THE EXPERIMENTAL DISCLOSURE OF LATENT NEOPLASTIC CHANGES IN TARRED SKIN

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PLATES 17 TO 20

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The growths which tar first calls forth on rabbit skin are all benign, and although true tumors (1) are wholly dependent for their continued existence upon encouraging local influences. Tarring itself will provide the needed encouragement, but when it is left off the growths disappear unless other favoring factors act to maintain them, as *e.g.* chronic pathological changes in the supporting tissues, or inflammation due to bacterial infection or to crowding. Where the tumors once were one finds only epidermis of ordinary appearance, yet if tarring is resumed some of them not infrequently reappear, even after months, and by reapplying tar at intervals they may be made to recur again and again (1),—clear proof that some cells in the apparently normal epidermis have retained their neoplastic potentialities. Usually each successive period of tarring brings out more growths than before, and they appear very soon, sometimes almost at once instead of after several months. It is as if new tumor cells had been merely waiting upon encouragement; and certainly in its lack the skin, unless markedly altered, remains devoid of growths no matter how long the animal lives. These facts taken together strongly suggest that tar may render many more cells neoplastic than ever assert themselves in tumor terms under ordinary circumstances. In the present paper this is shown to be the case.

Rationale

Our plan has been to tar the skin of rabbits repeatedly throughout a period somewhat less than is ordinarily required to elicit growths, and afterwards to subject it to non-carcinogenic stimulation. The stimulant utilized was wound repair; for it had been noted incidentally to previous experiments that the healing of holes punched in a rabbit's ear at a spot where a large tar carcinomatoid had once existed was attended by reappearance of the growth on several occasions (1).

It seemed possible that the punch-hole test would yield information

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incidentally as to whether the tumors evoked by tarring result from the proliferation of cells inherently peculiar, though present in normal skins, or whether they are the consequence of random cytological changes induced by the carcinogen. The healing of a circular hole in the ear is almost diagrammatic when there is no bacterial infection, the tissue extending in evenly from all around the periphery to a common center. The end result is a disc covered on each side with an epithelial sheet that has been formed by centripetal proliferation of cells at or near the margin of the original wound. If any of the elements multiplying to form these sheets were possessed of neoplastic potentialities, then their descendants should possess these also, for that holds true of tumor cells generally; and if these descendants extended inwards in anything like a linear way, one would expect that tarring of the eventual discoid surface would elicit tumors along one or more of its radii or even over an entire segment of it in case any considerable aggregate of neoplastic cells had been cut across when the hole was punched in the ear. Some preliminary observations supported this conception. In several instances tar papillomas were purposely cut across when the hole was made, and as it healed the growths were noted to extend inwards, providing their proportional share of the new epithelial covering, with result in segmental tumors (Fig. 1). In one case the tarred epidermis cut through when a hole was punched was noted to be gray at one small marginal spot, and from this spot pigment-forming melanoblasts extended in along a radius, together with the epithelium, as healing took place, with result in an almost black streak from periphery to center of the new disc. Later tarring disclosed the fact that the "tagged" epithelium possessed neoplastic potentialities, a sooty, radial papilloma forming on the streak (Fig. 3).

A circular hole 1.3 cm. across was punched in a rabbit ear which had been tarred on the inside throughout several periods of some weeks each, the last ending 159 days previously. The history of the animal (D. R. 4-48) is given *in extenso* in an accompanying paper (1). No growth was present on the outside of the ear at the time when the hole was punched in it, though the skin there was hyperkeratotic, and almost hairless. The hole healed symmetrically, and as this happened a narrow, superficial black streak was noted to be extending in with the new tissue along a radius from a dark spot at the periphery of the circle. It was situated on the outer surface of the ear, was very slightly raised, had a smooth surface, and as the hole closed so did it attain to the center of the new disc. Tarring was now begun again, twice weekly, and within a few weeks a growth had arisen along the streak, ridge-shaped, sharply defined, and about 1 mm. high (Fig. 3). It was punched out together with the surrounding tissue and cut in serial sections along its length. It proved to be a melanotic papilloma which had taken origin from a small spot at the edge of the original hole, where epidermis had been cut through which con-

tained pigmented melanoblasts. These were now present everywhere in the deeper layers of the papillomatous epithelium, while in the underlying connective tissue there were numerous chromatophores stuffed with pigment. Nowhere else on the disc was any melanosis visible.[1]

General Method

Adult, domestic rabbits of agouti breed were employed throughout. In most cases their ears were tarred twice weekly on the insides,[2] and stripped of the accumulated layer before every third tarring. 2 or 3 days after the final application the tar layer was removed, the animals were anesthetized with ether, and from four to eight holes were punched in each ear with sharpened, sterilized cork borers. Holes 1.1 cm. across were made in its basal half, and here they usually healed completely within a few weeks, while 6 mm. holes were punched in its further half, because the likelihood of complete repair had been found to diminish toward the ear tip. The total number of holes was determined by the size of the ears, which varied much from animal to animal. In some of the experiments it was essential for comparative purposes that the holes be punched in corresponding situations on the two ears of each rabbit. To make certain of this they were flattened against each other back to back with their edges absolutely corresponding, threads were run through them both with a sewing needle at each spot where holes were to be made, and these were severed leaving small pieces *in situ* to mark the centers of the discs of tissue, which were at once punched out. Ordinarily the holes were located to either side of the central artery about midway between it and the marginal vein, at relatively avascular situations that is to say, and they were spaced evenly from base to tip of the ear. Sometimes there was bleeding which had to be stopped, especially when the ears had been tarred. This was done by stuffing the holes with pledgets of sterile absorbent cotton, which were removed next day. Bacterial infection seldom ensued and when it did so the animal was discarded. Ordinarily a shallow, ring-shaped scab formed around the edge of the wound, and when it came away, after about 10 days, a smooth, healed edge was disclosed, from which extension inwards of the tissues took place rapidly and evenly.

The disc of new tissues thus formed had a smooth surface, which set it off sharply from the surrounding expanse, as did also its purply color during the first weeks. For many months it retained its original diameter, only very occasionally contracting and becoming distorted. At its center there sometimes persisted a narrow channel through the ear, lined with epithelium. All of the tissues participated actively in the healing, the cartilage rapidly extending in, and often proliferating excessively if the ear had been subjected to tarring (2), with result in lenticular or knob-like swellings or cups, protruding on one side or the other. The rate at which the holes closed varied much from individual to individual, and so too did the amount of hyperplasia. Microscopically the edge of the disc was readily to be told because here the cartilaginous sheet underwent abrupt change (Figs. 6 to 9). Thus a landmark was provided which proved useful after tarring was done later, since then the disc often became almost indistinguishable in the gross from

[1] These observations were made incidentally to the work of the accompanying paper (*q.v.*).

[2] Horizontal retort tar was used, from the Ostergasfabrik of Amsterdam. It was the gift of Dr. Karl Landsteiner.

the surrounding, rugose tissue. Tarring was never resumed until healing was completed or had come to a standstill.

The Response to Wound Healing of Normal and Tarred Epithelium

Experiment 1.—Tar was applied twice weekly for 4 weeks to the insides of one or both ears of seven rabbits. When one ear only was tarred the other was protected against accidental transfer of the material by covering it with a thin layer of gum dammar dissolved in benzol or acetone, a layer renewed as often as necessary. The gum was devoid of any inflammatory or tumor-producing effect, as the results showed. In some instances holes were punched in both ears immediately after they were last stripped of tar, though in other cases not until 5 weeks afterwards; and 4 to 11 weeks afterwards tarring was begun again and kept up for from 13 to 22 weeks. In several instances the holes had completely healed before the new tarring was begun, while in others those toward the end of the ear had not all closed entirely, for the reason that they had been too big originally.

Table I summarizes the findings. It shows that the first tarring elicited one growth in each of two animals. There were no signs of any in the others. While the holes were closing thirteen tumors appeared, seven of them where repair was under way and six elsewhere on the ears. They occurred in five of the seven rabbits and only on ears that had been tarred prior to punching. Six of the seven tumors on the healing surface were radial,—narrow, almost linear growths, extending from periphery to center of the new disc of tissue. The seventh was situated at the original edge of the punch hole, that is to say it was "peripheral," according to our nomenclature. All were low mounds or frank papillomas.

The tarring done after healing was completed had several objects,—to stimulate any neoplastic cells that might still be latent, to encourage the growth of the tumors already present (2), and to test the new epithelium as a whole for its responsiveness as compared with that of the rest of the ear. The applications evoked many new tumors, and they were more than three times as numerous where healing had taken place. The total epithelial area comprised in the recently formed discs was determined by planimeter measurements of tracings taken from the ears. It averaged for each organ 3.2 sq. cm., or only about 6.4 per cent of the whole tarred surface, this averaging 49.8 sq. cm. Yet of the total number of growths elicited by the second tarring 21.9 per cent (68 of 311 tumors) appeared on the new disc of tissue or at its edge.

As already stated, the growths which appeared while the new discs were forming occurred only on ears that had previously been tarred. The later tarring elicited about twice as many new tumors on these ears as on their fellows which served as controls. When both organs had been tarred beforehand, the yield of tumors was nearly the same on both.

Some specimen instances will be given.

TABLE I (*Experiment 1*)

Wound Healing and Tumor Incidence

Rabbit		Period of first tarring	Tumors elicited	Interval to punching holes	Further interval to second tarring	Extent of healing	Tumors appearing in interval	Period of second tarring	Total healed surface	Total ordinary surface	Tumors elicited by second tarring							
											On healed surface				Total		Transitory tumors	
											Peripheral	Radial	Eccentric	Central	On healed surface	On general surface	On healed surface	On ordinary surface
No.	*Ear*	*wks.*		*wks.*	*wks.*			*wks.*	*sq.cm.*	*sq.cm.*								
5	R	4	0	5	5	Complete		16	4	43	1	1		1	3	18 (1)*		2
	L											1			1	19 (1)		4
6†	R	4	0	5	5	Complete	1 radial	13	4	46	4	4		2 (1)	10 (1)	13		1
	L						2 radial				1	2	1	3	7	13		3
7†	R	4	0	5	11	Fair	1 radial 1 peripheral	16	2.5	49.5	5	2	1		8	40 (2)		6
	L			None	6		1 on general surface		3.1		2			3	5	27		8
9	R	4	0	5	6	Good	1 radial	14	3.2	52	1	2	4	1	8	9 (2)	1	3
	L		1	None	11		3 on general surface		3.1							13		1
10†	R	None	0	None	8	Complete	1 radial	15	3.5	38.5	5			2	7	11	2	2
	L	4					1 on general surface				2	1	2		5	22 (1)	3	16
12	R	None	0	None	4	Poor		22	2.3	45.5	1 (1)				1 (1)	6	1	
	L	4							2.8		5 (1)	1	(1)		6 (2)	20 (1)		7
13	R	None	0	None	5	Good		18	3.2	51.5	2				2	13		5
	L	4	1				1 on general surface		3.0		2	1 (1)		2	5 (1)	19 (2)	2	9
Average area, *sq.cm.*									3.2	46.6	Total...31 (2)	15 (1)	8 (1)	14 (1)	68 (5)	243 (10)	9	67
Percentage of whole									6.4		Percentage of total				21.9	78.1	11.8	88.2

* Growths recorded in parentheses were on outside of ear.

† See Charts 1 to 3.

Both ears of rabbit 6 (Chart 1) were tarred but holes were not punched in them until 5 weeks later. During the 3 weeks that followed three radial mounds were noted to be forming as the holes closed, although elsewhere on the surface of the ears,—which were rapidly reverting to the normal,—there was no trace of any tumor. Subsequent tarring demonstrated the neoplastic character of the mounds, these enlarging rapidly under its influence into vigorous papillomas or carcinomatoids. It also elicited other growths on the new sheets of epithelium, and they appeared sooner here and in far greater proportionate numbers than elsewhere.

Both ears of rabbit 7 (Chart 2) were subjected to tarring, and holes were punched in one of them immediately afterwards but not in the other until 5½ weeks had gone by. While repair was taking place of the ear first mentioned two tumors appeared, one peripheral, one radial, and elsewhere on its surface a third growth arose. This soon

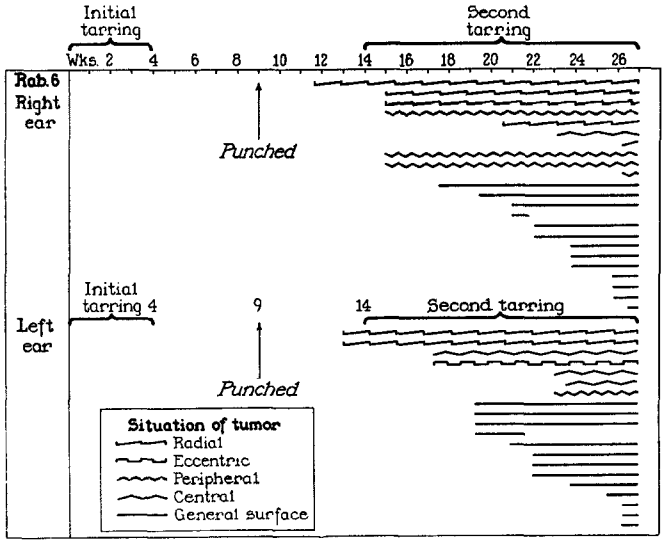

CHART 1 (rabbit 6). The effect of wound healing to elicit tumors on skin previously tarred.

disappeared, and failed to recur after tarring was resumed, whereas the peripheral tumor, which had also vanished, reappeared as a radial growth and, like the other growth of this character, enlarged rapidly. Many new tumors also put in an appearance. They arose sooner on the healed surface but were numerous elsewhere.

The results were different with the ear in which the holes were made 5½ weeks after the preliminary tarring. Healing failed to evoke tumors, and although the subsequent tarring brought out growths in considerable number relatively few of them were situated on the discs of new tissue, and these few appeared late and tended to vanish, as did many of those elsewhere.

The right ear of rabbit 10 (Chart 3) was normal when holes were punched in it, and the later tarring elicited growths no sooner where healing had taken place, though they occurred in greater proportionate number there. The left ear had been tarred beforehand, and a radial tumor appeared while healing took place, as also a growth elsewhere. The later tarring brought out more tumors in both areas.

One ear of each of the five animals of Experiment 2 was subjected to preliminary tarring while its fellow was protected with gum dammar. The tar was applied only three times, to find whether this short treatment would bring the skin into such a state that healing would elicit growths.

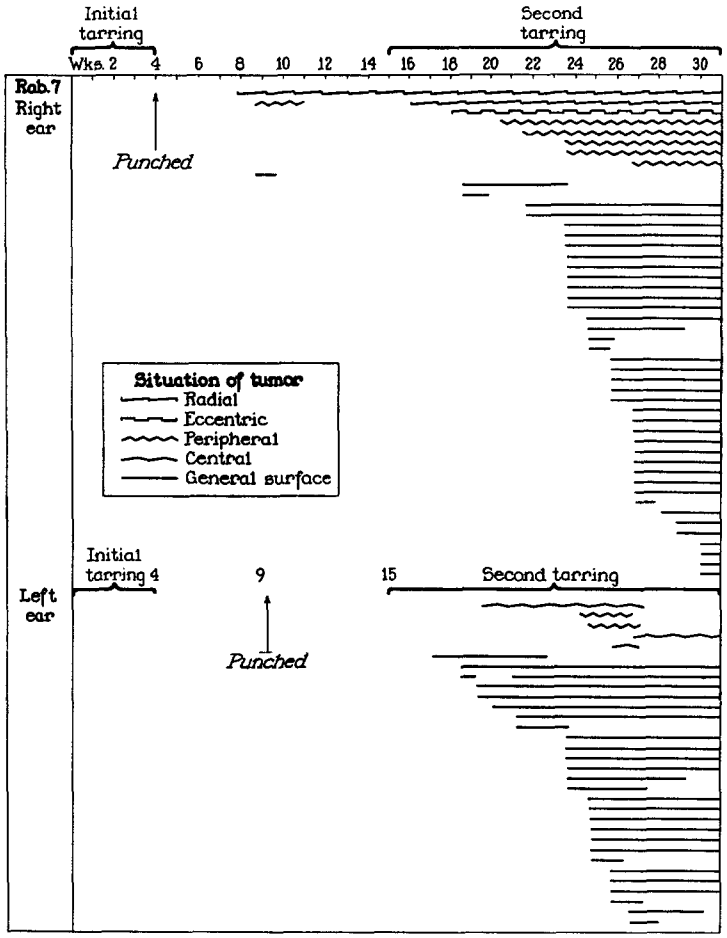

CHART 2 (rabbit 7). The effect of wound healing to elicit tumors on skin previously tarred.

In only one animal did this prove to be the case (Table II). Rabbit 6-45 developed a growth at the margin of a healing punch hole, none appearing elsewhere. The later tarring of the ears was begun 6 weeks after holes had been made in them: healing was then complete or had come to a standstill. Many more tumors arose on the ears previously tarred, 62 as compared with 39 on the control organs; and the growths were especially fre-

quent where healing had taken place. The surface area of the discs of new tissue was only 7.4 per cent of the whole, yet 24.8 per cent of the tumors (25 out of 101) appeared on their surfaces or at their edges.

In Experiment 3 (Table III) both ears of eleven rabbits were tarred three times, and holes were punched immediately afterwards. Tumors appeared in four animals, but only where healing was under way. After an interval of 4 to 9 weeks tarring was resumed. It brought out many more growths on or at the edge of the new discs of tissue. They had a total area amount-

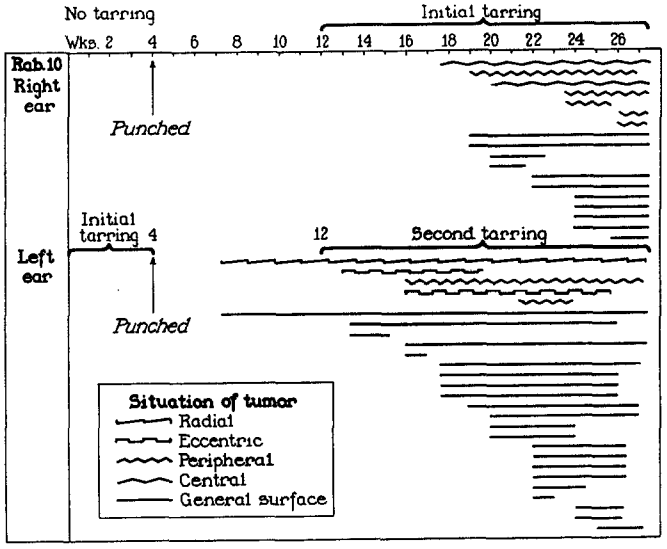

CHART 3 (rabbit 10). The effect of wound healing on normal and tarred skin.

ing to only 9.3 per cent of the whole expanse, yet 34.1 per cent of the growths (43 of 126) were associated with them.

Influence of the Preliminary Tarring on the Outcome

The healing of holes punched in normal ears never called forth tumors, whereas they not infrequently appeared when the organs had been tarred beforehand. Three applications were enough in some instances but tumors arose more often and were more frequent in animals subjected to eight previous tarrings. The number of growths which arose later on, after healing was finished and tarring had been resumed, varied from animal to animal and with how long the tar was applied. Tumors were more than three times as numerous, on the average, where healing had taken place, and they appeared earlier there,—a fact not discernible in the tables but

TABLE II (*Experiment 2*)

Wound Healing and Tumor Incidence

Rabbit		Preliminary tarrings		Interval to next tarring	Extent of healing	Tumors appearing in interval	Period of next tarring	Total healed surface	Total ordinary surface	Tumors elicited by second tarring							
										On healed surface				Total		Transitory tumors	
										Peripheral	Radial	Eccentric	Central	On healed surface	On general surface	On healed surface	On ordinary surface
No.	*Ear*	*No.*		*wks.*			*wks.*	*sq.cm.*	*sq.cm.*								
6-45	R	0	No tumors—Holes punched 6 days after last tarring	6	Complete	1 peripheral	13	3.5	41				2	2	3		
	L	3								1			2	3	8	1	
6-46	R	0		"	"		13	"	43.5			1	2	3	10 (2)*	1	
	L	3								5 (1)			1	6 (1)	18 (1)	1	2
6-51	R	0		"	"		21	"	43.5	2				2	5	1	3
	L	3								1	1		(1)	2 (1)	5	1	1
6-56	R	0		"	"		11	"	48.5	1			1	2	5		1
	L	3											1	1	10		2
6-59	R	0		"	"		17	"	41.5	1			1	2	5	1	2
	L	3								1			1	2	7		2
Average area, *sq.cm.*								3.5	43.6	Total....12 (1)	1	1	11 (1)	25 (2)	76 (3)	6	13
Percentage of whole								7.4		Percentage of total				24.8	75.2	31.6	68.4

* Growths recorded in parentheses were on outside of ear.

TABLE III (*Experiment 3*)

Wound Healing and Tumor Incidence

Rabbit		Preliminary tarrings		Interval to next tarring	Extent of healing	Tumors appearing in interval	Period of next tarring	Total healed surface	Total ordinary surface	Tumors elicited by second tarring							
										On healed surface				Total		Transitory tumors	
										Peripheral	Radial	Eccentric	Central	On healed surface	On general surface	On healed surface	On ordinary surface
No.	*Ear*	*No.*		*wks.*			*wks.*	*sq.cm.*	*sq.cm.*								
28	R	3	No tumors—Holes punched 3 days after last tarring	8	Complete	1 central	13	4	31	1				1	4		2
	L									1			2	3	2	1	
29	R	"		8	Complete		5	4	30	1			1	2	3		
	L												2	2	3		
31	R	"		4	Complete	(2) eccentric*	10	4	32	1				1	1		
	L											1 (2)*	(1)	1 (3)	6		
32	R	"		9	Almost complete	1 peripheral	12	3.9	34	2				2	1		
	L									4				4	2	1	
33	R	"		4	Complete		15	4	39	1	1		1	3	8		1
	L									1			2	3	10		
34	R	"		8	Almost complete		9	3.6	33			1		1	0		
	L							3.8				1		1	0		
35	R	"		8	Fairly good		13	3.5	29	(1)		1		1 (1)	2		
	L							3.5							2		

36	R L	"	No tumors—Holes punched 3 days after last tarring	5	Poor		11	1.8 2.0	48	1 (1)	2 (3)	1 1		4 (4) 1	7 6		1
37	R L	"		4	Fairly good Complete	1 central	17	4	32	1	1		2	2 2	4 5 (1)	1	2
38	R L	"		5	Complete		14	4	43			1	2	1 2	2	1	
39	R L	"		8	Fairly good		11	3.5 3.8	37	1 (1) 4		1	(1)	2 (1) 4 (1)	7 8	1 (1) 3	2 7
Average area, *sq.cm.*								3.6	35.3	Total....19 (3)	4 (3)	8 (2)	12 (2)	43 (10)	83 (1)	8 (1)	15
Percentage of whole								9.3		Percentage of total				34.1	65.9	34.8	65.2

* Growths recorded in parentheses were on outside of ear.

illustrated in Charts 1 to 3. Not infrequently growths arose on the outsides of the ears (Tables I to III), where the skin had become hyperkeratotic and more or less hairless as result of transferred tar. They tended to appear where healing was taking or had taken place. Nothing further need be said of them.

The tumors which developed in response to the stimulus of repair were only occasionally vigorous, and most of them tended to dwindle and vanish after it had been completed or had come to a stop. Renewed tarring, however, usually caused them to grow, and, like the other tumors then appearing, they enlarged far more rapidly than did the tumors situated elsewhere on the ear surface. Sometimes they rounded out into big tangential spheres at a time when tumors were still absent from the rest of the ear or were small (Figs. 16 and 17), and often they soon became so big as to cover the entire new disc. The rapidity of their growth was doubtless due to the labile state of the new tissues and the profusion of new vessels, these together ensuring a prompt formation of stroma[3] and an abundance of nutriment. Even the cartilage sometimes contributed to their support, extending up into them (Fig. 9).

Character of the Tumors

Whether situated on the discs or elsewhere on the ears, the tumors were of the common benign types (2), namely, typical tar papillomas (Figs. 4, 6, 8, 9), occasionally gray with pigment, or carcinomatoids (Fig. 7).[4] The latter are known to be mere expressions of factitious malignancy on the part of papillomas, consequent on the stimulus of tarring, and they change to growths of the latter sort within a few weeks after tarring is left off, or round up into cysts, or disappear (2). They were frequent at autopsy of the animals of the present work because tarring was kept up until within a few days of death. Those papillomas which were situated on the newly formed discs were often notably fleshy because of exuberant proliferation of the supporting connective tissue.

[3] Growths due to the rabbit papilloma virus are prone to enlarge with special rapidity where holes have been punched in the ear. Sections have shown that much of the increase in size is due to proliferation of the new-formed connective tissue (*vide* Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1938, **68,** 529, Figs. 1 and 2).

[4] A third type of benign tumor, the frill horn, is occasionally elicited on rabbit skin by tar. In the present work only one such growth was encountered, and it was part of a composite tumor which arose at the center of a healed disc (Fig. 11) after tarring had been resumed.

Situation of the Tumors

During the first few days after the holes had been made there was inflammation for from 1 to 2 mm. about them and a ring-shaped scab formed at their edges, as already stated. Only after this had come away did the holes begin to close, and it was then that tumors appeared on the healing surface or at its margin. At first they had the form of discrete rugosities or subepidermal mounds or hemispheres, like the generality of benign tar tumors on first appearing, and like these they often vanished unless tarring was resumed. Not infrequently it caused them to recur, and under the influence of the tar they enlarged into papillomas, or became indurated ulcers (carcinomatoids). Growths situated at the periphery of the new discs tended to encroach upon these more than upon the general surface of the ear, as might have been expected from the local conditions. In Experiment 1, carried out with ears submitted to a relatively long preliminary tarring, many of the tumors were radial, whereas after the briefer tarrings of Experiments 2 and 3, they were mostly peripheral or central.

The *peripheral* tumors appeared at the immediate edge of the original punch hole (Figs. 6 and 7), or in the narrow zone just outside where inflammation had been present during the first days of repair. They were by far the most frequent of the growths evoked by later tarring (62 tumors out of a total of 136). As already remarked, they often extended onto the disc of new tissue secondarily.

The *radial* tumors were usually easy to discriminate. As the holes began to close one or more low ridges or mounds formed on the surface of the new tissue, and these extended in *pari passu* as the new tissue advanced toward the center of the circle. Thus there came into being almost linear, radial, subepidermal ridges or narrow, segmental mounds reaching from the periphery of the new disc to its center, or to the edge of the persisting hole when closure was incomplete. The growth shown in Fig. 3, though formed under circumstances somewhat different from the ordinary, will serve as a representative instance.

Radial growths appeared only on ears that had been tarred prior to punching holes in them. Most of them appeared as the holes were closing but a few were evoked by the later tarring. Their incidence (20 of 136 growths) was doubtless less than it would have been had all the ears been tarred beforehand.

Next in frequency to the peripheral tumors were those that were *central* (37 of 136 growths). Developing as they did at a time when repair had been almost or quite completed, that is to say, when the stimulus of the reparative process was lessening, they usually took the form of subepidermal mounds which tended to retrogress if let alone. But when tarred they became carcinomatoids and papillomas of the ordinary sorts (Fig. 9). Though they often grew large their secondary extension was never radial. Sometimes when there was a central channel in the disc of new tissue, they extended through it to the outside of the ear.

Eccentric tumors, discrete growths situated somewhere between the periphery of the

disc and its center (Fig. 3), but having no evident connection with either, were relatively infrequent (17 of 136 growths).

The Stimulating Effect of Tar and Other Carcinogens

In previous work (2) we had occasion to observe that holes punched in tarred rabbit ears healed with extraordinary rapidity and often with overgrowth; but great differences from animal to animal were noted in these respects. Holes of the same size closed in some individuals within a few weeks whereas in others they never did so, all reparative processes ceasing while they still were of half or more their original diameter. The differences in the amount of overgrowth taking place later varied as considerably. Careful comparative observations were obviously called for, and the experiments of the present work were planned with a view to these. Holes of the same size were made at corresponding spots in the two ears and the rate of healing was carefully recorded, by means of tracings as well as notes. The ears tarred for 4 weeks (Experiment 1) were thickened, inflamed, and scurfy at the time the holes were punched, and these abnormalities had not entirely disappeared by the time they had filled in. It was found that as a rule the ring scabs came away 2 to 3 days earlier from an ear in this state than from the untarred ear of the same animal, and that the newly healed edge then exposed was thicker. The later healing too was considerably more rapid, and the discs of new tissue often bulged to either side and were lenticular in cross-section (Fig. 5), instead of having the thickness of the rest of the ear as in the controls. In Experiments 2 and 3 the ears were much less changed by the preliminary tarring and had usually resumed the normal aspect before healing was completed. During its early stages, however, the same differences could be perceived as in Experiment 1.

Very remarkable was the effect of the later tarring on the discs of new tissue filling the punch holes. Many of them thickened greatly instead of becoming slightly thinner as eventually happens when no more tarring is done. Some, as already stated, took on a cup or thimble shape, projecting 5 or 6 mm. beyond the general surface. Overgrowth of cartilage appeared to be the determining factor in their shape, and bone was sometimes laid down amidst the cartilage (Fig. 11). Usually, though not always, the cartilaginous overgrowth was symmetrical. Normal ears occasionally healed with a slight lenticular thickening or knobbing.

The early students of the changes in skin produced by tar concluded that it exerts a stimulating effect, and many of them supposed the benign tumors it elicited to be mere exaggerations of a general epidermal hyper-

plasia. Latterly, as attention has centered on the effects of the pure carcinogenic hydrocarbons, all this has been lost sight of. The pure hydrocarbons produce general tissue change only slowly, and many of them exert a repressant or "inhibitory" effect upon established tumors (3). These and other facts have led some workers to suppose that cell repression, not stimulation, is the essential precursor of the neoplastic change. Our observations just described, on the healing of wounds in tarred ears, constitute a reminder of the old finding that tar acts to stimulate markedly the tissues to which it is applied, doing so at the very period when tumors are arising. Yet it might be assumed that its carcinogenic ingredient actually exerts a repressant influence upon the cells which it causes to form tumors, but that this effect is obscured by a stimulation due to other ingredients exerted upon other tissue elements. Needless to remark, it would be difficult to disprove such an assumption. To obtain more data we have resorted to studies of the rate of healing of skin subjected to applications of the carcinogens, benzpyrene and methylcholanthrene. These agents effect changes in rabbit ears only during the course of months. Hence resort has been had to the ears of the mouse.

A drop of a 0.3 per cent solution of methylcholanthrene (Fieser) in benzene (C.P.) was placed every other day on the outside of the tip of one ear of fifty adult white mice, and a drop of benzene was put on the other ear as control. Both solutions extended some way on the inner surface and ran down onto the head. The animals were held until evaporation was complete.

Differences in the ears soon manifested themselves and became pronounced. After 27 days the organs receiving methylcholanthrene were mostly thickened, inflamed, and ruddy, and in many cases had become "cauliflower" ears, shortened, crumpled and thickened, red, and glazed. In a few instances they were ulcerated and scabbed, and the animals of which this held true were discarded as unsuited to further observation,—an unfortunate error, since a section taken by chance of one of the scabbed regions showed an unsuspected, eroding growth with the morphology of a carcinoma. The control ears were only dubiously changed, and the mice remained in good health. The skin of the head had lost its hair and become scurfy where the carcinogen had spread to it.

On the 27th day, with the animals under ether anesthesia, three holes were punched in each ear. Efforts were made to place them symmetrically and at the same distance (3 to 4 mm.) from the head, though in the case of the cauliflower ears absolute correspondence in situation was not possible. No. 16 gauge hollow needles, ground off to the shape of cork borers and sterilized in boiling water, served as the punches. Any bleeding soon stopped, a clot filling the hole; and this clot was removed next day. Every 2 to 3 days records were made of the rate of closure, in terms of the original diameter of the holes (hole ⅛ closed, ¼ closed, etc.). A dry ring scab formed, just as in the case of rabbits, and healing was largely completed during the 3rd week. While it was taking

place, no more methylcholanthrene or benzene was put on the ears and they rapidly resumed the normal aspect.

The rate at which healing took place was found to vary directly with the distance of the holes from the base of the ear, those near it closing swiftly whereas those within a millimeter or two of the margin frequently remained open permanently. Only such holes were compared as had been made at precisely corresponding points on the two ears.

The early healing was exuberant in the case of the methylcholanthrened ears, resulting in a thickened edge to the holes; and the repair was unusually rapid. The differences from the controls were most marked when the ears were most changed. Several animals were sacrificed to procure microscopic specimens and all showed similar findings. Figs. 12 to 15 show a "cauliflower" ear and its control, sectioned from base to margin through identical regions. The specimens were procured at the time methylcholanthrene was discontinued. As Figs. 13 and 15 show, the carcinogen has caused a pronounced thickening of epithelium, connective tissue, and the cartilaginous plate. The cartilage is bent at angles upon itself, and there are many new fibroblasts in the connective tissue, fairly numerous macrophages, and some lymphocytes. But the epithelial differences are most worthy of attention. On the outside of the ear, where the methylcholanthrene was directly applied, the epidermis does not consist of a single rank of cells, as on the control ear, but of a layer six to eight cells deep, which is undergoing differentiation to keratinized squames.[5] The basal edge of the epithelium is uneven and the hair follicles are much hypertrophied. At places where the carcinogen has spread to the inner side of the ear the same changes are present in less pronounced form.

The experiment was repeated on another fifty mice, using a 0.3 per cent solution of benzpyrene (Hoffmann-La Roche) in benzene. Though applied over a period of 52 days it caused much less reddening and thickening, and the holes could be punched at corresponding situations since there was no distortion. As soon as the ring scabs had come away the applications of benzpyrene were resumed and kept up until healing was complete. The new tissue which formed next to the scabs was thicker in the case of the benzpyrened ears than in the control organs and the later healing was definitely more rapid.

None of the mice receiving benzpyrene had developed a tumor when they were killed at the completion of healing. Tests on other mice of the same strain have shown that growths do not arise so soon.

Though these experiments prove that methylcholanthrene and benzpyrene can greatly stimulate epidermal cells and those of the other tissues of the ear, instead of exerting a generally repressant effect, they do not entirely exclude the possibility that a hidden, injurious influence of these substances on individual cells here and there may be the effective factor in carcinogenesis. Indeed, the observed hyperplasia might very well be secondary to cell damage. One may recall in this relation that necrosis

[5] Pullinger (*J. Path. and Bact.*, 1940, **50**, 463) has lately reported that a single application of methylcholanthrene results in a thickening of mouse epidermis, with an increase in mitotic figures and some differentiation of the epithelial cells.

occurs of the tissue immediately next encapsulated collodion capsules containing benzpyrene and methylcholanthrene, and that the sarcomas arising later appear further off in an envelope of new-formed connective tissue (4). Accumulations of polymorphonuclear leucocytes, such as bespeak a reaction to cell damage, can be seen at several spots in the deeper layer of the epidermis on the inside of the ear shown in Fig. 15.

DISCUSSION

Significance of the Situation of the Tumors

It seemed possible, as already stated, that the experiments would disclose whether tar tumors are due to a random effect of the carcinogen to cause normal epidermal cells to become neoplastic, or whether it acts upon elements predisposed by some peculiarity to become tumor cells under its influence. The findings will now be considered in this relation.

The tumors evoked by tarring rabbit ears are often several millimeters across when they first attract attention, and their size and rapid growth suggest that they may have originated from many associated cells. If these cells were possessed of primary peculiarities leading them to form tumors under the influence of tar, one might expect that when a spot was cut through where many were present, in making a punch hole, those left behind would proliferate toward the center of the hole as new tissue grew in to close it, and that in consequence radial or segmental tumors would appear as healing took place, or would arise when the disc of new tissue was tarred later on. Radial tumors did indeed develop on some of the discs of our experiments, but only when the ears had been subjected to tarring before the holes were made. It follows that large groups of cells inherently disposed to become tumor cells and differing in this respect from the generality can scarcely have been present in the normal epidermis. Yet this does not rule out the existence of such cells in small numbers or scattered singly. For Loeb (5) has found that epidermal defects are not repaired by strictly linear proliferation of individual elements but by complicated shifts of large numbers of cells, some advancing rapidly before the generality, others more slowly following after, and those in the upper and lower layers of the epidermis proceeding at differing rates. The multiplying elements generally remain attached to one another, and hence in an extending sheet of epidermis there are groups of cells whose descendants lag and never attain to the advancing edge while others are carried forward on it. If cells peculiarly responsive to tarring were scattered sparsely

through the epidermis they could scarcely proliferate inwards in series from the periphery to the center of a closing hole, as would have to happen if they were to give rise to a radial growth. The apparent origin of some tar tumors from spots of considerable size in the skin is doubtless to be explained by an unperceived preliminary multiplication of the cells first rendered neoplastic, with lateral extension of them in the loosened and inflamed connective tissue.[6] In previous papers (6) we have stressed the collateral effects of tarring to further tumor growth by encouraging cell proliferation and producing local conditions favorable to it.

Nothing can be inferred concerning inherent cell peculiarity from the peripheral, eccentric, or central situation of the tumors on the discs of new epithelium. True, it is possible to explain the growths as deriving from peculiar elements, some of which were present at the margin of the wound but took no part in the healing process, others lagging by the way during it, with result that they came to be eccentrically situated, while yet others attained to the center because they happened to be situated at the forefront of the host of proliferating elements. But these suppositions seem gratuitous. The eccentrically placed tumors were never linear in shape with their long axis along radii, as might have been expected had they been the result of the centripetal proliferation of peculiar cell families. They were always rounded in outline and usually they remained so as they enlarged.

The tarring after healing had been completed evoked much the larger number of tumors. Their special abundance on the new epithelial surface cannot be laid to any tendency of the tar to stick longer there, for it came away sooner if anything. There were other conditions though which may have made for great local effectiveness of it. The labile state of the tissues in and about the healed punch holes and the increased vascularization were favorable to the rapid multiplication of any tumor cells present. Those epithelial elements which attained to the center of the discs were under the stimulus of the reparative processes for the longest time, while furthermore they formed the most composite aggregate, converging as they did from all sides to a common point. If cells especially responsive to the carcinogenic

[6] The shape of many of the growths, which is that of low mounds, accords with this conception.

Methylcholanthrene and benzpyrene cause relatively little diffuse cutaneous change, when inducing papillomas on the ears of rabbits, and the tumors are always punctate when first noted (unpublished work with Dr. William F. Friedewald).

effect of tar were scattered in the skin they would certainly be most frequent at or about this point.

The Evocation of Tumors by Non-Carcinogenic Influences

In the tests here reported we purposely resorted to very sensitive indicators, namely, those benign growths which commonly appear when rabbit skin is tarred. Their status has been scrutinized in an accompanying paper (1) and proof has been brought that they are genuine neoplasms. Yet they are conditional in character, unable to maintain themselves without the aid that continued tarring or various other influences can provide. The stimulus we utilized to evoke them was non-carcinogenic in character, namely wound healing. Since it soon ceased it sufficed in only a few instances to establish the tumors that it evoked (Fig. 4), most of them disappearing. Even while its influence was presumably greatest, that is to say while the punch holes were filling in, it but seldom caused radial growths to extend laterally at the expense of the adjacent, non-neoplastic epithelium. The strictly segmental shape of the papilloma of Fig. 1 shows that under the circumstances of reparative stimulation the cells of preexisting tar tumors may be no more aggressive than those of the normal epithelium which is also taking part in the repair. Later tarring of the growth illustrated proved much more effective in disclosing the capacity for aggression of the neoplastic cells (Fig. 2).

The observation that injury may be followed by a tumor goes back to the earliest medical records; but only within the last few years have experiments been undertaken to determine its precise rôle, and the net result of them is uncertainty.

In the 1920's, soon after tumors were first purposely induced with tar, several investigators reported that warts and cancers appeared where tarred skin had been cut or excised (7). The instances described were merely episodic, and Deelman was the first to deal comprehensively with the phenomenon. In a series of papers, published between 1922 and 1927 (8), he brought what appeared to be convincing evidence that the repair of wounds in the tarred skin of mice is often accompanied by the appearance of papillomas and carcinomas at the healing edge or in the new scar. Many workers hastened to repeat his experiments but were unable to confirm them. Indeed, not a few reported that where the skin had been injured tumors arose with less frequency than elsewhere (9). So adverse were the findings that the "Deelman phenomena" lapsed from general attention. Several recent investigations of the influence of wounds to evoke tumors of skin treated with tar or benzpyrene have given indecisive results (10).

One can perceive several reasons for the conflicting experimental evidence on the relation of injury to tumors. Mice have been employed for most of

the work, and the skin of the back has been utilized, where scabbing and infection act to complicate the findings and cicatricial contraction rapidly reduces even large healed expanses to narrow scars. A similar scarring takes place in rabbits unless the local conditions are such as to prevent it. Recently we had occasion to remove an oval piece of tarred skin, 3 cm. by 4 cm. in diameter, from the neck of a rabbit after papillomas had been induced to appear there. The cut for the excision was made through several of the growths to see whether they would extend inwards during healing, as happens on the ears. But so great was the distortion of the denuded area as the epithelium extended into it, and so soon was the healing wound reduced to a narrow, jagged scar that little was learnt.

The differing consequences of mild and severe skin injury provide another factor making for confusion. Recent studies of the effect of irritants combined with carcinogenic agents have led to the recognition that intense inflammation, far from aiding in the evocation of tumors, may be detrimental to it. In some recent experiments of our own, a mixture of mustard and acetone called forth tumors on previously tarred rabbit skin when it produced only mild inflammation, whereas when this was marked existing tumors vanished and no new ones appeared (1). Most of the investigators who have followed up Deelman's work have not adhered to his methods. Some have repeatedly sandpapered the tarred skin of mice and have obtained a cicatricial atrophy instead of neoplastic phenomena.

In the present work we happened to use tar of the identical sort employed by Deelman, imported from Holland more than 18 years ago by Dr. Karl Landsteiner, and now no longer manufactured. But the positive outcome of the experiments cannot be laid to any special properties of this material, since the punch-hole method of evoking tumors is effective with rabbit ears painted beforehand with benzpyrene or methylcholanthrene, as one of us, working in collaboration with Dr. William F. Friedewald, has lately found. In the light of this fact it is curious that no growths appeared during the healing of the holes punched in the mouse ears that we submitted to the carcinogens. Evidently there are variables still uncontrolled.

In Deelman's experiments cancers appeared as well as papillomas where wounds were healing, while in our own work benign growths only were forthcoming. This difference is merely an expression of the differing response of mouse and rabbit skins to tar, cancers arising with great frequency in the mouse whereas in the rabbit they are rare, and appear very late as compared with papillomas and carcinomatoids.

When the tarring of rabbit ears is stopped after 4 weeks, the maximum

preliminary period that we employed, the organs rapidly regain a normal appearance, the growths already present on them dwindle and vanish, and none arise later unless the skin is disturbed in some way (2). Yet, as the punch-hole experiments show, the epidermis contains many cells having the character of tumor cells, and needing only the encouragement of wound healing to assert their capabilities. They are in the subthreshold neoplastic state described in a companion paper (1). No data are now available on how long they persist, though certainly some do for many weeks. In rabbits 28 and 32 of Table III the reparative process promptly evoked tumors, although 8 and 9 weeks had elapsed respectively since the last of only three tarrings. Such persistence of latent neoplastic cells is the more remarkable because the growths to which they give rise when stimulated (papillomas and carcinomatoids) are unable to maintain themselves without aid. In this connection it may be recalled that after such tumors have disappeared their cells may persist for 6 months or more as part of what appears to be ordinary epidermis microscopically, and retain the ability to multiply into tumors when again subjected to stimulation (1).

Tumor Inception versus Tumor Formation

The findings emphasize the need for a sharp distinction in thought between tumor inception and tumor formation, and between those factors which render cells neoplastic and those affecting their subsequent behavior. Instances which call for such distinctions are constantly occurring yet they are seldom made, the carcinogenicity of chemical substances, for example, being ordinarily reported without any discrimination of the influences rendering cells neoplastic from those determining their multiplication. True, the task is often difficult or impossible, for the necessary criteria may be lacking until a perceptible growth has formed, and by then the cells may have undergone further alterations entailing such gains in proliferative ability that encouraging influences affect them only slightly or not at all. This is the case with many of the cancers deriving secondarily from mouse papillomas. From the literature it seems plain that most carcinogenic agents not only cause cells to become neoplastic but in addition have adverse or encouraging effects upon them. Tar is a notable instance in point: not only does it initiate neoplastic change but it markedly furthers tumor formation.

The agents and influences which act solely by encouraging cells already neoplastic offer less considerable problems of interpretation. They are highly various in character (11). It has been the experience of everyone

who has applied a carcinogenic tar to mice for many weeks, during which they lost greatly in weight, that shortly after the cessation of tarring, as the animals became better nourished, tumors rapidly appeared and most of those already present grew faster. Here the improvement in bodily condition more than compensated for the adverse effects on the tumor cells of leaving off the tar. Twort and Twort have noted that experimental carcinogenesis with the pure hydrocarbons is more successful in sound animals than in sick ones (12). Clinical pathologists know that bacterial infection may stimulate tumor growth and increase malignancy. The Tworts have reported that "cells rendered abnormal by a few applications of benzpyrene quickly pass into the irreversibly cancerous phase when stimulated by oleic acid" (13). Experimental infection with a neoplastic virus, the Shope papilloma virus, not only spurs tar tumors to prodigiously rapid growth but brings about alterations in their morphology and renders some of them malignant which were previously benign (14).[7] Recently we have witnessed the effect of an irritant secretion from a cancer to evoke papillomas on skin suitably prepared. A tar carcinoma had extended to the outer, untarred side of the ear of a rabbit, causing a broad ulcer there from which a copious, thin fluid exuded which kept the neck to the right of the spine continually wet where the ear rested. Here, as at the corresponding situation on the left, the skin had lost its hair and become somewhat hyperkeratotic, owing to tar transferred from the ears during the course of several previous months. Where the exudate from the cancer kept this skin wet many papillomas arose in the course of a few weeks (Fig. 10) while none at all appeared on the opposite side of the neck, which served as control. It is possible, of course, that the exudate had itself a carcinogenic influence, though this seems unlikely. That it was irritant the microscopic sections showed.

These examples have been selected out of the great number available because they are so diverse in character. Evidently the influence exerted by the agents which stimulate the multiplication of cells already rendered neoplastic is essentially non-specific, save in exceptional instances such as that of the papilloma virus. The generality of encouraging agents would appear to act merely by producing tissue conditions favorable to the mul-

[7] The ability of the papilloma virus to stimulate and alter tar tumor cells, and the hidden presence of such cells in skin tarred but a few times may explain the observed effect of the virus (Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1938, **68**, 529) to call forth benign and malignant growths on briefly tarred skin devoid of tumors and on which none would appear later in the ordinary course of events.

tiplication of cells, irrespective of whether they are neoplastic. Yet this non-specificity does not lessen their practical significance, which can be crucial as our experiments have shown. These latter had to do only with rabbit tumors and benign, conditional growths at that, but evidence is not wanting that human cancers may owe their success to extraneous encouragement. The experience of surgeons with Roentgen ray tumors has provided some exquisite instances in point, occurring under conditions which nearly resemble those of our punch-hole experiments. It has been found that after the excision of cancers from expanses of rayed human skin wholly new malignant growths may appear at the edge of the wounds as healing takes place. Experimenters have noted that when rabbit ears have been rayed until perforation occurs papillomas and cancers not infrequently arise where repair is going on (15).

Trauma and Tumor

So many are the human histories in which a blow or other injury has been followed by cancer, and so striking is the relationship between the two that the term "traumatic cancer" is sometimes used in referring to such instances. According to Ewing (16) the possibility that normal tissues may react to trauma by malignant proliferation has always been regarded with skepticism because chronic tissue changes precede the appearance of most tumors. The findings of the present paper and of the one accompanying it (1) go some way toward simplifying the problem presented, showing as they do that cells may become neoplastic in considerable numbers yet never manifest themselves unless aided by extraneous influences. When one considers this finding in the light of the proven abilities of human cancer cells to persist for years without asserting themselves it becomes plain that the trauma which is followed by the prompt appearance of a tumor in tissue that had previously seemed normal may act merely by stimulating neoplastic elements already long present. A carcinogenic agent may have done its work years before and the cells it rendered neoplastic have remained ever since within their morphological context, incapable of asserting themselves until some intercurrent accident—a blow, a wound, a burn—stirs them to proliferation. The medicolegal bearing of these facts is obvious.

SUMMARY

A carcinogenic tar applied to rabbit skin renders many more epidermal cells neoplastic than ever declare themselves by forming tumors. They may be present in large numbers and persist for a considerable time after

brief tarring, yet give rise to no growths unless encouraged. The stimulus of wound healing will suffice to make some of them multiply and form tumors.

No evidence has been obtained, in experiments specifically directed to the point, that the cells which tar renders neoplastic respond in this way because they are possessed of peculiarities not shared by the rest of the normal epithelium.

The fact that non-specific stimulation (as *e.g.* wound healing) may act as the deciding influence in tumor formation brings out the need for a sharp distinction between the forces which induce neoplastic change and those which determine, or prevent, its realization in terms of a tumor. The distinction is vital to the appraisal of the many carcinogenic substances worked with nowadays.

The ability of tumor cells to lie latent for long periods and respond to non-carcinogenic stimulation by multiplying into growths provides an explanation of those clinical instances in which cancer appears rapidly after acute injury to tissue that had seemed normal.

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EXPLANATION OF PLATES

All of the microscopic specimens were stained with eosin and methylene blue.

PLATE 17

FIG. 1. To show the participation of tar tumor cells in the repair of a punch hole.

The recently tarred ear carried several small, discoid papillomas at the time holes were punched in it, and one of these was purposely cut through. As healing took place the growth extended in at approximately the same rate as the non-neoplastic epithelium and occupied a segment of the disc of new-formed tissue exactly proportional to its size at the periphery. × 1.

FIG. 2. The stimulating effect of renewed tarring on the tumor of Fig. 1.

After healing had been completed the growth showed no tendency to enlarge further, and hence tarring was resumed. It caused the tumor to extend rapidly at the expense of the surrounding epithelium, both new and old, as the picture shows. Not enough tarring was done to change the skin greatly and it brought out only one other wart. × 1.

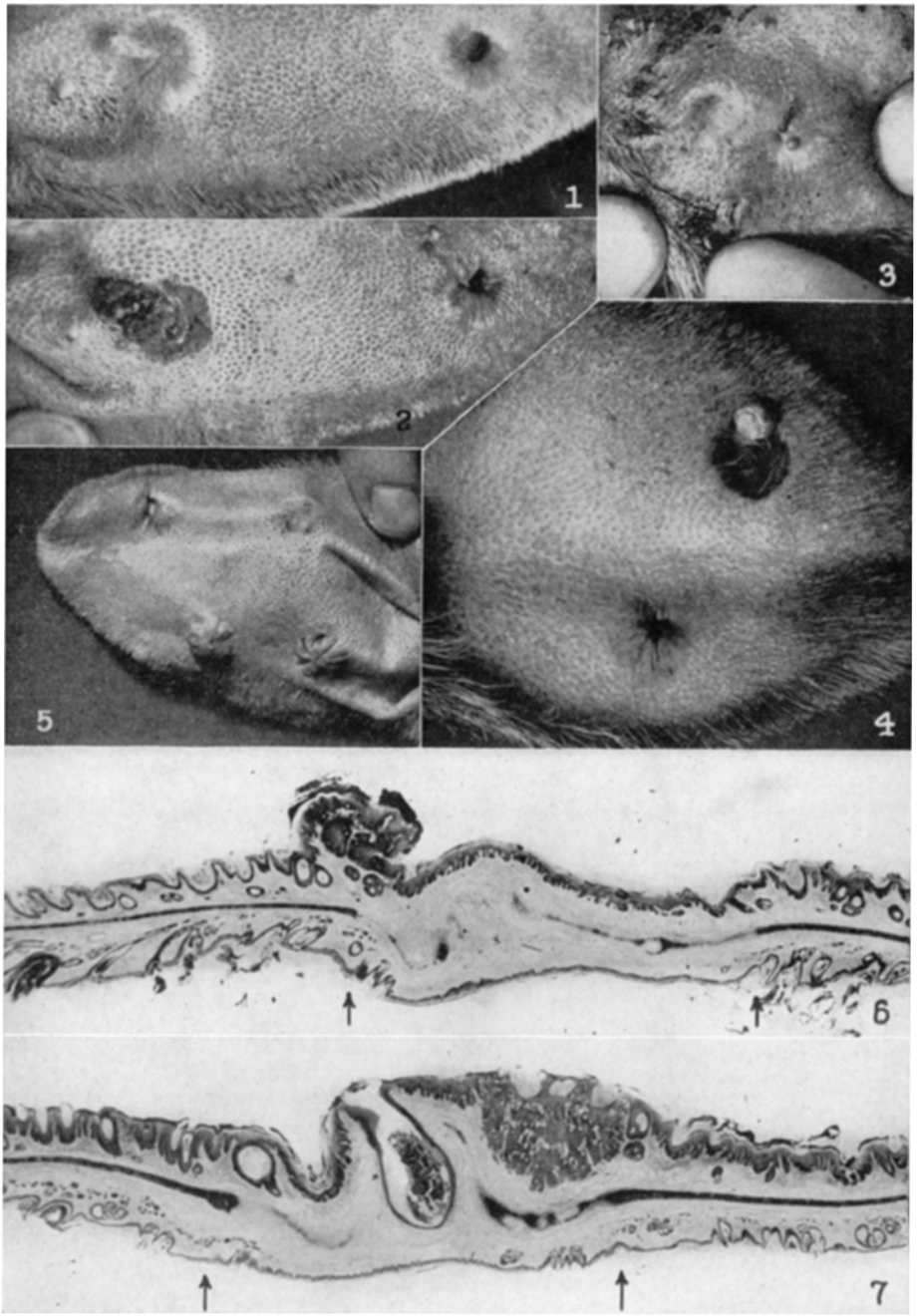
FIG. 3. A melanotic, radial growth due to the centripetal extension of neoplastic epithelium and melanoblasts as a punch hole healed. An eccentrically situated tumor is also present on the new disc of tissue. The outline of this last appears irregular because it overlaps scars due to the healing of holes punched previously in the same area (see text). × 1.

FIG. 4. A peripheral papilloma which appeared very soon after the healing began of holes punched in an ear tarred for 4 weeks. Though no more tarring was done the growth rapidly enlarged, becoming the sessile sphere shown here. × 1.

FIG. 5. Mounds due to excessive formation of tissue during the repair of holes in an ear previously tarred. There were mounds on the outside of the ear as well. They were largely due to hyperplasia of the cartilaginous sheet (see Fig. 11). × ½.

FIG. 6. A peripheral papilloma evoked by wound healing. The growth appeared soon after the hole in a recently tarred ear began to fill. The arrows in this and the succeeding pictures point to where the cutting edge went through the cartilage when the hole was punched. × 9.

FIG. 7. Another peripheral tumor which appeared under similar circumstances. Tarring had been resumed some while before the specimen was obtained, with result that the epidermis has become much thickened generally and the growth has the carcinomatoid form, being indistinguishable histologically from a squamous cell carcinoma. There is a persisting channel (here cut through slantingly) at the center of the disc of new tissue. × 12.



Photographed by Joseph B. Haulenbeek

(MacKenzie and Rous: Disclosure of latent neoplastic change)

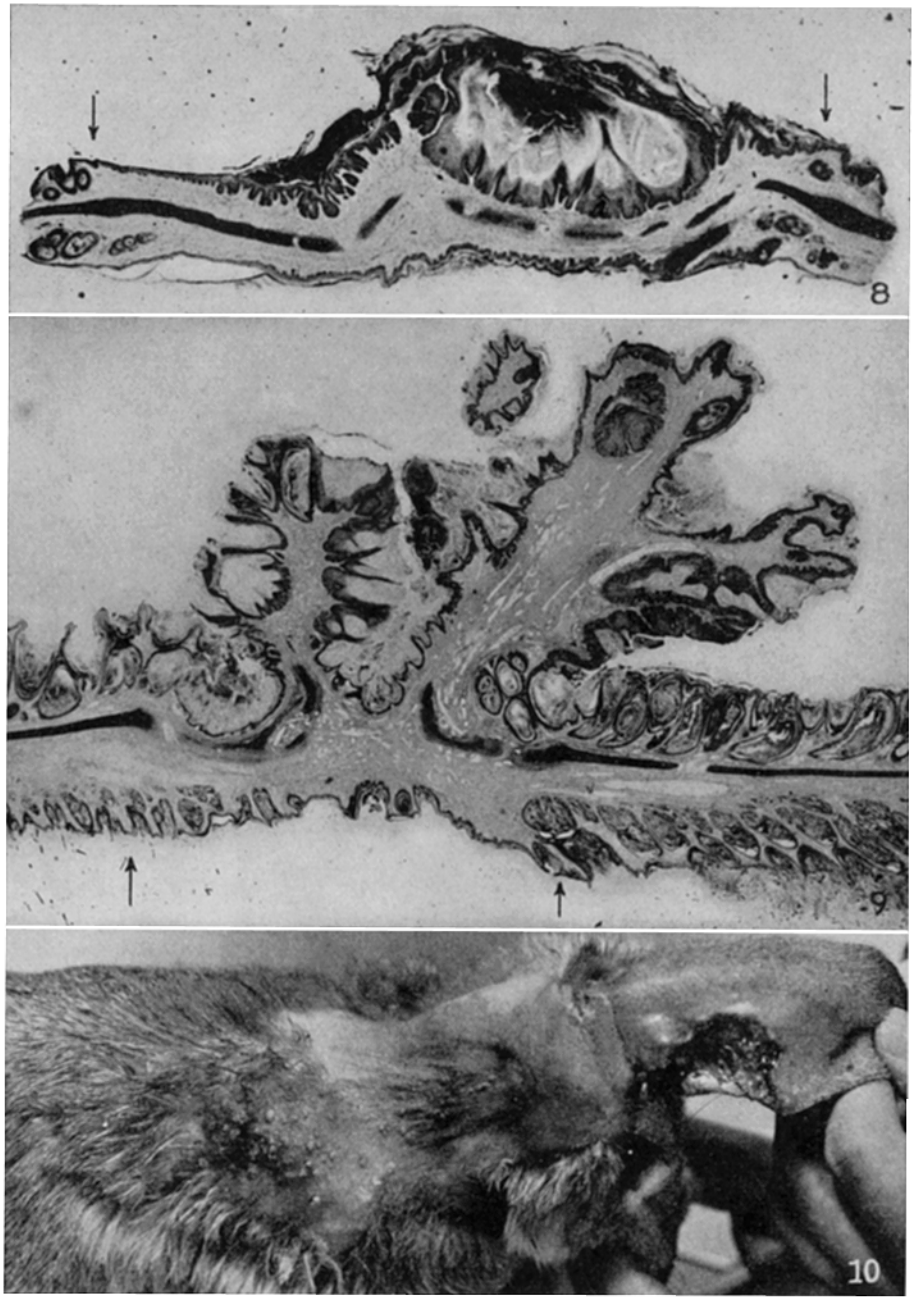
PLATE 18

FIG. 8. A radial papilloma which appeared when a healed disc was tarred. It was almost linear when it first arose and did not quite occupy the entire radius, falling short of the edge of the original punch hole. × 8.

FIG. 9. A central papilloma which grew large under the stimulus of repeated tarring. The growth extended in a ring around the actual center of the disc, with result that in cross-section it appears to be two tumors. The new-formed cartilage has extended up from the sides into the fleshy pedicle. × 6.

FIG. 10. Effect of an irritant exudate from a tar carcinoma to evoke papillomas on skin previously tarred.

The ear had been tarred only on the inside and no tarring had been done for many months, yet the cancer had grown and extended to the outer surface as here shown. The skin on the back of the neck, long previously rendered hairless and hyperkeratotic by transferred tar, had for some while appeared normal save that it remained bare. Recently, however, it had been kept constantly wet over a large region with fluid from the cancer. Here a multitude of papillomas developed, with none elsewhere. All the growths were benign, distinctively different from the carcinoma, as the microscope showed. × ½.



Photographed by Joseph B. Haulenbeek

(MacKenzie and Rous: Disclosure of latent neoplastic change)

PLATE 19

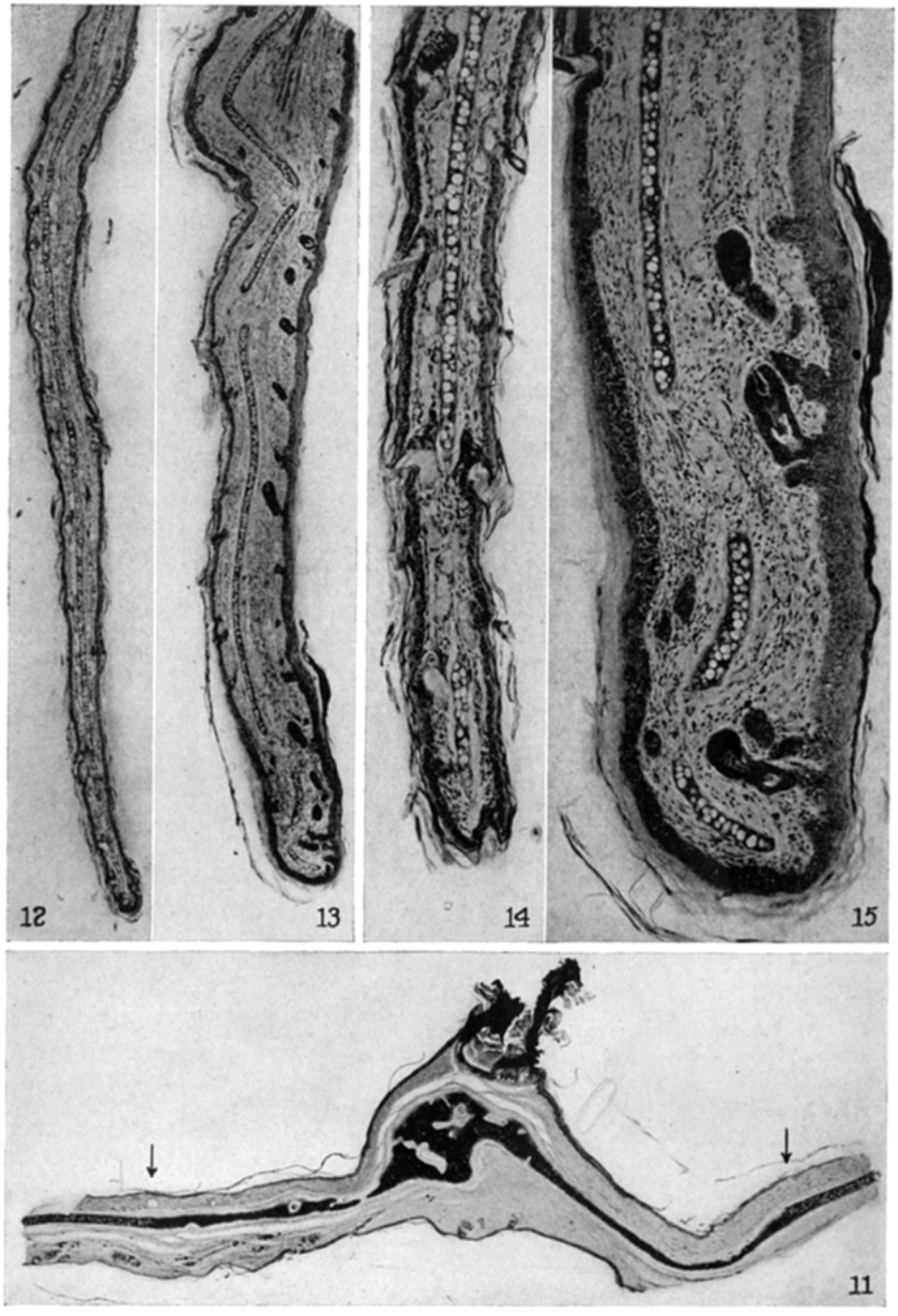
FIG. 11. A central growth surmounting a high mound of new-formed tissue, which consists for the most part of cartilage in which bone has been laid down. The ear had been repeatedly tarred after the hole had closed, but the specimen was not obtained until months later. The tumor has two components, one of frill horn type (to the right), the other ordinary papilloma. × 8.

FIGS. 12 to 15. The effect of methylcholanthrene to cause tissue proliferation.

Benzene had been dropped on the outside of one ear of mouse 28, and methylcholanthrene in benzene on the other ear (see text for details). The fluids spread to the inner surface of the organs. When the mouse was killed the ear submitted to the carcinogen was thickened, glazed, reddened, and crumpled,—a cauliflower ear,—whereas its fellow seemed normal. The outer surface of the ear is on the right in each picture.

Figs. 12 and 14, of the control ear, show a slightly scurfed organ with dubiously thickened epithelium.

Figs. 13 and 15, of the cauliflower ear, show by contrast great thickening due to hyperplasia of the connective tissue, cartilage, and epithelium. The thickened sheet of cartilage is bent upon itself, the connective tissue is much more cellular than is normal, and the living epithelium on both surfaces of the ear, especially on the outside, is many layered and differentiates gradually into keratinized scales. At two spots on the inner surface of the ear necrosis has occurred in the basal region of the epithelium and leucocytes have accumulated. The hair follicles show pronounced hyperplasia. Figs. 12 and 13, × 33; Figs. 14 and 15, × 105.



Photographed by Joseph B. Haulenbeek

(MacKenzie and Rous: Disclosure of latent neoplastic change)

PLATE 20

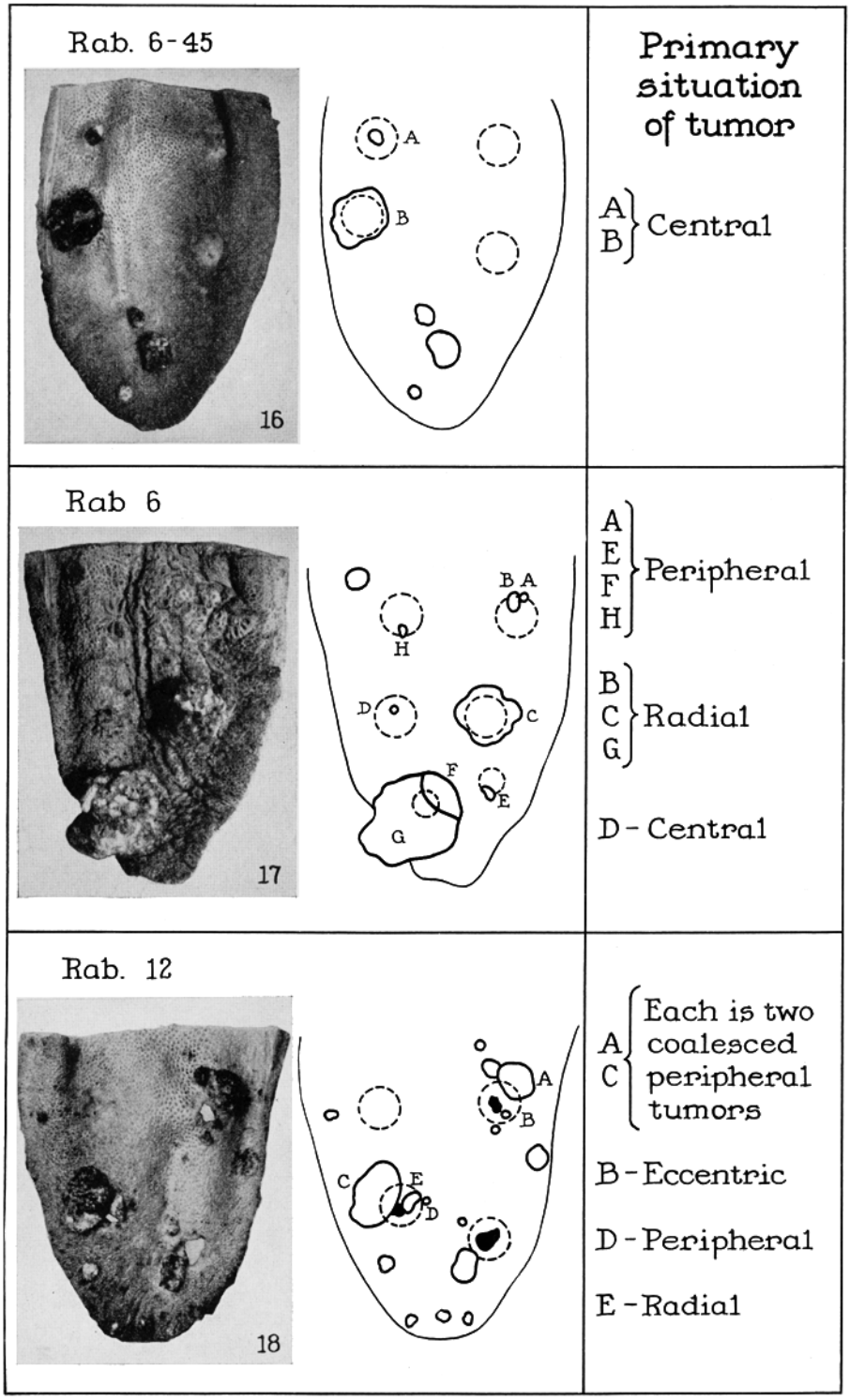
FIGS. 16, 17, and 18 illustrate the influence of healing to elicit tumors and the effect of tarring to encourage their growth. The size and position of the holes originally punched in the ears is indicated on the tracings by broken lines. × 7/10.

FIG. 16. Rabbit 6-45 (Table II). The ear had not been tarred before the holes were made, and no growths appeared while they were closing. The later tarring called forth tumors at the center of two of the four discs of new tissue but at only three other situations. One of the central growths had more than covered the disc by the time the picture was taken.

FIG. 17. Rabbit 6 (Table I, Chart 1). The ear had been tarred for 4 weeks before holes were punched in it, and while healing was under way one tumor (radial) appeared but none elsewhere. The later tarring caused the ear to become greatly thickened and hyperkeratotic. It brought out many growths on or at the edge of the discs of new tissue but only one elsewhere that was large enough to be charted at the time the photograph was taken. Two growths had become so large as to hide completely the discs on which they originated.

FIG. 18. Rabbit 12 (Table I). The ear was submitted to the same procedures as that of No. 6. No tumors appeared during healing and the reparative process came to a standstill before some of the holes were completely closed. They are shown in black in the tracing. The later tarring evoked many tumors, as the picture discloses, and these were proportionately much more numerous where the reparative process had taken place.

The persisting holes have been touched up with white in the picture to bring out clearly the situation of the tumors in relation to them.



Photographed by Joseph B. Haulenbeek

(MacKenzie and Rous: Disclosure of latent neoplastic change)