THE NEOPLASTIC POTENTIALITIES OF MOUSE EMBRYO TISSUES

IV. Lung Adenomas in Baby Mice as Result of Prenatal Exposure to Urethane*

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In previous papers from this laboratory (1) experiments have been described which were made to learn how soon in the life of the organism cells possess the ability to become tumor cells. The neoplastic potentialities of various mouse embryo tissues, procured in the latter half of gestation, were tested by transplanting them to adults, together with methylcholanthrene. Tumors arose swiftly and in great diversity, yet it was questionable whether the cells exposed to the carcinogen were still in the embryonic state when they underwent neoplastic change, since the interval before the growths first became perceptible somewhat exceeded the time until birth, had the embryos been left undisturbed. If it had been possible to utilize the cells of very young embryos perhaps tumors could have been obtained within this period, but they did not survive the requisite exposure to methylcholanthrene, even when this was circumspectly injected into the "beads" along the uterus. Obviously for a decisive test of the neoplastic potentialities of embryo cells these must be exposed *in utero* to a carcinogen acting so speedily that its neoplastic effects will be evident almost at once. Recent authors who have produced pathological changes in mouse embryos with the Roentgen rays (2) have made no mention of tumor formation, and the polycylic hydrocarbons fail to pass the placenta in effective quantity. It was recalled however that the injection of the highly diffusible hypnotic, urethane, into adult mice of strains liable to pulmonary adenomas in old age causes these growths to appear earlier than usual and in much greater number (3). We had employed such a strain of animals when testing with methylcholanthrene the neoplastic potentialities of transplanted fragments of embryo lung, and had noted that multiple adenomas formed within 2 to 3 weeks (4). Consequently urethane was now injected repeatedly into pregnant females, and their offspring were searched for adenomas. These were sometimes visible in 3-day-old animals, and often had attained a considerable size within 10 days, none appearing in controls. The urethane could have acted *in utero* for only a few hours, and it produced no visible damage in the lungs of

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the embryos exposed to it, which might have served as the basis for neoplastic changes occurring secondarily. All went to show that these changes were primary and took place prior to birth.

Law (5) has subjected embryonic tissues to the action of dibenzanthracene, injecting an olive oil solution of it directly into the amniotic sac of mouse embryos at the 15th day of gestation. The animals were killed when 200 days old on the average, and nearly all had lung tumors, in most cases multiple. While some of the growths may have been malignant the context of Law's paper indicates that the great majority were adenomas. The only other tumor developing was a fibrosarcoma of the skull in one instance.

Larsen has lately reported the presence of induced pulmonary adenomas in the 6-months-old offspring of A strain mice receiving urethane while pregnant (6). The work now described was done without knowledge of his. He found that urethane gave rise to few tumors in the young unless injected into the mother animals during the last 24 hours before parturition, and he noted that these latter showed many more growths than their young when all were examined together. His revealing observations will be considered in detail further on.

Materials and Methods

Mice of the C strain were mainly employed, as in the previous tests with transplanted embryo tissue. A large colony of the animals was available. In corollary several experiments were carried out with A mice raised in our laboratory.

The number of adenomas induced by urethane in adult mice is directly proportionate to the amount of the substance given (7). Hence it was injected into most of the pregnant females on several successive days, to a maximum of six, and in the greatest tolerable quantity. The precise duration of pregnancy was determined by the vaginal plug method in many instances, but palpation was relied upon in the majority to gain an idea of the age of the embryos. The injections were frequently begun as soon as these had pulmonary tissue for the urethane to act upon, that is to say round about the 12th day of gestation,[1] since our aim was to induce neoplastic changes as early as possible. Most of the females injected at that time either died or aborted as the injections were repeated, and our data have been procured in the main from those nearer term. Adult mice are known to differ widely in their tolerance of urethane.

At each injection the pregnant animal received, irrespective of its weight, 0.3 cc. of a 10 per cent solution of urethane in the subcutaneous tissue of the back. This produced 1 to 3 hours of apparent unconsciousness. The solution was very hypertonic because through a technical lapse the urethane had been dissolved in 0.9 per cent salt solution. Water bottles were used to supplement the fluid obtained from the diet of bread, milk, and lettuce. Not a few of the young born after the usual 21 days of gestation were puny and far below the normal weight, and these got their hair late and grew but slowly during several weeks. Ultimately

[1] We have been unable to find any account of when the lungs begin to form in the mouse. They are recognizable in embryos 12 days old. The tracheal anlage can then be seen pushing out from the foregut and ballooning into lung buds with primitive bronchi. It would be difficult to identify the lungs previous to this stage.

many litters were obtained however which resembled most of the controls in weight and vigor.

Some of the injected females were killed on the 18th to 20th day of gestation, and others on the 20th or 21st day, or when delivery had begun, in order to procure lungs which had not been exposed to postnatal influences. If the fetuses failed to breathe after removal from the uterus respiration was stimulated by dropping them repeatedly from a height of a few centimeters; and after they had taken a few breaths the chest was opened widely by lateral cuts, a flap including the sternum was everted, and the whole creature was plunged into acid Zenker's solution, for fixation of the pulmonary tissue *in situ*. Day-old mice were chilled in an ice box until insensible, and their lungs were fixed by the same procedure. Three-day- and 10-day-old young were killed by pushing in the cranium, after which a cord, already placed around the neck, was quickly tightened to prevent any aspiration of blood. The lungs were then dissected out and fixed as usual. By these procedures well distended pulmonary tissue was obtained, in which tiny adenomas could readily be perceived with the microscope. Mice 15 days old or older were chloroformed and the lungs were allowed to collapse to some extent prior to fixation, in order to reduce the number of sections to be examined. When the litters were large some of their members were killed 1 or 3 days after birth, in order to render the conditions more favorable to development of adenomas in the others; for the occurrence of these growths in adult mice is largely dependent upon sufficient food (8). Three groups of young animals, 3 days, 10 days, and 60 to 70 days old respectively, were searched intensively for adenomas, together with the appropriate controls. Many of the mother animals were killed and examined at the same time as their last surviving young.

Not a few of the 60 to 70-day-old offspring of urethanized mice had adenomas visible in the gross on the pleural surface, whereas none of the controls had any. They could not be seen on the pleura of 10-day-old animals, even at a magnification of × 17, and hence the lungs of these and of 3-day- and 1-day-old individuals were searched *in toto* in serial sections 7.5 μ thick, as were also those of fetuses near term. The pulmonary tissue of certain of the older animals was searched in the same way, notably when the incidence of tumors in mothers and young was to be compared. The searching was done, section by section, at a magnification of 120 diameters in the case of mice 10 to 70 days old, and at 180 diameters in that of younger creatures. This serial scrutiny proved crucial in the case of adenomas just forming, because they were often simulated in individual sections by cell groups cut through at the bend of a blood vessel or bronchiole, or were mimicked by pleural infoldings.

The choice of stains, methylene blue and eosin, proved fortunate, for they made the tiny adenomas of young animals stand out from their surroundings, ordinary lung parenchyma appearing purplish-pink save for the nuclei, whereas the adenoma cells were basophilic, sky blue to dark blue, according to the intensity of the staining.

Findings in the 60 to 70-Day-Old Offspring of Urethanized Mothers

Pure breeds of mice differ widely in their liability to spontaneous adenomas. These appear soonest and are most frequent in animals of the A strain, the C strain ranking next but far behind. Andervont found them in 20 to 30 per cent of C mice over a year old (9), as nodules visible in the gross on the pleural surface,—the "peripheral index"; but in our colony they almost never manifest themselves in this way in individuals less than 9 months old, much older ones often showing none. They are nearly always solitary. No one has tested the response of C animals to urethane, but to methylcholanthrene it is notably slow. The tumors were still absent from Shimkin's animals 13

weeks after an intravenous injection of this hydrocarbon, and there were only 4 to a mouse after 20 weeks, whereas in adults of the A strain they averaged 25 to an animal after 6 weeks, 31 after 13 weeks, and 47 after 20 weeks (10).

These findings gave no ground for the supposition that urethane would produce adenomas swiftly in the offspring of urethanized C mice; only their presence in implants of embryo tissue which had been acted upon by methylcholanthrene made early examination of the young seem worth while. In serial sections of the lungs of a 30-day-old animal of the first litter from a urethanized mother a typical adenoma was encountered, and in the other mouse of this litter, killed 60 days after birth, there was a growth on the pleural surface visible to the unaided eye. Consequently we now set aside many newborn mice and examined them in the gross 60 to 70 days later. The controls were in general killed when slightly older, mostly after 70 to 100 days, which tipped the scales somewhat in their favor because the longer a mouse lives the greater is the likelihood that adenomas will appear spontaneously. In a total of 97 controls examined when 60 to 104 days old, no tumor was to be seen on the pleural surface, whereas adenomas were present in 21 of 80 individuals 60 to 70 days old from urethanized mothers,—just such tumors both in the gross and microscopically, as develop spontaneously in adults. They averaged a millimeter in diameter, but a few were as much as 2 mm. across, and in occasional individuals two or three of them were present. The lungs were remarkably free from equivocal nodules.

In one young animal out of a group of seven killed when 30 days old, from four litters born to urethanized females, an adenoma had developed that was visible in the gross. Its character was confirmed with the microscope.

The Growths in Mice 10 Days and 3 Days Old

Adenomas proved fairly frequent on microscopic examination of the lungs of 15-day-old animals from urethanized mothers, and hence an intensive search was made for them in serial sections from still younger individuals. Tables 1 and 2 give the findings in groups 10 days and 3 days old respectively. It will be seen that 10 out of 16 of the 10-day-old sucklings had adenomas, in some cases two or three. In none of the lungs from 16 control animals 10 days old was any structure found that could be classed as an adenoma with certainty, though the organs were searched in serial sections with equal care.

Only characteristic adenomas (Figs. 1–3) have been given place in Table 1, of 10-day sucklings. Some were surprisingly large, as much as 0.2 mm. across, and not infrequently compression of the adjacent parenchyma attested to their rapid growth[2] (Fig. 1). They had a

[2] Some authors have remarked upon the fact that the induced or spontaneous adenomas of adult mice seem to be mostly situated at the pleural surface; but of the 15 adenomas found in animals 10 days old nine were well down in the parenchyma. It was noteworthy in this relation that the pulmonary tissue next the biggest tumors frequently showed compression on the side toward the pleura (Fig. 1), and occasionally there was an outward bulge over

TABLE 1

Adenomas in 10-Day-Old Mice from Urethanized Mothers

(Only indubitable adenomas are included)

Urethane injections	Interval to birth	Mother No.	Adenomas	Time from first dose	Weight at death	Remarks
No.	*days*			*days*	*gm.*	
		I	0	15	5	
4	1	II	0	"	5	
		III	0	"	8	
		IV	A-A A A 0	17	5.3 5 5 4.7	See Table 2 for litter mates
	1	V	A-A-A	"	4	" "
		VI	A A-A	"	7 6.6	" "
6	2	VII	A 0	18	5.5 "	
	3	VIII	A 0 A-A A	19	5.5 " 5 4.5	
		C I	0 0		5.2 3.9	See Table 2 for litter mates
		C II	0		6.8	
		C III	0 0		6 5.5	
		C IV	0		6	
		C V	0		5.6	
Controls		C VI	0 0		6.5 6	
		C VII	0 0 0		8.3 8.2 "	
		C VIII	0 0		4.5 5	
		C IX	0 0		4.5 4.7	

Each A means an adenoma.

close general resemblance to the adenomas arising spontaneously in adult C animals and those induced in A mice with methylcholanthrene or urethane, and like them were either wholly glandular (Fig. 3) or partially compact (3, 11) (Fig. 2), their older central portion then being adenomatous. At some marginal spots the neoplastic cells formed a single layer on the walls of alveoli, as if extending along them with local heapings up.

Although the growths could easily be distinguished for what they were,—a recognition validated by study of the specimens from mice killed in later weeks,—they differed significantly in detail from the adenomas of adults. This matter will be considered further on.

To recognize the tumors in 3-day-old animals proved more difficult. Only three out of 10 of them had indubitable adenomas (Table 2), a single growth in each instance, but three others had what seemed to be early stages, wholly resembling those reported by Nettleship, Henshaw, and Meyer as appearing in urethanized adult mice (3), and comprehensively described by Grady and Stewart in their study of how adenomas come about after the intravenous injection of a colloidal suspension of methylcholanthrene (11).

The tumors were much less well developed than in the 10-day animals, as would follow from the brevity of the interval since the first urethane injection. The largest was found in the individual surviving for the longest time after the first injection of the mother (14 days), the next largest after the next longest time (11 days), while in the case of the least advanced growth only 10 days had elapsed. The largest (Figs. 4 and 5) lay deep in the lung, was markedly basophilic, and stood out sharply from the surrounding parenchyma. It consisted of the characteristic cuboidal cells, enclosing and partially filling a number of small spaces. A venule lay amidst it, as frequently happens in the forming adenomas of adults. It appeared multicentric where cut across, and its aspect suggested an origin from bronchiolar epithelium, but serial sections proved it to be a single growth lying well away from any bronchus or bronchiole. The lining of these latter stained a much paler blue.

Fig 6 shows the subpleural situation and discrete character of the growth found 11 days after the first exposure of the mother to urethane, and Fig. 7 reveals its characteristic morphology. Its cells are ranged in the usual single layer about small "acinar" spaces, and already it protrudes from the pleural surface. The parenchyma round about looks normal; as yet the cells lining the alveoli have undergone little flattening.

The 3-day-old animal which showed a growth only 10 days after the first giving of urethane had six litter mates and four of these were let live until 10 days after their birth, that is to say until 17 days after the first injection. Two had then a well developed adenoma each (Figs. 1 and 3), and a third had two of them (Table 1, offspring of mother IV). The growth in the 3-day-old animal consisted of a clump of cells, for the most part compact but covering part of an adjacent alveolar wall with a single layer (Fig. 10). They were sharply set off from their surroundings by their strong blue color and large, round, vesicular nuclei (Fig. 9). Mitoses were present. The clump had no direct connection with the epithelium of any bronchiole but lay well down in the parenchyma, and there was no cellular reaction round about it.

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growths situated at some distance beneath it. The inference seems justified that a considerable proportion of the adenomas visible on the pleural surface of adult mice reach the surface secondarily as result of local pressure conditions as well as of enlargement. There is no need to invoke local differences in their initial distribution in the lung tissue.

The lungs of three other 3-day-old mice contained small discrete clumps of markedly basophilic cells with round, vesicular nuclei. They were solitary, and were situated in or on the wall of an alveolus. The instance pictured (Fig. 8) came from a sixth animal of the litter

TABLE 2

Adenomas in 3-Day-Old Mice from Urethanized Mothers

Urethane injections	Interval to birth	Mother No.	Adenomas	Time from first dose	Weight at death	Remarks
No.	*days*			*days*	*gm.*	
4	7	IX	A	14	2	
6	1	IV	0 A? A	10	2 1.8 1.9	See Table 1 for litter mates
		V	A?	"	2	" "
		VI	0 0 A?	"	2 1.9 1.9	" "
	2	X	A 0	11	1.9 1.9	
Controls		C I	0 0 0 0		1.7 1.6 1.6 1.5	See Table 1 for litter mates
		C X	0 0 A? 0		2 2 1.9 1.8	
		C XI	0 0		2.6 2.7	
		C XII	0		1.8	
		C XIII	A? 0		2 2.2	

A = characteristic adenoma. A? = dubious early stage.

of seven (Tables 1 and 2) dealt with in the last paragraph, the seventh proving negative. The clumps are queried in Table 2.

The Findings in Newborn Mice and in Embryos

From the relative size of the adenomas in 10-day- and 3-day-old individuals, it seemed likely that any encountered in still younger mice would consist of but

few cells. And in fact no indubitable adenomas have been come upon in 10 animals (from three litters) which were killed within 24 hours after birth, or in 14 embryos (from four litters) 18 to 20 days along. All the mothers had received six injections of urethane,—the last of them 1 to 3 days before parturition in the case of the young that came to term.

The lungs of mice less than 24 hours old have an alveolar lining that is but little flattened as yet, and often they are still partly atelectatic, a state of affairs rendering the recognition of adenomas almost impossible, as Tyzzer noted in his classical study of these growths in adults (12). The cells lining the alveoli of fetuses near term are basophilic and cuboidal, with round, vesicular nuclei, in other words closely resemble the cells of adenomas. It would manifestly be impossible to discern minute growths of this sort if situated in the parenchyma, but if they lay next the pleura or bulged it outward they might be perceived; and we have found what appears to be a forming growth (Figs. 11 and 12) in one of four fetuses removed from a female on the day after the last of six injections of urethane.

The mother animal had been selected by palpation as in mid-pregnancy, and hence the age of her fetuses was not precisely known; but the development of their lungs indicated that they were close to term, though they were only 22 mm. long as compared with 24 to 28 mm. for newborn mice from normal mothers. Mention has already been made of the frequent stunting effect of urethane on the embryos. Figs. 11 and 12 show that the problematic growth was discrete, bulging the pleura outwards, and that it consisted of cells with vesicular nuclei, ranged about an acinar (?) space. Nothing like it was found in the lungs of the other embryos, including three from the same litter, nor in those of any of the newborn mice.

The Findings in Sucklings of the A Strain

As already stated, adult mice of the A strain are far more liable to spontaneous adenomas than are those of the C breed (9), while furthermore the growths can be induced much more readily with methylcholanthrene (10). Hence we supposed that the offspring of urethanized A females might develop them earlier and in relatively great number. But on test an obstacle was met: the A animals injected during the latter half of pregnancy did very badly although much bigger than Cs at this time, and though only the standard amount of urethane (0.3 cc.) was given. Those receiving six injections either died or aborted or ate their young at birth, and so too with most of the mice which had four or five injections, the few that survived being so close to term at the time of the last as to give birth to their young on the day afterwards,—as did also the only animal that received three injections. It follows that the total interval between the first exposure of the young *in utero* to urethane and examination of them 3 days and 10 days after birth was, 7 to 9 days and 14 to 16 days respectively, that is to say was shorter than in the case of C mice. Furthermore the embryos had been exposed to much less urethane, both because of the fewer injections and because of the large bulk of the mother. Whether for these

reasons or no, the growths have been infrequent and tiny in individuals 10 days old (Figs. 13–15), actually comparable with those in 3-day-old C mice. For this reason no extensive study has been made of them. None was found in several A animals examined when 3 days old.

The Early Stages of Adenoma Formation

As already remarked, the early stages of the growths resembled those observed in adult mice given urethane or methylcholanthrene (3, 11).

First seen in the parenchyma were occasional discrete clumps of cuboidal or rounded cells situated in or on the walls of alveoli (Fig. 8), and with cytoplasm that stained an intense blue, sometimes very dark, contrasting so sharply with the hue of the normal parenchyma as to attract attention at low magnification. These cells had round or slightly oval vesicular nuclei, unlike those of ordinary alveolar elements, which are mostly dense, almost pyknotic, and more or less oblong. The clumps seemed punctate in origin and almost devoid of capillaries, unlike the richly vascularized alveolar tissue about them; yet their components looked very active. The growths of Figs. 9, 10, and 15 represent the earliest stage at which an adenoma gave definite signs of what it was. Here the cells have heaped up into a compact mass, but in Fig. 10 an adjacent alveolus is partly lined with them; mitotic figures can be seen. The tiny growth of Figs. 6 and 7 looks as if it had been adenomatous from the first. The tumor of Figs. 4 and 5 is expressive of further development. None of the growths was multicentric.

In late fetuses and sucklings up to 10 days old, of both the C and A breeds, spherical or discoid aggregates of cells were frequently found just beneath the pleura, which were at first taken for compact adenomas, and with the more reason because of the frequency with which the growths are found in this situation. They occurred irrespective of whether the mother had received urethane, and consisted of more or less cuboidal elements with round vesicular nuclei and cytoplasm staining blue,—cells not to be distinguished individually from those of adenomas. Sometimes the aggregates were spherical, lying tangential to the pleura amidst the lung substance, but more often they were discoid and protruded on its surface. Mitotic figures were frequent in them, far more so than in the lung parenchyma round about; and often the cells partially filled adjoining alveolar spaces. But it was noted that some of the masses, though appearing spherical in cross-section, were really fusiform, running through many sections, and furthermore that a thin membrane formed by reduplication of the pleura was attached to the surface either over them or near by; and the fact became evident that the masses were mere "mooring clumps" for membranes joining one lobe of the lung loosely to another. Fig. 16 shows a characteristic example. Frequently the clumps were situated at the sharp edge of a lobe or next it, that is to say at sites where adenomas are prone to occur; but now and then one was on the shoulder of a lobe or its rounded convexity. Occasionally a small lenticular mass of blue-staining cells like those composing a mooring clump was to be seen closed in between the layers of the membrane, well away from the lung surface. Even where the cells of a clump filled alveolar spaces, they were never ranged in the glandular pattern of mature adenomas; yet the fact remains that if no membrane had been attached next them they could not have been distinguished with certainty from compact tumors of such kind. For this reason we have accepted no subpleural growth as an adenoma unless it had typical features, and hence undoubtedly some have been omitted from Tables 1 and 2. The pleural nodule of Figs. 11 and 12 lay on the convexity of a lobe, had no membrane inserted over it, and an acinus seemed to have formed within it; yet because of the existence of "mooring clumps" its status is problematic. These are not infrequent in embryos.

Solitary, almost spherical giant cells 30 to 40 μ across, containing two or three vesicular nuclei, were present now and again on the alveolar walls of 3-day and 10-day animals, never more than two or three of them to a lung. None was found where adenomas were forming, and they seemed to have no relation to this process. Quite often in both C and A sucklings,—from control as well as urethanized mothers,—there were what may be called "polymorphonuclear balls,"—small, sharply demarcated, spherical aggregates of cells staining blue like adenoma cells, but with nuclei of horseshoe shape or resembling those of the blood polymorphonuclears, though coarser and less pyknotic. The cells had no granules, showed no division figures, and the balls formed of them appeared in excellent state, without sign of central necrosis. Usually they lay just outside some capillary in the alveolar wall, which appeared patent and normal. Wholly different in aspect from adenomas, their significance is not clear and their ultimate fate has not been traced. None has been come upon in embryos.

The Findings in Control Animals

It is singular that the literature contains no observations on how soon spontaneous adenomas begin to form. The scarcity of mitoses when they first come to attention in adult mice makes plain that their recent growth has been exceedingly slow, and it may well be that they originate early in life. Hence our diligent search for them in the control young of the present work,—the more careful because the tumors would doubtless be small, and perhaps solitary.

The lungs of one of 13 normal C mice 3 days old, examined *in toto* section by section, had a discrete clump of dark blue cells, in the parenchyma far from any bronchiole (Fig. 22); but both the cells and their nuclei were smaller and denser than those of the adenomas found at this time in the offspring of urethanized females. In another control mouse 3 days old several adjacent alveoli were almost filled with clumps of blue cells. Their nuclei were smaller than ordinary and oblong in some instances, yet the resemblance to a forming adenoma was great and at one spot the cells were ranked in two parallel rows (Fig. 23), as often happens in such growths. In the lungs of three out of 16 normal animals 10 days old small compact clumps have been found of basophilic cells wholly resembling the one pictured in Fig. 8 as coming from a 3-day-old mouse of an urethanized mother. There was only one such clump in each animal.

In sum, the findings give some support to the possibility that adenoma cells may have been present in the pulmonary tissue of the young normal mice. Their existence could not have been excluded in any case, since it would be impossible to identify them if scattered singly. This much is certain however, that the lungs of the controls contained no adenomas identifiable as such.

Origin of the Adenomas

Some workers are convinced that the adenomas called forth by urethane in adult mice arise on the basis of chronic inflammation of the pulmonary tissue, due either to the substance itself or to intercurrent infection. No sign of any such course of events has been observed in the young animals of the present work. Orr believes that the preliminary inflammation may have disappeared by the time the adenoma is well formed (13).

Urethane is known to produce ascites and to cause injury to several organs, largely in consequence of capillary damage. Orr reported that it set up chronic pulmonary inflammation in the outbred stock mice employed in his experiments, and Winchester and Higgins (14) found that it induced more or less pulmonary edema in animals of the C strain. The quantity of urethane injected into the pregnant females of the present work frequently exceeded the tolerable maximum, many of them dying; and often the fetuses and young of the surviving animals were abnormally small, as already mentioned, while occasionally the development of the lungs seemed retarded; yet nowhere were any local cellular anomalies other than adenomatous change perceptible in the pulmonary tissue. Furthermore there was no cellular reaction about the forming growths, as the figures sufficiently demonstrate.

The lungs providing material for the conclusion that adenomas are secondary to inflammation have generally been the subject of consolidating infections. The C strain is no exception in this latter respect. Mature animals of our colony often develop a consolidation which slowly involves most of the pulmonary tissue and eventually proves fatal. The disease is obviously infectious, spreading rapidly amongst cage-mates; but its cause is still undetermined. The lesions it produces have no resemblance to adenomas. The bronchioles seem to be first affected, cellular exudate accumulating within some of them; more and more are implicated; large and small mononuclear cells accumulate round about them and about the blood vessels; and the alveoli become filled with swollen, desquamated elements. The gross result is consolidation. In all these respects the findings are like those in the "grey virus disease" described by Andrewes (15). Some of the urethanized mother animals, killed with their young 60 or 70 days after parturition, had developed the malady and hence were discarded together with their offspring, but the lungs of the great majority appeared normal, save for adenomas, and those of the embryos examined and of the 1-day- and 3-day-old sucklings gave no microscopic evidence of the disease. In a single 10-day-old animal its beginnings were found.

Manifestly the best method to determine whether the consolidating disease prepared the way for the adenomas was to try to induce the latter in the young of a breed with healthy lungs. This was one reason for the tests of A mice. Our colony of them is remarkably free from pulmonary inflammations and the "lung disease" in special has never been found. Nevertheless adenomas arose in the young of females urethanized while pregnant. There was no cellular reaction about the growths (Figs. 13–15).

These facts, considered with what has already been said, prove that the adenomas induced in young mice were not secondary to pulmonary inflammation but primary in origin. Nettleship, Henshaw, and Larsen decided that the growths they induced with urethane in adult animals ordinarily arose from tissue which had undergone "little or no injury," and Grady and Stewart found no trace of preliminary pulmonary disorder in connection with the early stages of the adenomas due to methylcholanthrene.

Derivation of the Growths

Opinions have differed widely on whether pulmonary adenomas originate from alveolar cells or from the epithelium of the bronchial tree. None of the growths in sucklings had any direct connection with this latter; they were everywhere surrounded by parenchyma. All were arising, or appeared to have arisen, either from the alveolar wall or from elements of alveolar character lying

next the pleura, and the resemblance to bronchial epithelium was but slight. In the mother animals on the other hand, examined 70 days after parturition but of indeterminate age, and doubtless having in some instances "spontaneous" adenomas antedating the urethane injections, growths were not infrequently encountered which were composed of elements like those lining the small bronchioles (Fig. 17) and staining nearly as pink. The morphological resemblance was complete, except that the tumor cells were seldom markedly cylindrical, a difference which might have been consequent on pressure factors. In both instances a large proportion of the nuclei were round or slightly oval, and vesicular, as in the growths of sucklings, but many others were oval or oblong and almost pyknotic, often notably big and sometimes then with much more cytoplasm than usual about them. When oblong they were frequently ranked with their long axes parallel. All gradations between the two types were present. The resemblance to bronchiolar epithelium can be seen in Fig. 18. The growth providing this figure, made for ease of comparison, was exceptional in protruding into the lumen of the adjacent bronchiole; but other adenomas with identical features (Fig. 19) were wholly isolated amidst the lung parenchyma, as serial sections proved.

Amount of Exposure to Urethane as Determining the Incidence of Tumors

In Table 3 are listed all those cases in which the urethanized females were examined, as well as their remaining offspring, 60 to 70 days after parturition. The age of the females was not known, but they were sturdy multipara, several months old at the least, and some must have been well on the way toward having "spontaneous" adenomas, as has just been remarked. They, not their embryos, bore the immediate brunt of the urethane. On both these grounds one might have supposed that they would have had adenomas more often than their young, when all were eventually killed. And this was so, 10 of 14 mothers showing them in the gross on the pleural surface as compared with 10 of 49 young. Yet they were few at most in the mothers and usually solitary. Repetition of the injections had but a slight effect; in only two of five mother animals which had received four or six injections were there definitely more tumors than in those which had received but one.

Larsen noted that the number of adenomas present after 6 months in the offspring of A mice receiving urethane while pregnant largely depended upon when the substance had been given (6). There were at least five times as many when it had been injected during the final 24 hours of gestation as when it had been administered earlier, and furthermore every animal had them, instead of a large proportion. The reason, Larsen concluded, was that more blood carrying urethane reached the fetal lungs in the final hours before birth. No such differences with time of injection have been perceptible in the present experiments. The microscopic findings in 10-day-old animals carry some weight in

TABLE 3

Peripheral Adenomas in Mothers Urethanized While Pregnant and in Their Offspring

(Findings 60 to 70 days after parturition)

Urethane injections	Interval to birth	Mother	Offspring		Adenomas		Remarks
			Age	Mouse	Presence	Size	
No.	*days*		*days*			*mm.*	
1	2	A			0		
			70	a	+	1	
				b	0		
				c	+	1	
				d	+	1	
				e	+	1-1	
		B			0		
			70	a	+	2	
				b	+	1-1	
				c,d	0		
	6	C			+	1	
			67	a,b,c,d,e,f	0		
		D			+	1	
			67	a	+	0.5	
				b,c,d,e	0		
2	1	E			0		
			70	a	0		
		F			0		
			70	a,b,c,d,e,f	0		
3	1	G			+	1	
			70	a	+	1.5	
				b,c	0		
		H			+	1-1	
			70	a	0		
		I			+	0.5	
			70	a,b,c,d,e	0		
4	1	J			+	0.3	
			60	a	+	0.3	
		K			+	1-1-1-1-1	
			70	a,b,c,d	0		
	4	L			+	1	Carcinoma in other lung
			70	a	+	1	
				b	0		
		M			+	1-1	
			70	a	+	2	
				b,c,d,e	0		
6	2	N			+	1-1-1-1-1-1	
			70	a	0		

this relation because their entire lungs were examined microscopically. It will be seen (Table 1) that the incidence of adenomas in the litters from mothers getting six injections of urethane was only dubiously larger when the last had been given within 24 hours of parturition than when it had been given 2 or 3 days previously. The findings in the young killed 60 to 70 days after birth stress the same point. The offspring of females receiving four injections, the last within 24 hours of parturition, had no more tumors visible in the gross than those from mothers receiving the final injection 4 days previously (Table 3).

Nor did repetition of the injections into the mothers make adenomas any more frequent in the young. Actually the incidence of these growths in the offspring of the four mothers receiving urethane only once was considerably greater than it was in the young born to the eight animals getting it three to six times, seven out of 20 young mice in the first category having tumors visible in the gross as compared with four out of 22 in the second.

Mention has been made of the fact that a large proportion of the pregnant mice receiving urethane several times did badly and gave birth to young that were far under weight. Here was a possible reason for the differences just brought out. For Tannenbaum has shown that the appearance of spontaneous adenomas in adult animals can be checked or even prevented by underfeeding them (8); and the offspring of mothers getting urethane three to six times might have been more poorly nourished than those from females receiving it only once. But all the young of Table 3 appeared healthy when they were killed 60 to 70 days after birth, and while some were unusually small the two groups did not differ significantly. Of course early differences crucial to the development of adenomas might have been ironed out by this time, but the findings in the 10-day-old mice of Table 1 speak against the influence of any such differences. It will be seen from the weights there given that the individual with most growths (offspring of mother V) was the smallest of the seven killed 17 days after a single injection of urethane into the mother,—weighing 4 gm. as compared with a maximum of 7 gm. An animal having three peripheral adenomas, each a millimeter across when it was killed 72 days after birth from a female repeatedly injected with urethane weighed only 13 gm. at this time instead of the normal 20 to 24 gm., and it had weighed only 2.8 gm. when 10 days old and been almost devoid of hair then. It is not listed in Table 3 because its mother was not examined at the same time.

A better reason why repetition of the injections failed to result in more growths is perhaps to be had in the difficulty of inducing adenomas in adult animals of the C strain. No previous observations with urethane are on record, but methylcholanthrene gives rise to the growths very slowly in C mice, as already mentioned, and the number is small for a long time. Only toward the 20th week after intravenous injection of the hydrocarbon did they appear

in Shimkin's test, whereas in A mice there were more than 24 to an animal by the 6th week (10). They were never many in our C animals, and hence chance must have entered largely into the gross findings of Table 3. Yet the results cannot be wholly accounted for on the basis of these facts. The C embryos exposed to urethane in the mothers receiving but a single injection of it proved so responsive that adenomas followed in a large proportion of instances (Table 3), the growths arising very rapidly (Tables 1 and 2). Multiple injections failed to elicit them in any greater number. One injection into two pregnant females 2 days before parturition resulted in adenomas in six of their nine offspring, whereas three and four injections into five mothers, the last given within the final 24 hours of gestation, yielded growths in only two of 14 young (Table 3). These data, which go against all experience with adult mice, strongly suggest the existence of litter differences in the potentialities for adenoma formation. No sex differences were observed.

One might have supposed that the young of the two females receiving most urethane and themselves developing multiple adenomas in consequence (mothers K and N, Table 3) would have had growths most frequently. Actually their young had none,—at least none visible in the gross on the pleural surface. But the pleural index must sometimes be misleading when pulmonary tumors are few. Nevertheless Table 3 in its entirety seems to indicate that the offspring of individual C mothers developing adenomas in the gross after urethane injection possessed no greater tendency to such growths than the young of females in which none appeared. Using the pleural index Lynch was unable to obtain any evidence of a maternal influence on the incidence of pulmonary adenomas in tarred mice (16), and Bittner and Little found none in the case of the spontaneous growths (17).

Age as Affecting the Adenomas

Nearly everyone studying the adenomas of adult mice microscopically, whether spontaneous or induced, has remarked upon the scarcity of mitoses. Yet in the growths of the 10-day sucklings they were so abundant that often nearly every section showed them (Fig. 1), five being visible in a single high-power field of the microscope in one instance. There were other signs too of pronounced cellular activity. The adenomatous pattern was often ill-defined, and the individual cells were less differentiated and more basophilic than in the growths of adults (Figs. 1 and 2). The nuclei were almost uniform in size, round and vesicular, with little chromatin and this mostly marginated; the cytoplasm was relatively scant.

The tumors of the mother animals killed 60 to 70 days after parturition were far more various. All were frankly adenomatous in arrangement and mitoses were few or wholly lacking (Table 4); many of the growths stained purple and now and then the color was pink. Occasionally one consisted

entirely of cells with vesicular nuclei like those of 10-day-old mice, but in most instances a considerable proportion of the nuclei stained a dark, even, almost pyknotic blue (Figs. 17–19), and often then they tended to be relatively large, and oval or oblong instead of round. Not infrequently when this was the case they had an unusual amount of cytoplasm about them (Fig. 17). Some of the cells with these features may have been intrusive, but the majority were ranged side by side with the ordinary elements having round, vesicular nuclei, and there were all gradations between the two. The differentiation of the neoplastic elements,—for such it obviously was,—culminated in cells which resembled those lining the bronchioles, though not quite so acidophilic (Fig. 17). Often they were in rows like these.

In sum, it was plain that the adenomas of 10-day-old mice consisted of actively multiplying cells, which for that reason, at least in part, were undergoing but little differentiation, whereas those of the mother animals were indolent and had largely differentiated.

A corroboratory indication of these contrasting states was found in the relative size of the growths encountered in mothers and young respectively after 60 to 70 days. Although the mothers were by then well along toward the age when some might have had spontaneous adenomas visible in the gross, and although they had received urethane directly, the largest of their tumors were only half as big as those in certain of the young (offspring of mothers B and M, Table 3). Indeed the average adenoma in these latter was as big as any in the mothers. Active proliferation must have kept on during some weeks for them to have reached such a size.

In order to learn whether cell multiplication was still continuing in any important degree comparative counts were made of the mitotic figures in some of the growths of mothers and young. The undertaking was not comprehensive; just enough was done to answer the question posed. The findings are given in Table 4.

It will be seen that no mitoses were present in the two adenomas, each a millimeter across, of mother M of Table 3, nor any in a smaller growth disclosed on serial section of the lungs. There were a few in the 2 mm. adenoma of one of the young from this mother, as also in another growth of considerable size which lay deep in its lung parenchyma. But evidently cell division had almost ceased, and this was the finding also in the notably big adenomas of other young animals, the progeny of mothers A and G. Some of the growths in these young showed no mitoses whatever, and their maximum frequency was no greater than in several of the tumors of mother K. It may be recalled that this animal was one of the two which had multiple adenomas visible in the gross, after the repeated injection of urethane, and that none of her four young showed any tumors (Table 3).

The conclusion seems warranted that proliferative activity in the adenomas

TABLE 4

Mitoses in the Adenomas of C Mice Urethanized While Pregnant and of Their Young

(Findings in growths present 60 to 70 days after parturition)

Days	Urethane injections	Mother	Young	Size of tumor	Cells examined	Mitoses		Remarks
						Actual No.	No. per thousand cells	
				mm.	*no.*			
		A			3,000	0	0	Whole tumor searched
			a	1	24,000	0	0	
	1		e	2	42,000	2	0.05	
			"	1	40,000	1	0.03	
70			"		18,500	1	0.05	Whole tumor searched
			"		10,400	0	0	Tumor partly lost
		G			9,400	1	0.1	Tumor partly lost
	3		a	1.5	44,500	0	0	
		J		0.3				
60	4		a	0.3	24,500	5	0.2	Growth in mother lost
			"		22,600	3	0.13	Whole tumor searched
		K		1	33,400	4	0.12	Whole tumor searched
		(Her four young		1	20,500	2	0.1	Tumor partly lost
		were negative		1	19,200	3	0.16	Whole tumor searched
		in the gross.)		1	18,800	0	0	" " "
				1	15,700	3	0.19	" " "
					14,000	4	0.29	" " "
					8,400	2	0.24	" " "
70	4				4,350	3	0.7	" " "
		M		1	63,000	0	0	Whole tumor searched
				1	33,400	0	0	" " "
					12,100	0	0	
			a	2	598,400	19	0.03	Whole tumor searched
			"		61,200	2	0.03	" " "

The growths for which no size is recorded were either microscopic or had lain hidden in the parenchyma. All recognizable stages of mitosis were counted.

of the young animals had almost ceased by the time they were 60 to 70 days old, being by then nearly on a par with that in the mother animals. And there was substantial evidence to this effect in the morphology of the growths, which now exhibited not infrequently a differentiation as complete as that in

the tumors of adults. It was marked in some of the biggest adenomas of the young (Figs. 20 and 21). Growths in the same individual often differed widely in such respect, just as happens in adults.

DISCUSSION

The main aim of the present work, like that of other experiments previously reported (1), has been to learn whether the cells of embryos possess the ability to undergo neoplastic change. On first inspection the facts seem to leave no doubt of this for the lung, yet they cannot forthwith be accepted as proof.

First one must know whether the growths appearing in the offspring of urethanized mothers are true pulmonary adenomas, and if so, whether the latter are really tumors. On both points the answer is yes. The growths have been followed through every stage to the form found in adults, and they exhibit the familiar, distinctive characters of pulmonary adenomas. All workers with these latter are now agreed that they are genuine neoplasms. Both the spontaneous and the induced growths have been transplanted successfully (18), and while some have become carcinomatous on passage others have retained their initial traits. Adenomas are the first and most frequent tumors to arise from pieces of mouse embryo lung implanted with methylcholanthrene in the leg muscles of adults of the C strain (4), and several obtained in this way have been transplanted. One has now been propagated in five successive groups of adult hosts, and it has retained its original adenomatous morphology although in the course of months it forms huge tumors, killing the hosts. So convincing is the evidence that the adenomas are genuine neoplasms that we have deemed it unnecessary to try to transplant those obtained in sucklings. Doubtless for the same reason Larsen has reported no transplantations of the adenomas present in 6-months-old A mice from urethanized mothers (6).

So rapidly is urethane excreted that its direct effect can only have been exerted upon the embryos *in utero*, organisms that is to say which are protected from many extraneous influences, notably most of the infections. This state of affairs has provided opportunity to gain light on several moot points.

Much uncertainty has existed as to whether the adenomas arise from alveolar cells or from bronchial epithelium. Tyzzer, who first studied them comprehensively, concluded that they could be of either origin (12). Those we found in sucklings were wholly surrounded by alveolar tissue, and the evidence of early stages was all to the effect that they had originated from alveolar elements, like those induced in adults by methylcholanthrene (11). None had any direct connection with a bronchiole. But needless to say, as an adenoma grows large it must often fill the space into which a bronchiole opens and may even project into its lumen. The cells of the tumor of Fig. 18, in which this was the case, were not joined to those lining the bronchiole, yet a union might on occasion take place secondarily since experiment has shown that elements as widely

different as those of regenerating epidermis and of a carcinoma of prostatic origin can join in a layer (19). Furthermore the cells of pulmonary adenomas in adults sometimes resemble the epithelial elements lining the bronchioles (Fig. 17). It seems likely that these phenomena have provided the grounds for the belief that the growths often take origin from the bronchiolar lining,—the more so as the evidence on this point has been mostly obtained through studies of large, well established growths under conditions complicated by bacterial infection, atelectasis, and consolidation. If adenomas do arise now and again from the epithelium of the bronchial tree the occurrence must be highly exceptional.

The derivation of the cells lining the alveolar wall has long been the subject of controversy, some investigators holding that they are epidermal in nature and others that they are mesodermal (20). The changes taking place secondarily in the adenomas developing in the young of urethanized mothers provide evidence in this matter. Though the growths originated from the alveolar wall and had no direct connection with the bronchiolar epithelium, their cells not infrequently took on a resemblance to the latter within 60 to 70 days (Fig. 20). Occasionally in the growths of the mothers the resemblance was absolute (Fig. 17). Recently one of us (21) has found that the cells of the alveoli formed after the transplantation of fragments of mouse embryo lung to adult hosts may undergo metaplasia to stratified squamous epithelium if exposed to methylcholanthrene. The change may occur at spots distant from bronchiolar epithelium, as Passey has noted in rat lungs chronically inflamed by bacterial infection (22). These facts, taken together, make plain that the cells of the alveolar wall are pluripotential despite their specialization, and that they are epithelial in character. The hypothesis might be put forward as alternative that bronchiolar elements lie scattered amongst the alveolar ones, all undiscerned, and that the adenoma cells and squamous epithelium derive from these. But if this is the case why do not adenomas arise often from the bronchiolar lining instead of rarely if at all?

Certain workers with the spontaneous adenomas of adult mice and those induced with urethane believe that both arise on the basis of inflammatory lesions. If this were the fact the tumors occurring in the young of urethanized females could have resulted from neoplastic changes taking place after birth, in animals living long enough; the 6 months' existence of Larsen's mice would have provided abundant opportunity for this sequence of events. The present findings exclude it. Neither in the embryos removed from females receiving urethane repeatedly nor in their recently born offspring did any pulmonary inflammation occur which might have provided a basis for later adenomatous change; and no reactive proliferation or accumulation of lymphocytes or macrophages took place about the growths in the young animals (Figs. 1–14). The conclusion seems warranted that the neoplastic changes which found expression

in the adenomas of sucklings were not secondary to inflammation but primary in origin.

The action of urethane to elicit tumors has excited much comment, for it has appeared peculiar in several respects. The simplicity of the substance as compared with most oncogens has seemed to set it apart,—though subcutaneous injections of sugar cause sarcomas to arise in the mouse, and hydrochloric acid thus introduced will do so in rats. The finding has also seemed remarkable that urethane induces no growths except pulmonary adenomas,—though ergot gives rise to neurofibromas only, and Scharlach R to hepatomas, examples which need no longer be cited, now that urethane has been shown to elicit hepatomas as well as adenomas (23). The fact that a single exposure to the substance suffices for neoplastic change has also aroused speculation,—although a single painting of mouse skin with methylcholanthrene results in cancer in some strains of animals. More extraordinary is the finding that pulmonary adenomas arise in the absence of any visible tissue damage. Grady and Stewart (11), noting this of the adenomas induced with methylcholanthrene, were led to ask whether the chronic "precancerous" tissue alterations, which so generally precede the occurrence of tumors as almost to enter into any definition of them, are really essential to their origin. The question is worth asking again.

There remains to consider, of the peculiarities of urethane, the rapidity with which it induces neoplastic change as indicated by the present work. Here a distinction must be made between the preliminaries to such change and its actual occurrence. Though the preliminaries frequently extend over a long time the change itself takes place rapidly, according to all observation with oncogenic agents; it is as if a trigger had been pulled. The generality of agents press gradually upon the trigger; only its eventual click is abrupt. Urethane would seem to pull it hard. But another possibility exists, that the substance merely stimulates the proliferation of cells already neoplastic. Several authors have thought that this is its mode of action. The occurrence of problematic cell clumps in our young control mice might be viewed as supporting such a conception, and the extraordinary rapidity with which adenomas arose in the animals urethanized *in utero* accords well with it. Observations on the point are under way.

According to an axiom now well authenticated through experiment, the greater the natural liability of mice to this tumor or that the more readily can the growth be elicited by the application of an oncogen. Everything that is known of the response of various breeds to urethane falls in with this generalization. Nettleship, Henshaw, and Meyer (3), who first demonstrated that the substance induces pulmonary adenomas, noted also that it elicited many more of the tumors in A mice, the breed most prone to them spontaneously, than it did in C3H animals. Cowen (24) obtained data in R III, CBA, and C57 mice

which accord with this finding as do also the present results with adults of the C strain. Henshaw and Meyer (7) believed that the adenomas began to form within 1 to 2 months after the injection of urethane into 6 to 8-weeks-old animals of the A strain.

From all this one might have inferred that adenomas could scarcely have developed in animals of the C strain only a few days old. But the conditions of test were exceptional. Urethane is so diffusible that it must have reached the embryos in quantity. It was given in the maximum amount tolerable and the exposure of the pulmonary tissue to it was intensive; mice have not previously been injected with it day after day. Furthermore the tumors arose under conditions making for their rapid enlargement; embryos and newborn animals are notably favorable hosts for implanted neoplasms (25), the growing organism providing in abundance the stroma and vascularization that tumors need. And there was a deeper reason why the adenomas appeared so soon and contained mitoses in profusion, namely the state of the cells engaged in producing them. They were already vigorously proliferating to form the lung when they came under the influence of urethane. That they were behaving in this way because of an innate urge has been decisively proven by Cohn and Murray for the chick embryo (26) and is sufficiently attested for the mouse by the continuing growth of fragments of embryo lung after implantation in adult hosts. The adenomatous change superimposed upon the cells such further activity as enabled them to multiply even more rapidly than their normal fellows,—an advantage which became increasingly manifest when the proliferative activity of the latter fell off after birth.

These findings bear on what is implicit in the neoplastic state. Widely various examples are on record of the extraneous stimulation of tumor cells; numerous substances have been found to promote the growth of tumors produced experimentally in animals and one often sees the phenomenon clinically, *e.g.*, after a growth becomes infected with pus-producing organisms, or when testosterone urges on the prostatic carcinomas of man. All such happenings are comprehensible because the agents urging the growths on stimulate normal tissues as well; they act as adjuvant influences merely, and when they are no longer present their influence lapses. The happenings after adenomatous change has taken place in the young of urethanized females stand in a different category. In their case stimulation resulting from neoplastic change is superimposed upon a proliferative activity natural to the cells of the very young organism. Yet again the relationship is not enduring. As the animal becomes mature and its normal pulmonary cells almost stop multiplying, the tumor cells cease to nearly the same extent. Now their only advantage is that which the neoplastic state itself brought with it, and under the circumstances obtaining in the adult animal this suffices for but the slowest proliferation. The tumor cells have remained susceptible to the normal ageing influences. No longer

active, they differentiate until they more or less closely resemble the normal bronchiolar epithelium.

These facts appear to provide a partial answer to the question whether the period in the life of the organism at which cells become tumor cells has any importance for the neoplastic process set up within them. The period has indeed an influence in the case of the growths under discussion. When adenomatous change takes place in the very young organism it increases the activity of cells already possessed of a natural tendency to divide; and for this reason, as well as because of their highly favorable environment, they proliferate more rapidly than the adenoma cells of adults. But the relationship which has this consequence is no more than additive; and the most effective of its factors, those due to youth, soon wane and are gone.

Although the urethane acted upon embryos and the resulting adenomas were perceptible within the first days after birth, this does not mean necessarily that the pulmonary cells became tumor cells *in utero*. The relative size of the growths in the 10-day- and 3-day-old animals, and the failure to find any with certainty in fetuses and animals just born prove that their formation took place almost wholly after birth. But it could scarcely have been otherwise. The longest interval from the first urethane injection to parturition was 11 days (Table 2), and during the first 2 of these the lungs had barely begun to form. Nevertheless when the animal was killed, 3 days after birth, or after 14 days in all, an adenoma of considerable size had developed (Figs. 4 and 5). In no other instance of the sort was the elapsed time so long. The interval before birth in a second 3-day animal with a smaller tumor (Figs. 6 and 7) was 8 days, the total elapsed time 11 days; and in a third mouse, with a still less developed growth (Figs. 9 and 10), 7 days and 10 days respectively. The length of postnatal life was constant in these instances, only the prenatal interval varied. Yet much cannot be made of this circumstance as bearing on the size of the tumors, since the urethane may not have brought about adenomatous change until it had been given several times.

While the evidence is strong that neoplastic conversion was consummated *in utero* the possibility still must be considered that the urethane merely rendered the young animal susceptible to the influence of some extraneous tumor-producing agent reaching it immediately after birth. One thinks of an agent like that responsible for mammary cancer in the mouse, which is passed on from mother to young in the milk. But the data of Table 3 provide no support to this conception, disclosing as they do no correlation whatever between the incidence of adenomas in mothers and their young. The results of four or six injections of urethane are especially noteworthy in this regard; though the mother animals in some of these instances developed multiple adenomas, their young showed almost none. This is the more significant in view of the cumulative effect of urethane to elicit adenomas in adult animals, the rapidity with which these de-

clared themselves in the gross in young animals; and the fact that the offspring of females receiving urethane only once often showed them (Table 3). It would appear that, for reasons unknown, potentialities for the growths were slight or lacking in the embryos of those mice that happened to be many times injected. The existence of small islands of cells resembling the first stages of adenomas, in some of the control baby mice but not in others, may have a bearing on why the growths appeared in only a proportion of the individuals exposed to urethane instead of in all of them.

As already stated, the observations here recorded were made, like others from this laboratory, to learn how soon in the life of the organism cells possess the ability to become tumor cells. Growths arise so rapidly and in such diversity from mouse embryo tissues, after transplantation with methylcholanthrene to adult mice, as to make it well nigh impossible to suppose that extraneous actuators resembling those now known (the tumor-producing viruses), and entering after birth, are responsible for the generality of neoplastic changes. The facts all have indicated that the cells of the embryo possess the ability to become tumor cells (1). The present demonstration that the injection of urethane into pregnant mice causes adenomatous changes to come about so quickly in the young they carry that tumors are perceptible almost at once after birth makes it difficult to avoid the conclusion that the pulmonary cells of mice in mid-fetal development are capable of being neoplastic. This capability would appear to exist earlier in the life of the organism than the one for mammary tumors, which is conferred at the first nursing. Evidently each type of neoplasm must be studied for itself in such relation.

The relative share of the intrinsic and the environmental in neoplastic change is amongst the most deep-going questions in cancer research. Yet only the environmental factors have been inquired into with particularity thus far for the good reason that practically all observations have been made of necessity upon growths coming to attention after birth,—upon those developing in organisms which had passed out from the protection of the uterus into a world in which they were beset by oncogenic agents. Even the hereditary and familial tendencies to tumors, manifest in inbred strains of mice, have been perceptible only in terms of postnatal happenings. Gideon Wells could find but 66 valid instances of tumors in newborn infants, none of them carcinomas (27),—a negligible number, it may be remarked, in comparison with the instances of disease due to viruses reaching human embryos *in utero*. True, hydatidiform moles and chorio-epitheliomas are due to neoplastic changes in a tissue of embryonic origin: but the chorionic cells are not shielded by the natural contrivances whereby the embryo itself is protected; on the contrary they are even more exposed to environmental factors than the majority of the cells of the mother, bathed as they are directly in her blood. The cells composing embryonic rests and the frank embryomas and teratomas from which tumors

take off in later life are not only subject to the same environmental influences as the cells of the host but are exposed to special hazards, often becoming the seat of pathological disturbances which may render them exceptionally liable to neoplastic change.

SUMMARY AND CONCLUSIONS

The observation that adenomas develop very rapidly in the pulmonary tissue of mouse embryos implanted together with methylcholanthrene, in adult animals, has led to tests of the neoplastic potentialities of this tissue *in utero*. C strain females in the latter half of pregnancy were injected with urethane and the lungs of their young were searched for adenomas. None could be perceived with certainty in embryos at term or in mice just born, but they were several times found 3 days after birth and they were frequent and much larger in 10-day-old animals. The controls showed none. After 60 to 70 days they were often visible in the gross. Corroboratory findings were obtained in A mice. No parallelism could be perceived in the incidence of the tumors in mothers and offspring.

The adenomas arose from tissue devoid of any sign of preliminary local disturbance. Mitoses were abundant in them and they grew rapidly for a while, but within 2 months cell division had almost ceased. By this time however many of the neoplasms were as big as any adenomas in the urethanized mother animals and in some instances twice as big. While growing fast they underwent little differentiation, but this took place when proliferation slowed and in consequence the tumors came to have the morphology of the spontaneous and induced adenomas of adults.

The neoplastic cells were derived from alveolar elements, yet in proportion as differentiation of them occurred they came to resemble the epithelial cells lining the small bronchioles. Occasionally the resemblance to bronchial epithelium was complete, save that the cytoplasm of the tumor cells was slightly basophilic.

The following conclusions seem justified:—

1. The injection of urethane into pregnant female mice of the C strain frequently initiates the development forthwith of pulmonary adenomas in the young she is carrying.

2. Some of the pulmonary cells of mouse embryos well along toward term possess the ability to be neoplastic.

3. The adenomatous change finds swift expression in young creatures because of conditions implicit in their youth. The rapid proliferation of the tumor cells is almost entirely due to these conditions, not to the neoplastic state as such.

4. Adenomatous change prior to birth is intrinsically the same process as that occurring in the adult creature.

5. The adenomatous state does not prevent the cells of young mice from undergoing the maturation that takes place in normal elements of the same sort as the organism grows older. Though the proliferative activity natural to youth and the unnatural activity consequent on neoplastic change are summated in the young organism, they still are separable.

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EXPLANATION OF PLATES

The photographs were made by Mr. J. A. Carlile.

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

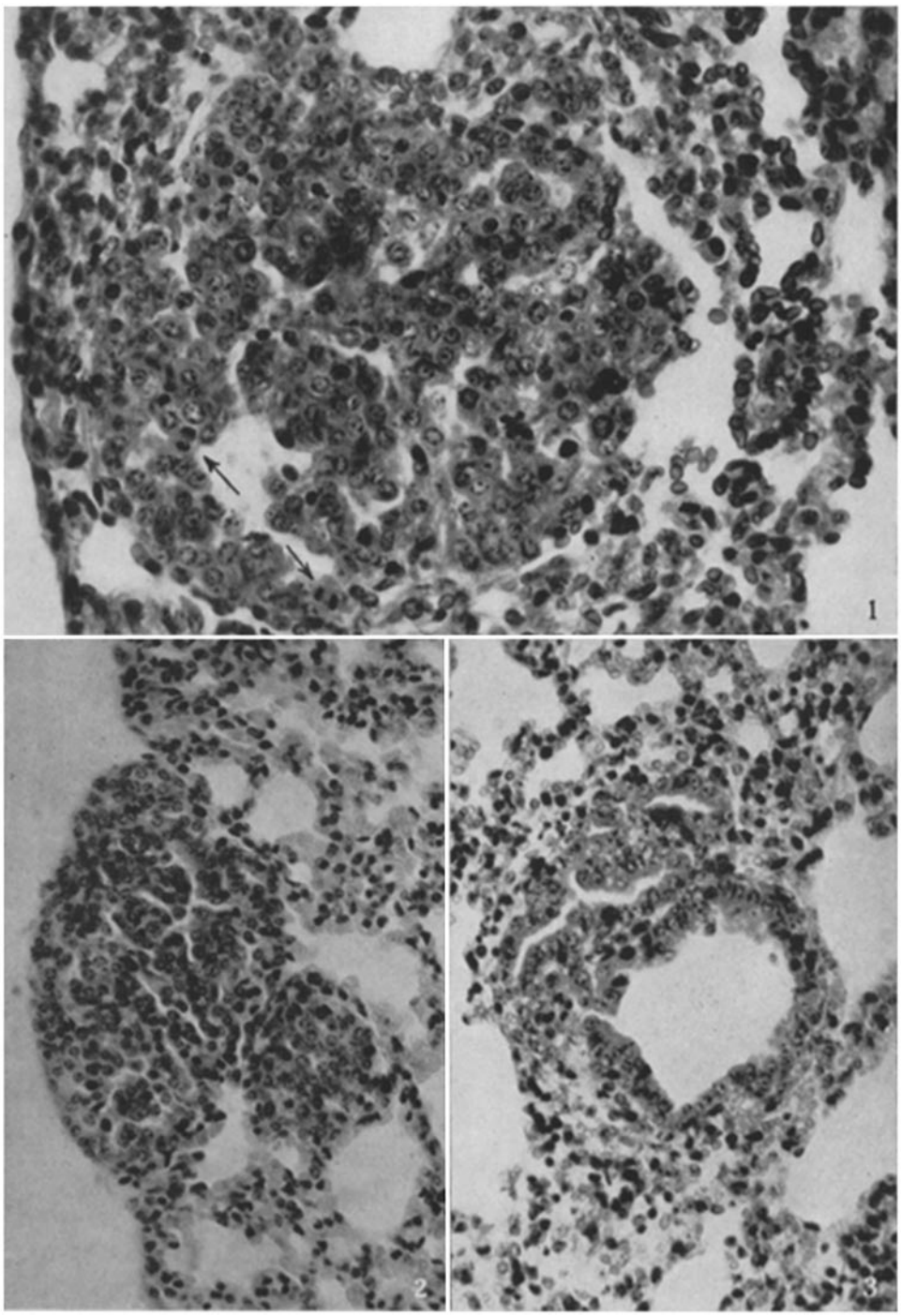
PLATE 24

FIG. 1. Adenoma in a 10-day-old mouse from a mother that had received six injections of urethane, the last within 24 hours of parturition (mother IV of Table 1). The growth lies near the lung surface and has compressed the parenchyma between it and the pleura,—seen on the left. Its cells are more basophilic than those of the parenchyma about them and they nearly all have large, round vesicular nuclei. The arrows point to two of the many mitotic figures. × 505.

For other growths in animals of the same litter see Figs. 3, 9, and 10.

FIG. 2. Adenoma in a 10-day-old animal from another mother getting six injections, the last of them 3 days before parturition (mother VIII of Table 1). The markedly basophilic growth protrudes on the pleural surface. It has filled several alveoli with compact masses of cells but its central portion is adenomatous. Other sections showed mitoses to be fairly numerous in it. × 294.

FIG. 3. Adenoma in a 10-day-old sibling of the mouse furnishing Fig. 1. As in the case of the other early tumors there is no reactive proliferation or cellular accumulation about the growth. It looks as if it had originated from the epithelium of the bronchial tree, but serial sections showed it to be isolated amidst parenchyma. × 321.



(Smith and Rous: Neoplastic potentialities of mouse embryo tissues. IV)

PLATE 25

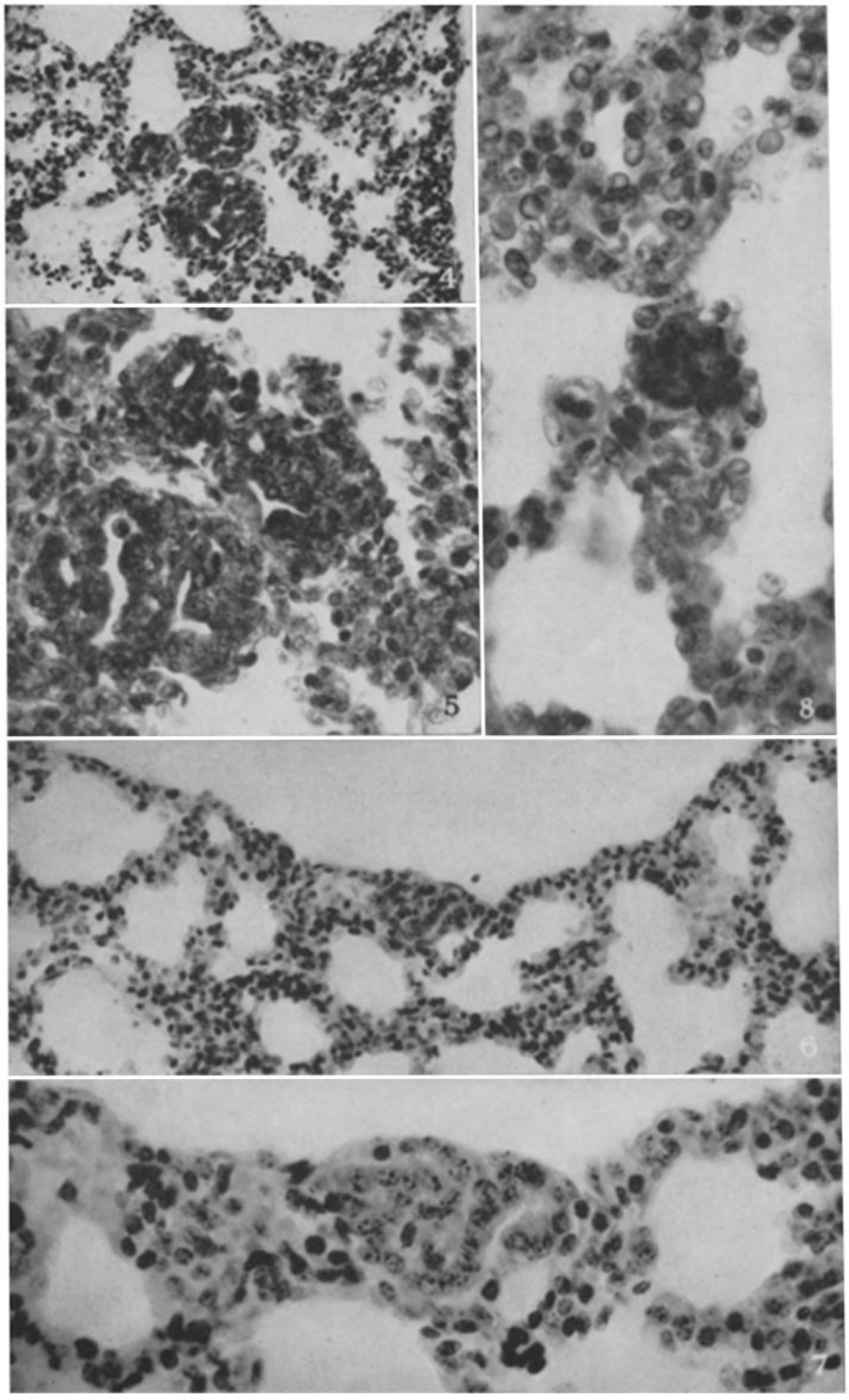
FIG. 4. Adenoma in a 3-day-old mouse born 7 days after the last of four urethane injections into the mother (mother IX of Table 2); total period since the first injection, 14 days. At the level here pictured there appear to be three separate islands of neoplastic proliferation, but they were parts of a single growth. It lay in the parenchyma far from any bronchiole and stained a much deeper blue than the epithelium lining the latter. × 186.

FIG. 5. Higher magnification of the same tumor at another level,—to show its "glandular" arrangement and cuboidal cells. × 401.

FIG. 6. Subpleural adenoma in a 3-day-old mouse born 2 days after the last of six injections of urethane into the mother (mother X of Table 2); total period since the first injection, 11 days. The growth is discrete. × 250.

FIG. 7. The same tumor at higher magnification. It is of typically adenomatous character. There is no cellular reaction about it. × 569.

FIG. 8. Clump of deeply basophilic cells with vesicular nuclei; lung of a 3-day-old litter mate of the animals providing the growths of Figs. 1, 3, 9, and 10. Similar clumps of cells have occasionally been seen in normal C strain mice 10 days old. × 569.



(Smith and Rous: Neoplastic potentialities of mouse embryo tissues. IV)

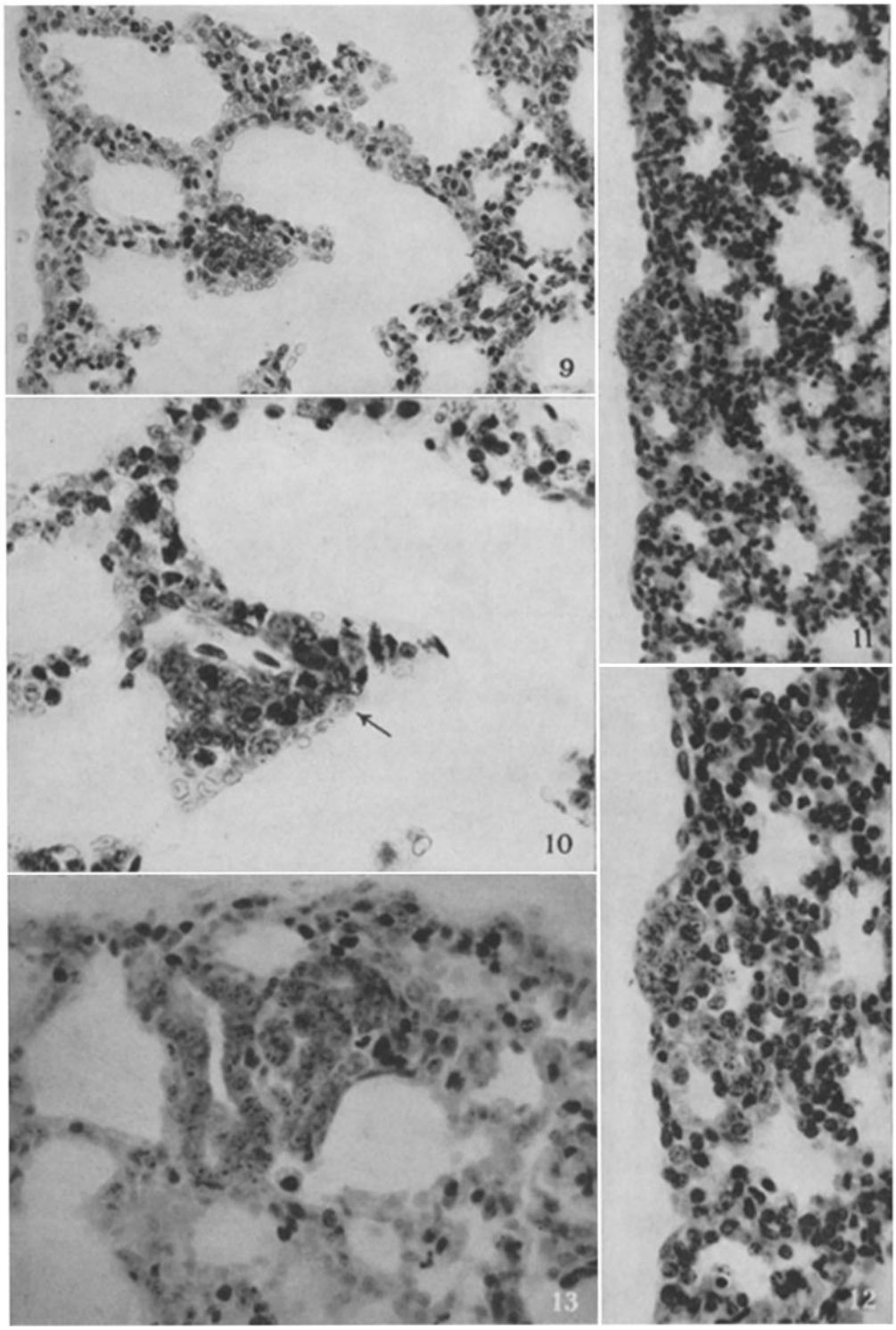
PLATE 26

FIG. 9. Adenoma in a 3-day-old litter mate of the animals furnishing Figs. 1, 3, and 8. The growth lies in the parenchyma and is of the compact type. It stained bright blue against the general pink. × 277.

FIG. 10. Section through the edge of the growth of Fig. 9, where it covers part of an alveolar wall with a single layer of cells. The arrow points toward a mitosis; there were three in the tiny growth. × 527.

FIGS. 11 and 12. Adenoma (?) in the lung of an embryo near term. The mother had received six injections of urethane, the last one 24 hours before the embryo was procured. The growth bulges on the pleural surface, appears to have some acinar arrangement, and its cells occupy part of a neighboring alveolus. The embryonic state of the pulmonary tissue is evident. Fig. 11, × 280. Fig. 12, × 449.

FIG. 13. Adenoma of the glandular type in a 10-day-old mouse from a mother of the A stain which had received three injections of urethane, the last within 24 hours of parturition; total interval since the first injection, 14 days. × 449.



(Smith and Rous: Neoplastic potentialities of mouse embryo tissues. IV)

PLATE 27

FIG. 14. Another "glandular" adenoma from the same animal; there were only the two growths. The tumor is markedly basophilic, as in the previous instance. Some atelectasis is present owing to delay in fixation of the lung after it had been removed from the animal. × 422.

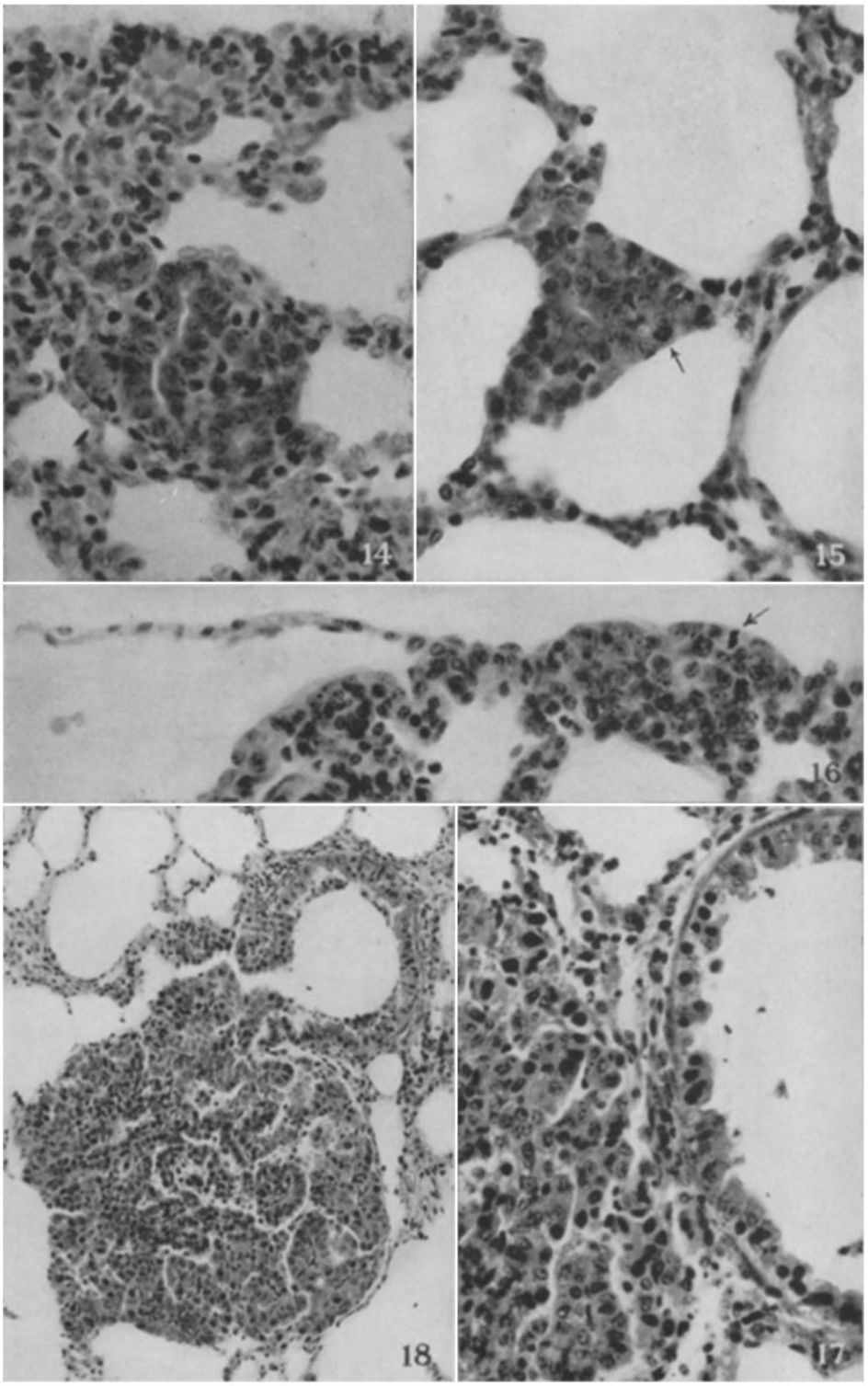
FIG. 15. Compact adenoma in a 10-day-old mouse from another female of the A strain injected thrice with urethane, the last time within 24 hours of parturition. The growth shows the usual basophilia. The arrow indicates a mitotic figure. × 422.

FIG. 16. Mooring clump with membrane attached near by; from a day-old mouse of a C strain mother. Serial sections showed the clump to be spindle-shaped and almost 300 μ long. The arrow points to a mitosis. × 422.

FIG. 17. Peripheral zone of an adenoma, with part of an adjacent bronchiole, in a mouse killed 70 days after birth of her offspring. She had received three injections of urethane during pregnancy.

The growth differs considerably from those in sucklings. Interspersed among cells with round, vesicular nuclei, such as make up the tumors in these latter, are many with nuclei that are oval or oblong and almost pyknotic, often relatively large and sometimes with more cytoplasm about them than ordinary. In one spot near the lower edge of the picture nuclei of this sort lie with their long axes parallel, and the cells appear cylindrical, as if from lateral compression. The epithelium of the bronchiole has precisely the same general morphology as the component elements of the tumor except that some of its cells are more cylindrical. × 278.

FIG. 18. Low magnification of the same tumor, to show where it has filled a space into which the bronchiole opens. Though its cells closely resemble the epithelium of this latter the two are not joined. At the center of the growth, where degeneration was under way, the cytoplasm had a blue cast on staining, but at the periphery it was almost as pink as the bronchiolar epithelium. × 136.



(Smith and Rous: Neoplastic potentialities of mouse embryo tissues. IV)

PLATE 28

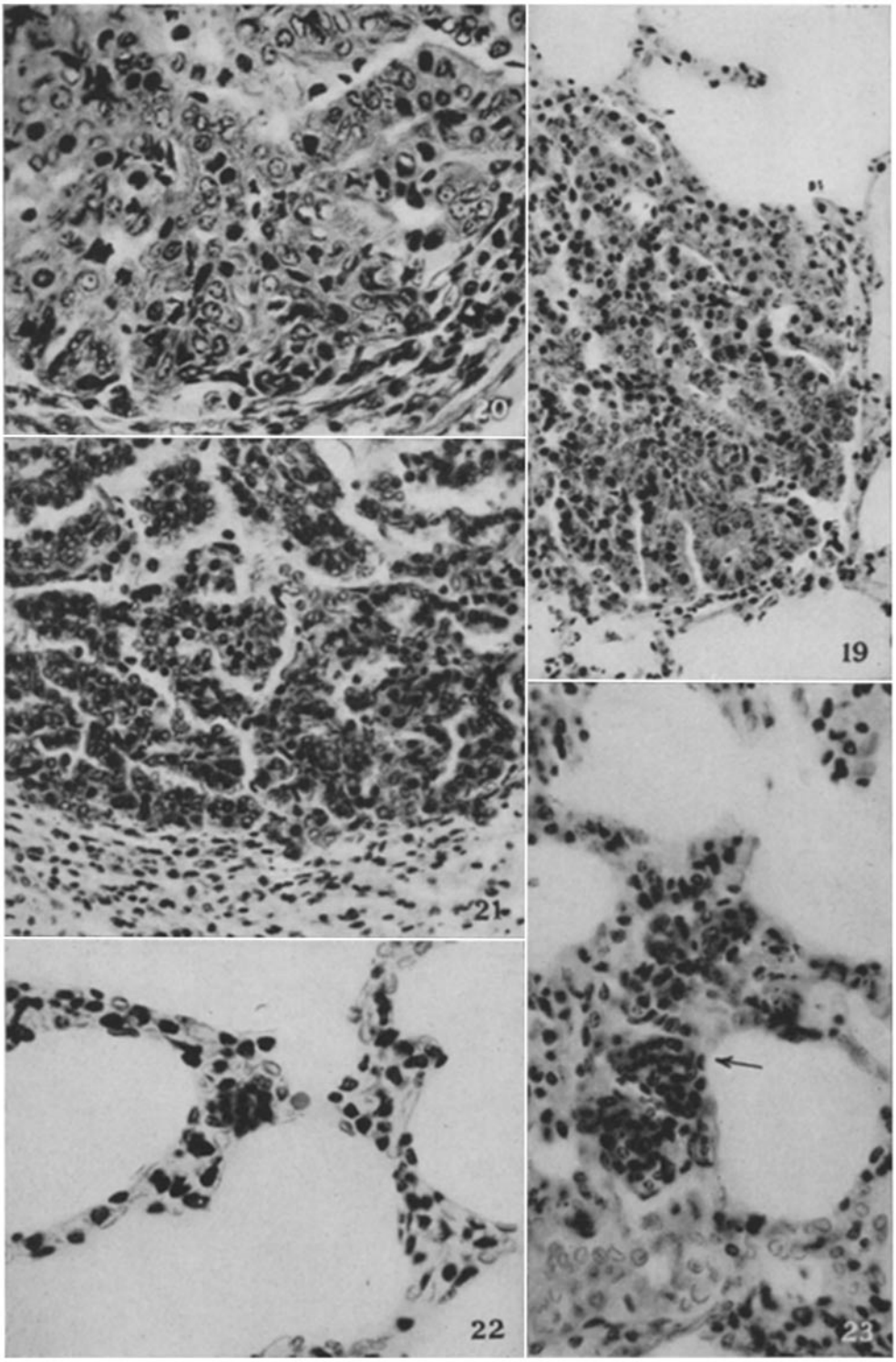
FIG. 19. Another adenoma from the same animal as that of Figs. 17 and 18 and with the same cytological features. It was not directly connected with any bronchiole. × 243.

FIG. 20. To show the "bronchiolar" character of the cells of an adenoma found in an animal 70 days after birth from a urethanized mother (mother G of Table 3 and 4). Though unusually large (1.5 mm. in diameter) the growth was devoid of mitotic figures. The tissue next its border is compressed. × 449.

FIG. 21. Edge of another exceptionally large adenoma (2 mm. across) in a 70-day-old mouse from a urethanized mother (mother A of Tables 3 and 4). The growth is markedly basophilic and differentiation is not as far advanced as in the tumor of Fig. 20 yet many dark-staining nuclei are present, of the sort found in mature adenomas. Mitotic figures were rare (Table 4). × 277.

FIG. 22. Clump of cells in an alveolar wall of a normal 3-day-old mouse of the C strain. The cells are notably basophilic, as in the case of adenomas, but the nuclei were smaller than in these latter and were not vesicular. × 527.

FIG. 23. Dubious growth in another 3-day-old control of the C strain. The cells are basophilic and at one spot they lie in two parallel rows (arrow), but many of the nuclei are oblong and they are unusually small. × 449.



(Smith and Rous: Neoplastic potentialities of mouse embryo tissues. IV)