumor 2, Table I). A third animal had a cyst 28 mm. across after 100 days, where the implant with *OSSM* had been put. Its wall was lined with hyperplastic epidermis and studded with papillomatous growths, 2 of them keratinizing squamous cell carcinomas microscopically; and just outside the cyst was a nodule of spindle cell sarcomatosis. Tissue from one of the carcinomas was transplanted to 7 mice and grew progressively at 9 of 14 sites, regressing at the others (Tumor 6, Table I).

In the third experiment, the tissue was implanted together with *OSM*. In one host killed after 35 days a squamous cell carcinoma was already present, in another killed on the 68th day a cancerous cyst 15 mm. across had formed, and in a third, examined on the 80th day, there were multiple tumors in both thighs,—malignant papillomas, squamous cell carcinomas, and a spindle-cell sarcoma. Several mice died of lung disease while the implants were small, and sections of these have not been made.

The minced tissues of 7–8 mm. embryos gave rise to masses somewhat larger than those produced by the skin of fetuses near term, and the nodules arising from the control implants with *OSS* underwent no flattening but became plump and nodular or sausage-shaped. Bone, cartilage, thyroid, salivary, nerve, and lymphoid tissues in excellent state were found within them, together with numerous small cysts lined with epidermis or with mucosa from the glandular portion of the stomach or the large or small intestines. All lay in a matrix of connective tissue, which was much more abundant than in skin implants and may very well have derived from the embryo. Some of the cysts of gastric or intestinal origin soon became a centimeter across, owing to secretion into them of mucoid or thin fluid. Occasional small cysts with a lining of cylindrical or cubical cells of indeterminate origin were also encountered, and in one instance a tooth.

Fewer tissues survived in association with *OSSM* or *OSM*,—chiefly epidermis, bone, and cartilage, and sometimes gastric and intestinal mucosa. The conditions were unfavorable to encystment of the dye-stained oil, most of it remaining scattered in the profuse connective tissue; and only occasionally did one of the cysts have a pink or ruddy hue.

The tumors appeared later than from implants of the skin of older embryos, as might have been expected under the conditions, and often one could not be sure that they had taken origin from the epidermis, though this seemed likely when the cysts from which they arose contained hairs. Secretion of fluid by the implanted gastric and intestinal mucosa also introduced a

difficulty, the sudden enlargement of a cyst often meaning no more than that this fluid had accumulated rapidly.

The big cyst in a mouse of *Experiment 18* which began to grow fast soon after the 26th day and showed multiple cancers on the 44th day, contained keratin but no hairs, and owed its enlargement mostly to distention with a thin cloudy fluid. It had a patch of gastric mucosa on a wall lined elsewhere with stratified squamous epithelium; and this epithelium, from which the carcinomas had arisen, might have come either from the squamous portion of the stomach or been epidermis that had joined to the gastric epithelium to form the cyst. Cancers rapidly develop after pieces of embryo stomach are implanted with *OSSM*, as will be shown in the accompanying paper.

Results with 10–11 Day Embryos (3–3.5 Mm. Long).—During early gestation the relation of the embryo to the uterus is such that it cannot be freed from maternal cells. Dr. George D. Snell of the Jackson Memorial Laboratory has kindly reviewed his mouse material for us, and he reports that only after more than 10 days do the embryos float in a well formed amniotic sac (8). Those from C females pregnant for 10½ to 10¾ days have a sac large enough to be opened with the cautery, as we have found; and the embryos (3–3.5 mm. long) were readily procured. To lessen the chance of injury to the implanted tissues mouse fat was substituted for olive oil as solvent for the methylcholanthrene. One per cent of this was dissolved in it, but no Scharlach R or only enough for a telltale.

The fat obtained by grinding the adipose tissue of mice with sand in a warm mortar was passed through a Buchner filter, and refiltered in the warm through cotton. It was faintly yellow, water-clear, and remained fluid and thin at room temperature, though some fine white particles came out which redissolved on gentle heating. After methylcholanthrene had been added it was distributed in tubes and autoclaved.

The preparation was first tested on the skin of 13 mm. embryos. A litter yielded so little of the tissue that it had to be suspended in much Locke's solution. The fat containing methylcholanthrene was warmed to dissolve all particles, 0.025 cc. was taken up into the syringe, then the same amount of skin suspension, and injection was done rapidly into each thigh of 11 mice. In 3 killed after 15, 27, and 34 days small fluorescing cysts were found. A nodule in a fourth animal suddenly enlarged between the 26th and 33rd days, and autopsy on the 42nd disclosed in both legs cysts containing tumors,—a branched benign papilloma, a papilloma which appeared malignant, and at many spots small invasive carcinomas. Of the 7 remaining animals 1 was lost and the others were killed or died of intercurrent causes after 85 to 124 days. All had large tumor cysts in one or both legs, some partly full of papilloma tissue, the others with carcinomas or papillomas, or both, on their walls. There were unencysted fat droplets amidst the muscle, and some of these still fluoresced in ultraviolet light when examined after 55 days.

Sections from the implant procured after 15 days showed a less active extension of the epidermis to the outlying droplets of methylcholanthrene-containing fat than in instances in which *OM* had been used with the skin of older embryos, but it had occurred at some spots, and with mimicking of carcinomatosis. The epidermal lining of the cysts seemed less injured than by *OM*, a finding corroborated in the later specimens. In the animals killed after 27 and 34 days preneoplastic changes had taken place in the epidermis.

Experiments were now undertaken with 3–3.5 mm. embryos. They had been dated by the vaginal plug method. A spud or pipette was used in handling and washing them, and they

were eviscerated under a dissecting microscope, minced, and suspended in a relatively large bulk of Tyrode's solution.

The technic in *Experiment 28* was like that in the test just described except that injection was done slowly and into only one thigh of 5 young adult mice. During the following weeks most of these gained no weight or else lost it, owing to a chronic pulmonary disease, and for a long while the nodules remained tiny (1–3 mm. across) at every site. One began to grow after the 78th day and on the 89th day a spindle cell sarcoma was found. It was transplanted to 6 animals and did well in all (Tumor 17, Table I). In a second mouse a nodule enlarged shortly after the 82nd day and again a sarcoma was found (95th day), while autopsy of a third at this time disclosed another. In all 3 hosts there were tiny scattered cysts also at the injection sites, creamy and opaque, or translucent. The remaining 2 animals had merely these latter after 110 days.

For *Experiment 29* Scharlach R had been added to the mouse fat containing methylcholanthrene. The fat was mixed with an equal amount of the suspension of embryo fragments, and 0.05 cc. of the mixture was injected rapidly into both thighs of 3 mice and into one thigh of 2 others. Again the animals developed pulmonary disease and the nodules remained minute, except for one which enlarged from the first and by the 76th day had become a cyst 8 mm. across, lined with intestinal mucosa and containing a greenish fluid. Next to it were several small, creamy, flattened cysts. The other 4 animals had tiny cysts after 72 to 106 days, none of them colored with Scharlach R.

Fewer tissues survived than in the tests with 7–8 mm. embryos, and only occasionally was there any sign that the methylcholanthrene had been encysted. Some of the cysts were walled with intestinal villi and were full of mucus, while others contained keratin and hairs and had a lining of adult epidermis equipped with follicles and sebaceous glands. In a mouse of *Experiment 28*, killed on the 89th day, a cyst 1 mm. across, containing keratin, had a lining of stratified squamous epithelium which was in the preneoplastic state, with the cells encroaching at one spot on the connective tissue. In another animal killed after 95 days the lining of a similar cyst was frankly carcinomatous everywhere and had begun to invade (Figs. 34 and 35). There were some dubious hairs amidst the keratinized contents of the cyst. It was surrounded by reactive tissue compressed as result of its enlargement and showing many lymphocytes and some small hemorrhages. Nearby were some big fat droplets and further away a cyst lined with normal epidermis and containing many hairs.

The conditions provided by these experiments were unfavorable in several respects. Not only is methylcholanthrene relatively ineffective in mouse fat (9) but the droplets were seldom encysted by the embryo epithelium, most of them lying at a distance from it. Little of the implanted tissue survived and this little did poorly. Most of the host mice had lost weight, and under such circumstances the proliferation of embryo implants (4) and of tumors (10) is often held up. Nevertheless in one instance the stratified squamous epithelium lining one of the tiny cysts had become carcinomatous everywhere, and in another animal the change seemed well under way. The probabilities are that with a better technic and healthy hosts tumors can be induced early and often.

Character of the Epidermal Tumors

The growths arising from the embryo epidermis were like those appearing on the skin of adult mice after exposure to methylcholanthrene, except that none

was of sebaceous gland origin for the very good reason that the methylcholanthrene had prevented any differentiation of such glands. Some of the papillomas were pedunculated or had a fleshy core (Fig. 31). They tended to appear somewhat sooner than the carcinomas,—which in most instances were primary, though some took origin from papillomas. Occasionally branched growths of the latter sort, covered with hyperplastic epidermis were found within the first 3 weeks (Fig. 41); but it is uncertain whether these were true neoplasms. None occurred in control cysts, but they might have been due merely to the stimulating influence of *OSSM* as distinguished from its carcinogenic effect: for not infrequently oil droplets were present in the connective tissue cores of the papillae. The growths represented the least neoplastic deviation from the normal, if tumors they were. The other growths were indubitably neoplastic, and when not interfered with grew progressively and proved fatal. Often they involved the skin, causing it to become necrotic, and whenever this happened the mice were killed forthwith to avert the complexities of ulceration. Large growths frequently extended around the femur (Fig. 26), and often the malignant cells replaced the voluntary muscle fibres individually. Extension of the tumors from the cyst wall soon took them beyond the zone of lymphocytes, and these cells did not collect about them later unless they were doing badly, when a reactive tissue formed crowded with lymphocytes and macrophages, like that appearing about the epidermal tumors of adult mice under similar circumstances.

In two respects the tumors differed from those of adults:—The great majority were primary carcinomas instead of papillomas, and even the most malignant of them failed to metastasize. Whenever they had been present long, sections of the lungs were searched, and of the iliac glands also whenever these were enlarged, but with negative results.[2]

The preneoplastic alterations described some pages back were often present everywhere on the cyst wall, yet the growths were always local in origin even when many. This fact was most evident in specimens procured from ill-nourished hosts. The injection of *OSSM* had no obvious effects on health, even of mice weighing only 10–12 gm., but a chronic pulmonary disease, perhaps that described by Nelson[3] (11), was prevalent in our colony and often asserted itself a few weeks after the implantations, the lungs becoming widely consolidated and the animals losing weight. Most spontaneous mammary tumors of the mouse do poorly in animals thinned by underfeeding (10), and even slight reductions in weight brought about in this way will prevent tumors from appearing on skin treated with the chemical carcinogens, although the latter are as effective as ever in producing neoplastic changes (12). As might have been expected from these facts the findings in our healthy animals and those wasted by disease differed to some extent. In the former the tumors which arose first often grew fast, and most of the cyst wall was soon occupied by them. In sick hosts on the other hand not only did the cysts enlarge more slowly, but sometimes one of them while still small had as

[2] A pulmonary metastasis has been found in a recent experiment (see footnote 10 of Paper II).

[3] No significant bacteria could be procured from the lungs by culture in a variety of media, and Nelson's coccobacilliform bodies could not be found.

many as 10 distinct and various neoplasms on its lining, with more just beginning to manifest themselves. There were exceptions of course. In some healthy mice the cyst lining was studded with separate papillomatous verrucae and fleshy carcinomatous discs, or several growths had coalesced into a thick fleshy wall.

As the cancerous cysts became big, owing to fluid accumulation, the malignant epithelium proliferated laterally and kept the inner surface lined,—unless enlargement was very rapid,—and wide expanses became covered in this way with frankly carcinomatous elements (Fig. 36) or with a stratified squamous layer that aped the normal. Had the course of events not been known one might have supposed in some instances that a wholesale conversion had taken place of normal epithelial cells into carcinomatous elements of a single kind. Serial sections of the tiny cysts occasionally showed them to be lined with epithelium which was everywhere carcinomatous yet only just beginning to invade. Figs. 34 and 35 provide an instance in point. Doubtless in its case the cancerous change had taken place while the cyst was exceedingly minute: for the compression round about it at autopsy was indicative of rapid recent growth as were also the many mitoses in its epithelial lining.

Much could be inferred from the dead material contained in the cyst. Frequently one could trace in this the entire history of a growth, its punctate beginning, primary character, subsequent broadening and change in the direction of greater malignancy, as surely as one follows geological events in successive strata. Near the center of the cyst oil droplets and necrotic fragments of the original implant were present, and concentric layers of keratin had been laid down about these at first by the hyperplastic epidermis lining the wall. But after tumor formation had got some way along, one found necrotic papillae overlying papillomas, or whorled or jumbled keratinized elements over a squamous cell carcinoma (Fig. 42 of Paper II), or loose necrotic debris and scattered polymorphonuclear leucocytes where the cancer had been anaplastic and its cells had died without differentiating. The pattern of the dead material showed that not a few of the growths had been well ordered and benign in the beginning, and had later become disorderly and aggressive, ending as wild neoplasms occupying the greater portion of the cyst wall. But inferences like this were justified only when fluid had not disordered the contents of the cyst, the usual happening after it had pushed out into the connective tissue. Often its further wall then consisted merely of compacted connective tissue, the laterally extending epithelium having failed to keep pace with the enlargement.

The Occurrence of Sarcomas

Whenever the methylcholanthrene had been well encysted tumors arose from the epidermal lining of the cyst sooner than from the surrounding connective tissue; but when most of the carcinogenic material lay free epithelial tumors appeared late if at all and sarcomas often complicated the findings. They took origin either from the tissue around unencysted oil droplets or from the wall of cysts, or where the needle had passed in attempts to inject more *OSSM* into these latter (*vide* Experiment I). The implantations of minced embryos 3.5–7.8 mm. in length provided conditions especially favorable to sarcomas because the bulk of the carcinogenic material remained scattered in the profuse connective tissue matrix of the nodule.

The earliest sarcoma was come upon at autopsy after 81 days; they seldom appeared in the gross until after 100 days or more.[4] A sudden enlargement of the implant signalized

[4] In a control animal injected with nearly twice the usual amount of *OSSM* but no embryonic tissue, a sarcoma recognizable in the gross had arisen by the 77th day.

their presence, as in the case of epidermal growths, but it took place so late,—after 73 days at the least and more than 90 days ordinarily,—as to be almost pathognomonic. The sarcomas were solid and fine-textured, with yellow flaring necroses when large, and they were mostly of spindle-cell type though some were pleomorphic and others myosarcomas. Slicing often disclosed embedded epidermal cysts, usually small and creamy, often dead, but sometimes ruddy with Scharlach R and lined with epidermis that had undergone, or was undergoing, neoplastic change. A sarcomatous element was often present in the composite growths resulting from the secondary coalescence of multiple epidermal tumors that had arisen long previously; and sometimes the malignant, connective tissue cells served as stroma for the epithelial tumors, with result in papillo- or carcinosarcomas (Table I, Tumor 14).

The Results of Transplantation

As already mentioned the epidermis of C embryos did well in control implants, irrespective of whether it was transferred separately or as part of a minced embryo mass; and the lining of the cysts formed by it nearly always remained in an excellent state for months. Occasionally it died, as already mentioned, leaving a lump of concentric keratin, but its flourishing condition in neighboring cysts nearly always made plain that it had not succumbed to any adverse host reaction but to local conditions, the pressure of accumulating keratin in special. As a whole the findings demonstrated the complete homogeneity of the C strain animals. The fact is important because the transplanted tumors frequently proved unable to survive.

The first tumors to be transplanted had grown large before this was done and sometimes the grafted tissue contained several intermixed growths. As a rule they all appeared in the derivative tumors, together or severally (Table I, Tumors 11–16). The growths utilized later on had existed for a much shorter period and were still so discrete that they could be transplanted individually, with result in tests of representatives of all the main types. Usually the grafts were placed in both thighs of the new hosts; bacterial infection never followed. The findings were notably consistent. The tumors either (*a*) grew progressively; (*b*) formed nodules which later regressed; (*c*) survived; or, (*d*) failed, becoming necrotic. Those neoplasms tabulated as merely surviving were vascularized by the host and lived, but failed to proliferate into a tumor mass.

Neither of the two *benign papillomas* grew after transplantation (Table I, Tumors 1 and 2), though one survived at 7 of 10 sites, forming minute cysts in which was living papillomatous tissue (Figs. 29 and 30). The microscope disclosed a minute, anaplastic squamous cell carcinoma amidst the second tumor (Fig. 31), and this cancer grew at 2 of the implantation sites whereas at 13 others the grafts failed, presumably because only papilloma had been placed there. The mouse furnishing the material for transfer was 9 days old when implanted with embryo skin and *OSSM*,—in an experiment described in Paper II (13).

All of the *malignant papillomas* tested gave rise to nodules which enlarged swiftly during the first weeks yet regressed later in most instances. This course of events was especially remarkable in the case of Tumor 5, which produced nodules 4–15 mm. across at 22 sites during the first 2–3 weeks, only to have them all become necrotic soon after. The primary growth

ahd appeared active and aggressive (Fig. 37), and it retained its malignant aspect when regressing (Fig. 38).

The results were better with *carcinomas* transferred individually, though they differed from growth to growth. One tumor which had been anaplastic in the original host grew at every new situation (Table I, Tumor 7) whereas another with nearly the same morphology (Tumor 8) failed at 9 sites and barely survived at a 10th (Figs. 32 and 33), and this although a composite growth (Tumor 13) from the opposite leg of the same animal did excellently on transfer. Tumor 9, a moderately anaplastic carcinoma, succeeded at most sites whereas another growth of almost identical aspect from the opposite leg, Tumor 10, failed or gave rise only to regressing growths.

Most success was had with the *composite tumors*, these regularly doing well (Table I), with every component of the original material represented in one or another of the growths produced (Figs. 39 and 40). Any sarcomatous tissue present in the grafts tended to outstrip and suppress the carcinomatous after transfer and hence was often found alone in hosts killed late (Tumors 15 and 16). The pure *spindle-cell sarcomas*, Tumors 17 and 18, grew progressively at every site.

The epidermal tumors which grew after transplantation always formed cysts containing keratin, necrotic debris, and thin fluid, sometimes stained with hemoglobin or its derivatives; and as the cysts enlarged they "pointed" in the subcutaneous tissue. Here their wall sometimes consisted of connective tissue only, whereas within the muscles it was thickened by tumor tissue and ragged and necrotic on its inner surface, the resemblance to the original growths being in these respects complete. But nowhere was the cyst wall lined with non-neoplastic epidermis, as in the case of the latter, and lymphocytes were absent unless the tumor was faring ill. The transfer of composite growths frequently resulted in multiple cysts in a matrix of neoplastic tissue (Fig. 1). None of the growths was transferred in series.

The phenomena of regression call for special comment. Usually the tumor had formed a considerable nodule during the first 2 to 4 weeks, but within another week or so it became a mere thin-walled bag, full of fluid and yellow debris, with shallow patches of unhealthy neoplastic tissue surviving for a few days more on its thin wall (Fig. 38). Sometimes lymphocytes and macrophages accumulated round about it, but far from always. The bag was very gradually resorbed.

SUMMARY

A method has been devised whereby the transplanted epidermis of mouse embryos can be selectively exposed to the action of a chemical carcinogen. Scharlach R was dissolved in olive oil with the aim of stimulating and attracting the epidermal cells, methylcholanthrene was added to the solution, and numerous fine globules of it were injected into the thigh muscles of adult mice together with fragments of embryo skin. Much of the oil underwent primary inclusion in the resulting cysts, and the proliferating epidermis, while forming them, extended to not a few of the outlying droplets with result that they too were added to the cyst contents. During these activities the methylcholanthrene came into direct contact with many of the epithelial cells, and later on the layer lining the cyst was continually exposed to the influence of the carcinogen.

The epidermis underwent neoplastic changes with great rapidity; often in less than 4 weeks papillomas and carcinomas had arisen like those deriving from adult epidermis. The growths were punctate in origin and usually multiple. Many were transplanted to adults of the same homologous breed of mice that furnished the embryo material (mice of C strain). The grafts did not uniformly succeed as was the case with those of normal skin of embryos of the same stock,—which regularly grew at first in the new hosts and remained alive long after. The benign papillomas failed to live or barely survived, and the apparently malignant papillomas, though rapidly forming nodules of considerable size, usually regressed later. Some of the carcinomas also regressed or wholly failed, while others gave rise to progressively enlarging tumors. The best results were obtained with grafts in which several neoplasms were intermingled, these flourishing together in the new hosts.

Methylcholanthrene in olive oil exerts an influence on epidermal cells like that of Scharlach R, stimulating them to multiply, attracting them, and causing them to mimic carcinomatous elements.

The implications of the findings will be discussed in the accompanying paper.

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EXPLANATION OF PLATES

PLATE 33

All of the sections were stained with methylene blue and eosin.

The photographs were made by Mr. Joseph B. Haulenbeek.

FIG. 1. Tumor due to implanting in the left thigh muscles of an adult the skin of 20 mm. embryos together with olive oil supersaturated with Scharlach R and containing 1 per cent of methylcholanthrene (*OSSM*). The superficial discolorations and erosions are due to the barium sulfide used to remove the hair. The animal was killed 110 days after implantation (Experiment 1, see text). × 1.

FIG. 2. Margin of one of the carcinomas composing the tumor. It is almost completely anaplastic, shows many mitoses, and is actively replacing the muscle fibres. × 180.

FIG. 3. A growth resulting from the transplantation of the tumor of Fig. 1 to the thigh muscles of new hosts (Tumor 14, Table I). Although but 30 days had passed it occupied the entire upper leg. There were 5 different neoplasms in the mass, 4 carcinomas and a spindle-cell sarcoma. Several large cysts can be seen. × 1.

FIG. 4. Epidermal cyst with many tumors on its wall, as result of the implantation of 20 mm. embryo skin together with *OSSM*. The host animal was killed after 55 days (mouse 14, Experiment 7). Nine distinct papillomas and carcinomas were found microscopically but not all of them are shown here. Between them the cyst was lined with hyperplastic epidermis, save in one region where its wall consisted merely of reactive tissue. It was full of ruddy pultaceous matter, with lamellated keratin in some regions. × 12½.

FIG. 5. Results on the 75th day in another animal of the same experiment (mouse 6, Experiment 7). No tumors have arisen as yet from the epidermal lining of the cyst on the left, but it was in a preneoplastic state as higher magnification showed. The dark zone around about it at a little distance consisted of lymphocytes. The cyst to the right was so packed with malignant papillomatous processes as to appear almost solid, but there was fluid and pultaceous matter in some of the clear spaces shown. Much of the crowded neoplastic tissue was still living. × 11½.

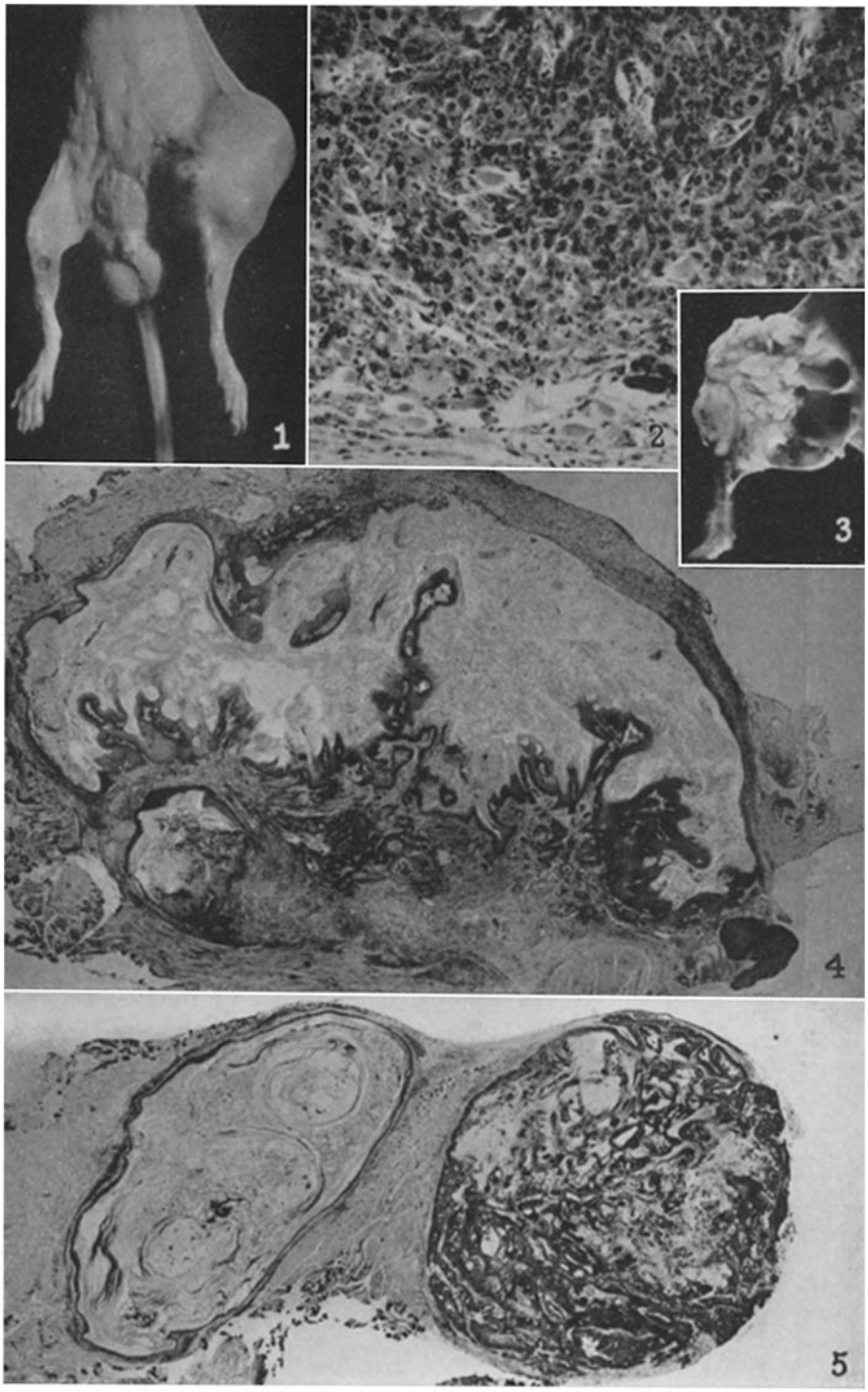

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PLATE 34

FIG. 6. Squamous cell carcinoma which took origin from the wall of an epidermal cyst due to implantation of the skin of 18 mm. embryos together with *OSSM*. The mouse was killed on the 26th day because of rupture of the cyst during palpation, and the cancer was found next the break (mouse 8, Table III). The growth has resulted in a local thickening of the epidermal layer, which has a well defined stratum granulosum consisting probably of non-neoplastic elements overlying the carcinomatous cells. These stain less deeply than the merely hyperplastic epidermis to either side. Where they have extended into the profuse reactive tissue they are wholly anaplastic. Many mitoses can be seen (arrows). × 240.

FIG. 7. Papilloma on the wall of an epidermal cyst from an animal receiving olive oil containing 1 per cent of methylcholanthrene (*OM*) together with fragments of the same skin as that from which the carcinoma of Fig. 6 derived: 29 days had passed since the implantation (mouse 9, Table III). In some regions the basal epidermis of the layer covering the papillae is crowded and appears columnar. × 80.

FIG. 8. Another tumor originating from the wall of the same cyst. A moderately anaplastic squamous cell carcinoma has extended from amidst droplets of *OM* into the surrounding tissue, where it is replacing the muscle fibres individually. Many mitoses can be seen. × 180.

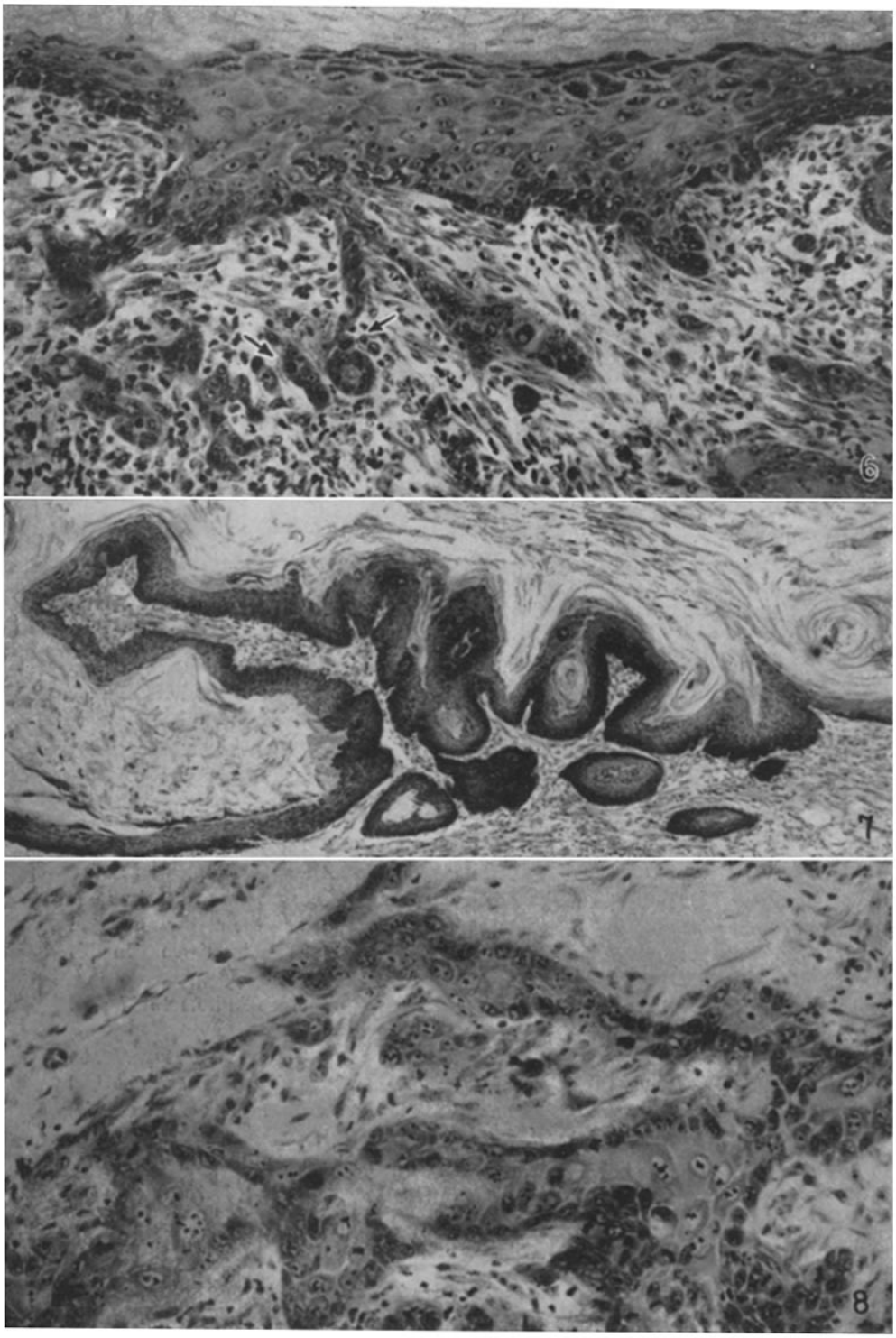

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PLATE 35

Fig. 9. A third tumor from the same specimen. Its cells are much more basophilic than those of the epidermal layer from which it has originated, most of them are smaller, and the overlying keratin stains with methylene blue, unlike that round about. Invasion of the connective tissue has begun, and toward the left the neoplastic cells have extended far out along the bottom of a layer of merely hyperplastic epidermis. × 170.

FIG. 10. Anaplastic carcinoma with giant multinucleated cells, which has extended laterally in the connective tissue next the epidermal lining of an implantation cyst (the blurred layer at the top of the picture). The skin of 18 mm. embryos had been implanted 34 days before, together with *OSSM* (mouse 11, Table II). × 230.

FIGS. 11 and 12. Transverse sections of the skin of the back of an 18 mm. embryo (17 days old). Some rudimentary hair follicles are present, the corium is loose and cellular, and there is no recognizable adipose tissue. Fig. 11, × 18; Fig. 12, × 140.

FIGS. 13 and 14. Results, after 11 days, of implanting in one thigh the skin of 18 mm. embryos together with an equal quantity of Tyrode's solution, and in the other the same skin with olive oil containing 1 per cent each of Scharlach R and methylcholanthrene (*OSM*). The embryos from which the skin came were litter mates of the one furnishing Figs. 11 and 12. × 18.

The skin with Tyrode's solution gave rise to several cysts (Fig. 13), not all of which are shown: a part of one that was multilocular can be seen at the top of the picture. They are lined with epidermis much thinner than when it was taken from the embryo (Fig. 11), and the hair follicles have differentiated and hypertrophied but as yet hairs are absent from the contents of the cyst. The follicles are embedded in a thick layer of fatty tissue and have extended through this to impinge directly on the muscle of the host, where some have become clubbed and bent.

The state of affairs in the implant with *OSM* is very different (Fig. 14). This consists of a single cyst, larger than any of Fig. 13 and containing many dead fragments. There are some empty spaces amidst the keratin where *OSM* was dissolved out during preparation of the section. The epidermal layer lining the cyst is nearly or quite as thick as that of the original embryo (Fig. 11) but hair follicles are few and their differentiation has been abortive. No fat has been laid down, and the cyst is separated from the surrounding muscle merely by a shallow layer of reactive tissue. In this instance there were no outlying droplets of *OSM*.

FIG. 15. Specimen procured 13 days after the implantation of skin from 16–17 mm. embryos together with olive oil.

The epidermal lining of the cyst is still thick and of stratified squamous type. The follicles have begun to form hairs and some of them, becoming huge, have pushed through the well defined fatty tissue and become bent where they met the muscle. Here rounded lacunae can be seen (arrows) where oil droplets were present. The follicle epithelium immediately next these latter has been wholly uninfluenced by them. × 35.

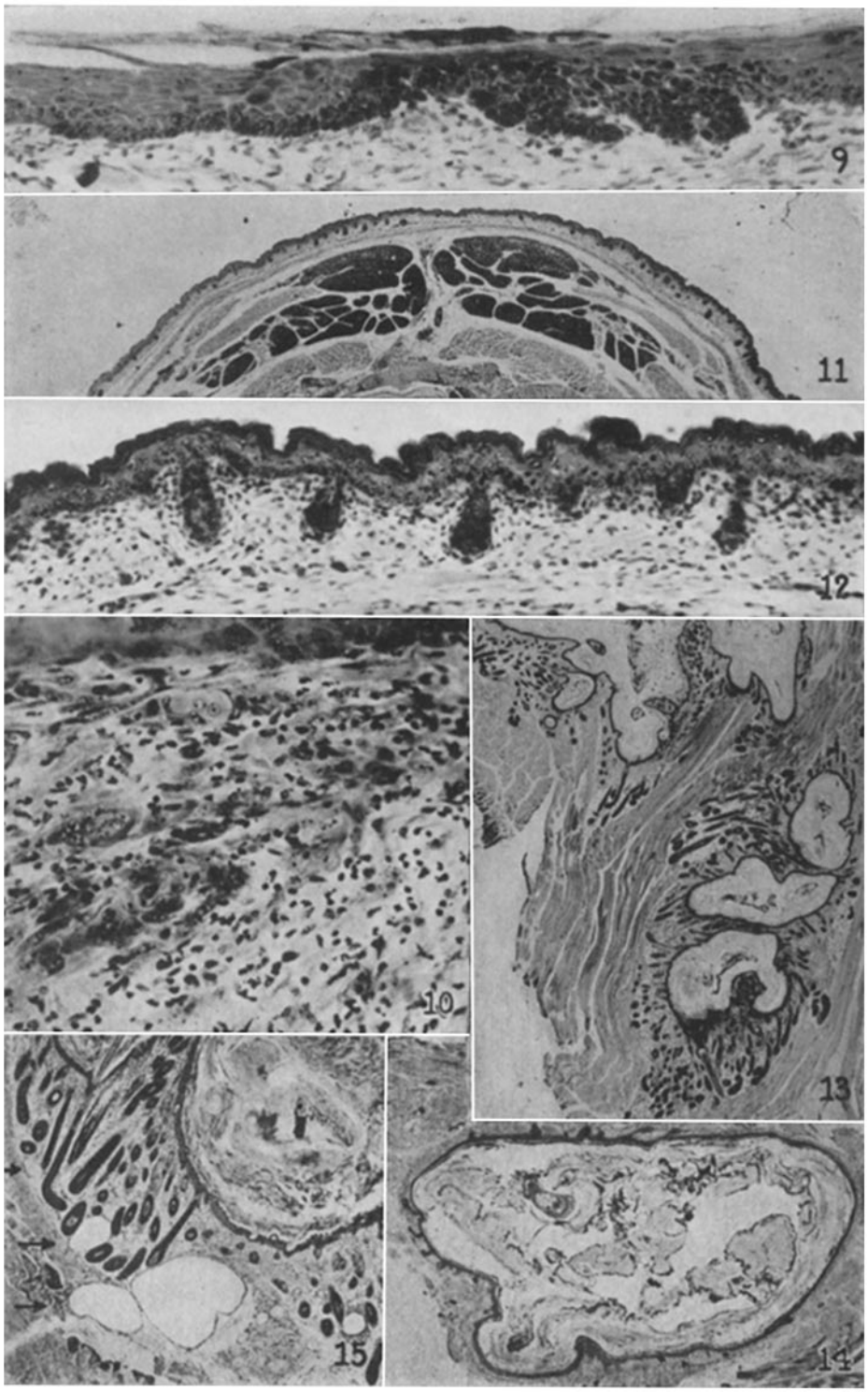

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PLATE 36

FIGS. 16 and 17. Findings 21 days after implantations of the materials of Figs. 13 and 14 in another mouse. × 18.

The multiple cysts due to skin with Tyrode's solution (Fig. 16) are only a little larger than on the 11th day and the epidermis lining them is now very thin; higher magnification showed that it had attained the adult form. Within the cysts there are numerous hairs, seen mostly in cross-section. The corium has become dense and the adipose tissue is more abundant than on the 11th day. The cysts were flattened in the plane of the muscle fibres and the section has been cut in this plane.

The single cyst resulting from the implantation with *OSM* (Fig. 17) has enlarged considerably, compressing the muscle (top of photograph), and is now rounding out. Much of its epidermal lining is still as thick as the original embryo epidermis, and the follicles, with a single exception, appear undifferentiated, have been stretched wide, and are surfacing. Yet the presence of numerous hairs,—in cross-section amidst the keratin near the middle of the cyst,—attests to previous differentiation and functioning. Numerous lacunae can be seen in the muscle, where *OSM* droplets had lain scattered. The epidermal lining of the right side of the cyst is thin, and here there are no follicles,—a condition due to secondary extension of the epidermis to surround the dead material, as a study of early stages has shown.

FIGS. 18, 19, and 20. Stages in the encystment of olive oil supersaturated with Scharlach R (*OSS*).

In Fig. 18 the cells of the embryo epidermis have grown out amongst the globules of *OSS* from a stratified layer which is markedly granular. A few of the smallest globules have already become enclosed in the epithelium. × 80.

Fig. 19 represents a later stage in the encystment. Many globules are in course of passage through the lining wall of the cyst, which has a well defined stratum granulosum. The epithelium surrounding one of the outlying globules (arrow) has differentiated with the formation of a granular layer. × 62.

Matters are further along in Fig. 20. Here active extension to the oil droplets has ceased. × 55.

All of the specimens are from the same implant of the skin of 17 mm. embryos with *OSS*; it had been in place 17 days.

FIG. 21. Late results of implanting the skin of 20 mm. embryos together with *OSS*. The epidermal cyst shown was much flattened but it has been cut in the plane of the flattening with result that it appears rounded. It had "pointed," bulging out into the connective tissue, and in one region (arrow) its wall was exceedingly thin and devoid of epithelium. Elsewhere this was of the adult type. In the older, intramuscular portion of the cyst many hair follicles in good condition can be seen, as also numerous hairs amidst its keratinized contents. Part of a similar cyst is visible at a corner of the picture. × 22.

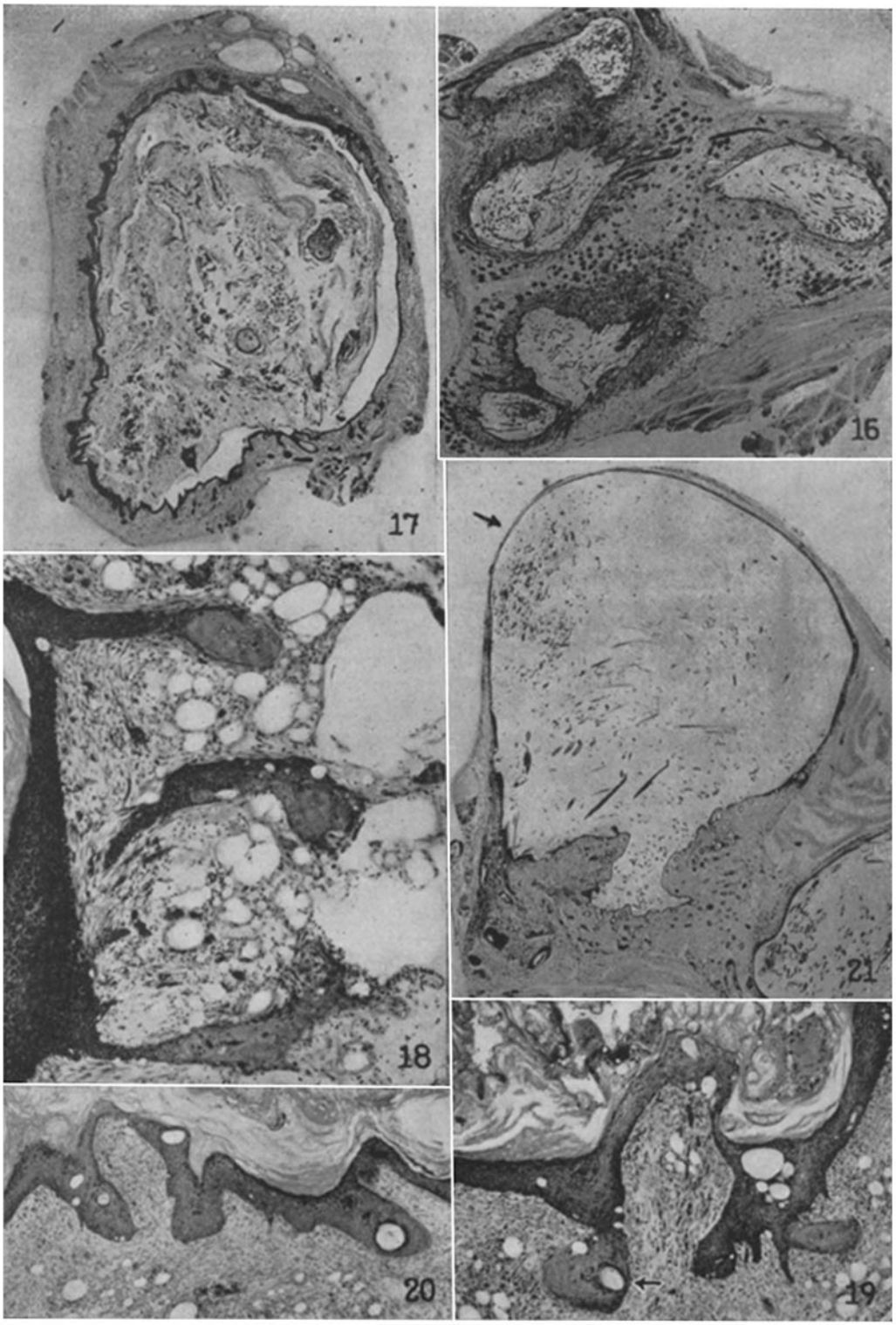

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PLATE 37

FIG. 22. Part of a cyst forming by the extension of the epidermis from skin fragments implanted together with *OSSM* 6 days previously (Experiment 14, 18 mm. embryos). The epidermal cells show few signs of proliferation but are migrating to include the oil droplets. Other droplets are already present in the cyst, as also dead fragments of skin. × 140.

FIG. 23. Lining of a cyst due to implantation of the skin of 18 mm. embryos with *OSSM* 9 days previously. There are many mitoses now and the cells of the stratified squamous epidermis show great irregularities of size and shape. A stratum granulosum is almost wholly absent, and the basal cells appear to lie separate at several spots, perhaps owing to edema. The line of demarcation between them and the very cellular connective tissue is ill-defined. × 300.

FIG. 24. Part of a cyst due to implantation in another mouse of the materials furnishing Fig. 22 (Experiment 14): 10 days have elapsed. The epidermal layer is now very thick, notably basophilic, and practically devoid of hair follicles. Numerous round lacunae bespeak the presence of oil droplets. Some have been included in the epidermal layer or are in process of inclusion, but there are many others amidst the exceedingly cellular connective tissue separating the cyst from the muscle. No fatty tissue developed from the implant. × 21.

FIG. 25. Another part of the wall of the cyst furnishing the carcinoma of Fig. 6 after 26 days: from the same experiment as Fig. 22. The epidermis is notably hyperplastic and at several spots its cells are darkly basophilic, crowded, and appear to be extending down. Whether they are carcinomatous is uncertain. An immense number of lymphocytes have collected in the connective tissue. × 130.

FIG. 26. Tumors in the thighs of a mouse implanted with the skin of 18 mm. embryos and *OSSM* (mouse 15, Table II). The animal was killed on the 97th day because the growth on the right had begun to involve the skin. It had wholly surrounded the femur. The microscope showed the presence of 5 distinct carcinomas and a carcinosarcoma, all of which were successfully transplanted (Tumor 13 of Table I; see Figs. 39 and 40).

The growth on the left appeared earlier (after 45 days as compared with 59) but enlarged more slowly. Like the other it "pointed" toward the rear of the thigh. Though it was an invasive anaplastic carcinoma 9 grafts of it failed on transplantation and the tenth barely survived (Tumor 8 of Table I). × 1.

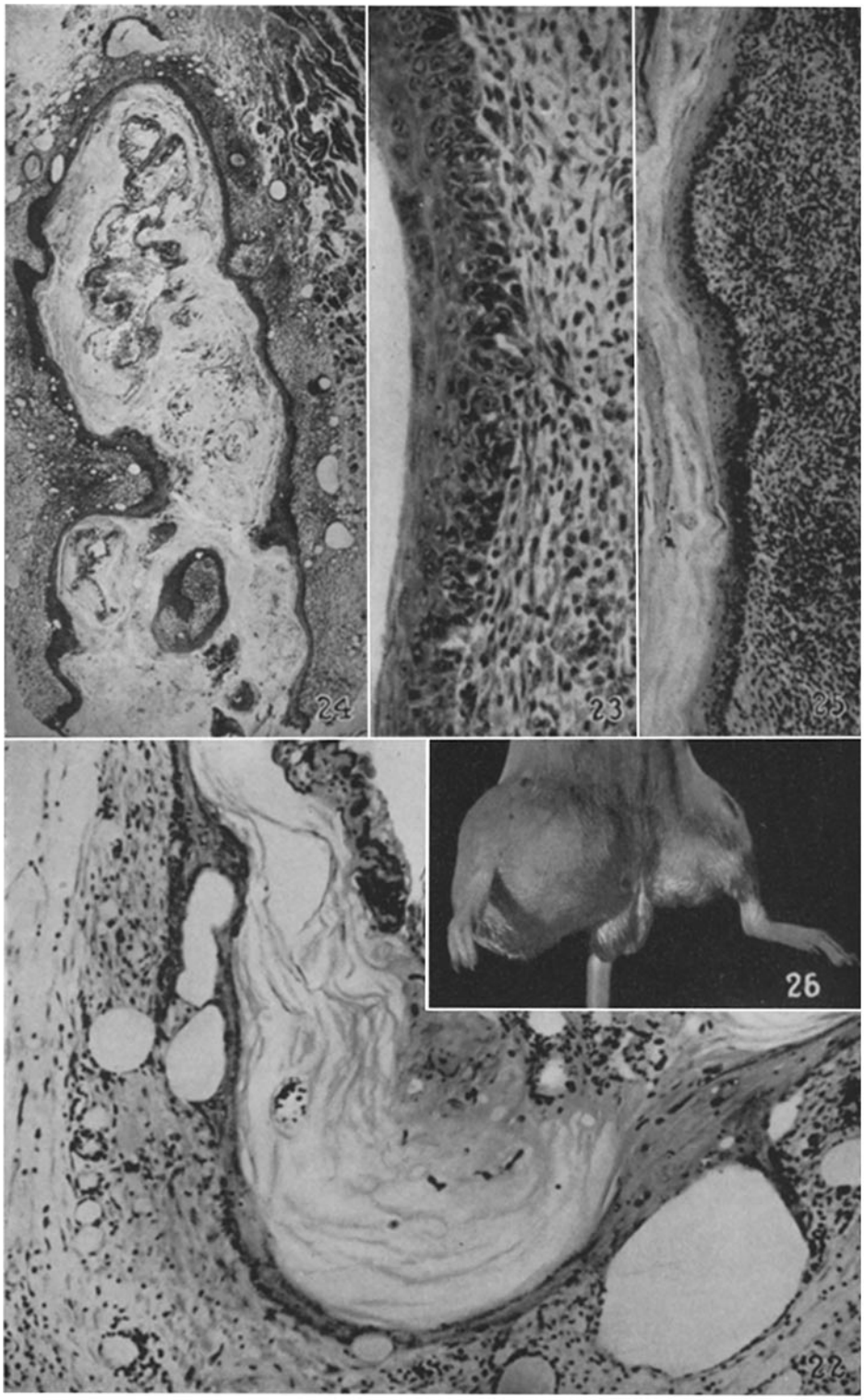

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PLATE 38

FIG. 27. Epithelial extension to droplets of *OSSM* from the wall of a cyst due to an implantation 13 days previously (skin of 16–17 mm. embryos). The droplets of oil lie amidst reactive tissue, with many more leucocytes about them than ordinarily. Some of them have already been surrounded by the epithelial elements, which show great irregularities of size and form. To be compared with Fig. 15 illustrating the response of the same epidermis to olive oil as such. × 100.

FIG. 28. Results of the implantation of skin from 18 mm. embryos together with *OM*; 15 days. The epidermis is extending from the cyst wall to nearby globules, just as in the case of *OSS* and *OSSM*. The aspect of the cells and the local accumulations of them suggest that some may already have undergone neoplastic change. × 125.

FIG. 29. Benign papilloma situated on the wall of a cyst lined with stratified squamous epithelium. It had resulted from the implantation 70 days previously of the minced tissue of 7–8 mm. embryos together with *OSSM* (Tumor 2 of Table I). × 13½.

FIG. 30. The same papilloma 39 days after transplantation. The growth has established itself but done no more, failing to extend laterally to cover the wall of the tiny cyst in which it lies. × 12.

FIG. 31. Benign papilloma with a fleshy core, which had arisen in 45 days where the skin of 11–13 mm. embryos had been implanted in a mouse 9 days old together with *OSM*. Some of the epidermis which lined the cyst containing the growth can be seen to the left. The papilloma failed to survive on transplantation (Tumor 1, Table I), though an anaplastic carcinoma grew which was present at one spot in it as other sections showed. × 35.

FIG. 32. Carcinoma resulting from the implantation 97 days previously of the skin of 18 mm. embryos together with *OSSM* (mouse 15, Table II, left leg). Highly invasive. Most of its cells are seen to be completely anaplastic but at some spots differentiation has occurred (arrow), with the formation of lumps of keratin. × 160.

FIG. 33. Results of transplanting the carcinoma of Fig. 32 (Tumor 8 of Table I). The grafts failed to give rise to tumors at any of the 10 sites to which they were transferred, and all became wholly necrotic except the specimen here pictured which still has some differentiating neoplastic cells after 35 days (arrows) like those of the primary carcinoma. × 160.

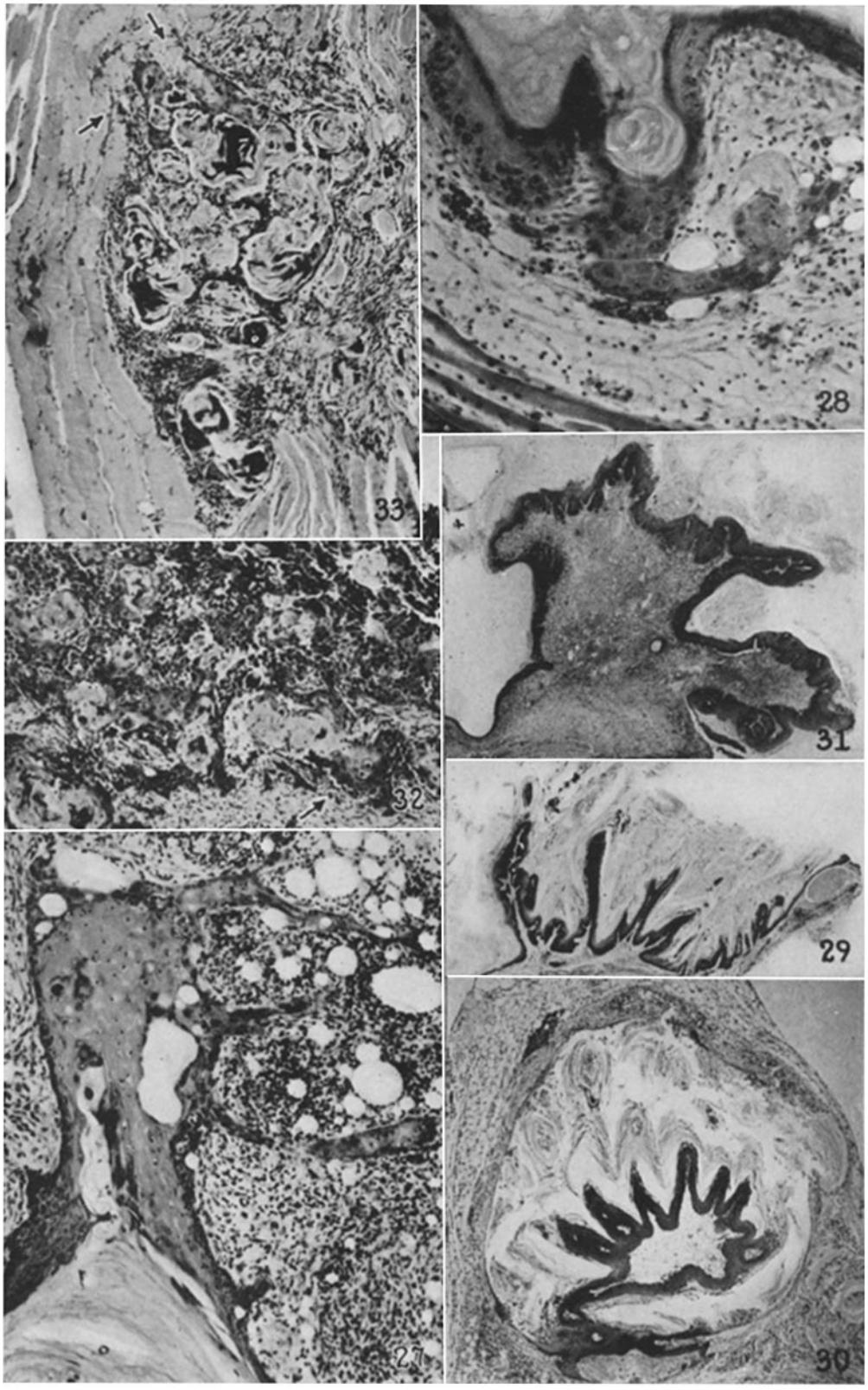

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PLATE 39

FIG. 34. Part of a nodule removed 95 days after the implantation of the tissue of eviscerated and minced embryos $10\frac{1}{2}$ days old (3.5 mm. long) together with mouse fat containing 1 per cent of methylcholanthrene (*FM*). To the right, part of a bone can be seen containing red marrow, as also half of a cyst lined with epidermis of adult type and full of keratin and hairs. Here there is no sign of influence of the carcinogen.

Toward the left is another cyst with many lacunae near it where globules of *FM* were present. The epithelium lining its wall is irregularly thickened, and higher magnification showed it to be everywhere disorderly and carcinomatous and just beginning to invade (see Fig. 35). The surrounding connective tissue was very cellular, containing many lymphocytes and a few fresh hemorrhages, and it was somewhat compressed as if by sudden enlargement of the cyst. × $31\frac{1}{2}$.

FIG. 35. From another of the serial sections of the cyst shown in Fig. 34. The neoplastic epithelium is invading the reactive connective tissue. Several mitotic figures can be seen. × 300.

FIG. 36. Carcinomatous extension along the wall of an epidermal cyst which had resulted from the implantation 40 days previously of skin from 15 mm. embryos together with *OSSM*. The cells are so abnormal in this instance that the malignant character of the layer can be readily discerned; they are dying without differentiation and some are multinucleate. × 180.

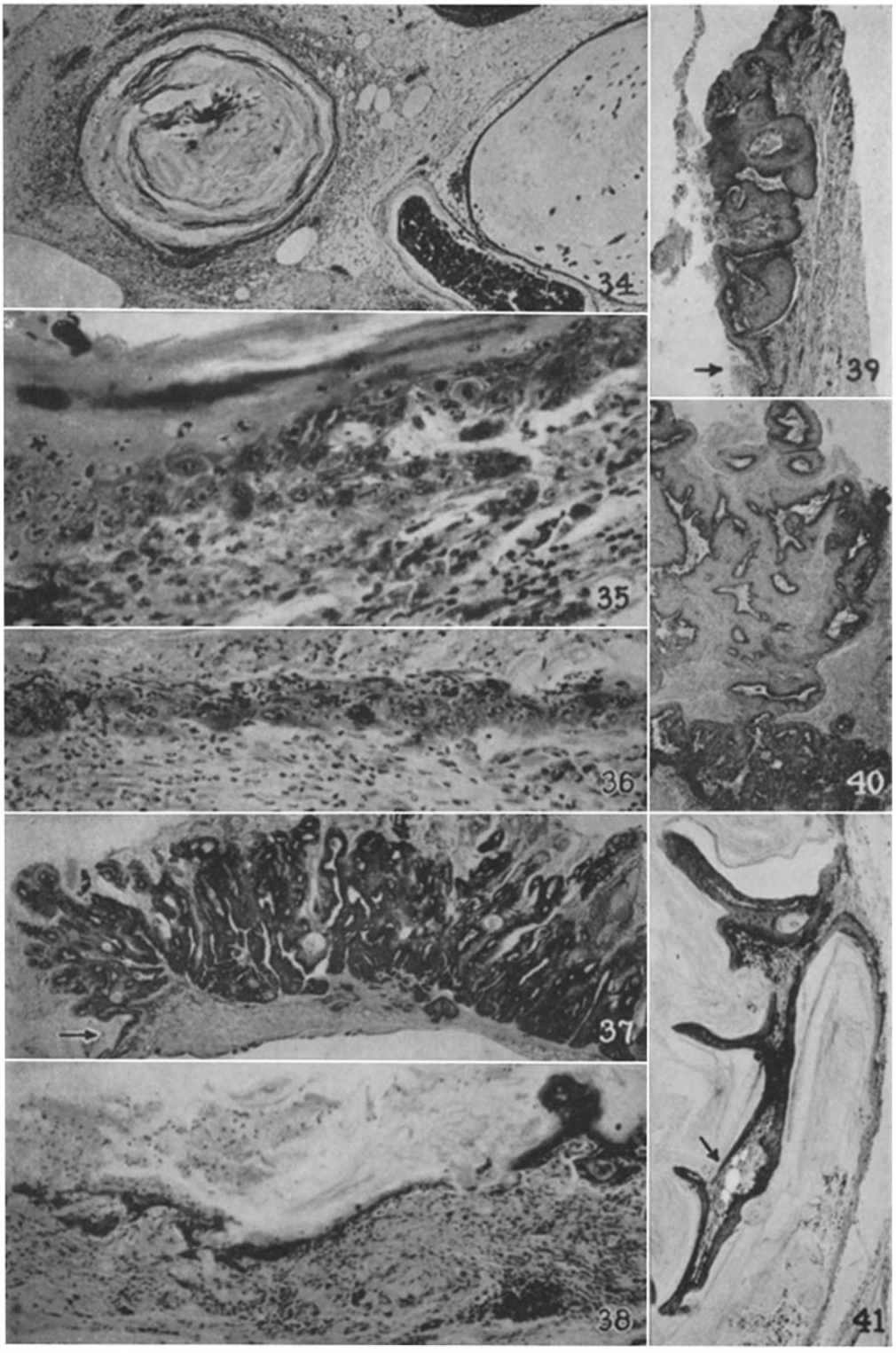
FIG. 37. Part of a malignant papilloma on the wall of an epidermal cyst due to the implantation 91 days previously of the skin of 18 mm. embryos together with *OSSM* (mouse 14, Table III). The growth has invaded the connective tissue. The epidermal layer lining the cyst can be seen on the left (arrow), the papilloma falling off sharply to it. × 13.5.

FIG. 38. The tumor of Fig. 37 regressing after transplantation (Tumor 5 of Table I). The 22 grafts grew swiftly at first at every site, giving rise in 12 days to growths 5 to 15 mm. in diameter, but thereafter they began to dwindle, either at once or within the next 2 weeks, and by the 36th day when this specimen was procured the smaller growths had vanished and the larger were mere bags of necrotic material with some living remnants of the tumor in a few cases. This picture illustrates the latter state of affairs. The papillomatous tissue has retained its malignant aspect although it is now reduced to a mere skim, underlain by reactive tissue, on the wall of a cyst full of pultaceous matter. × 105.

FIG. 39. Part of the wall of a cyst forming the major part of the big tumor shown in Fig. 26 (mouse 15, Table II),—to show 2 of the tumors present in the composite mass. One appears to be a benign papilloma, while that next it (arrow) is frankly malignant. × 21.

FIG. 40. The same neoplasms after transplantation (Tumor 13 of Table I); both are flourishing. In every host the grafts gave rise to huge tumors. The animal furnishing the present picture was killed after 55 days. × 14.

FIG. 41. Branched papilloma covered with hyperplastic epidermis: from the wall of a cyst due to the implantation 18 days previously of 18 mm. embryo skin together with *OSSM* (mouse 9 of Table II). Several droplets of *OSSM* were present in the connective tissue core of the papilloma (arrow). × 57.



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