AGE OF THE HOST AND OTHER FACTORS AFFECTING THE PRODUCTION WITH URETHANE OF PULMONARY ADENOMAS IN MICE

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The discovery that urethane induces pulmonary adenomas in mice of breeds liable to such growths spontaneously (1) has led to extensive work. The themes studied have lain mainly in three categories: genetic as conditioning the neoplastic response, tumor-inducing power of urethane as influenced by modes of administration, and comparison of its effects with those of related substances. Behind all these investigations, needless to say, has stood the problem of how urethane acts. The present work is concerned mostly with factors affecting the neoplastic response as they bear on this basic question. Among other facts it will be shown that the age of the mouse has a large determining influence on the incidence and yield of tumors; that urethane does not act by promoting the multiplication of cells already in the adenomatous state, but acts solely by inducing this state; and that a second exposure to the substance after a considerable interval results in a greater number of adenomas than if the effects of the two had been merely summated. Colchicine, though checking cell division and causing various nuclear abnormalities, fails to influence the yield of tumors to urethane given concurrently. Fasting is also devoid of effect, despite its well known mitosis-inhibiting influence.

Materials and Methods

Animals of the Swiss breed, originally procured from the Swiss stock of Dr. Clara J. Lynch and since bred in the Rockefeller Institute, were mainly employed. These mice show nearly as high an incidence of spontaneous adenomas as the A strain, which was also used (2). The A strain has the greatest spontaneous liability to adenomas of any known and is the one most responsive to urethane. The C strain employed in previous work on adenomas in this laboratory (3) was utilized for corroboratory experiments. Its incidence of spontaneous adenomas is quite low, animals 6 months old having none, and only about one year-old animal in six showing any, usually a solitary growth, rarely two or three. Large colonies of all the strains were available.

The mice were fed bread and milk and Purina fox chow, with carrots and lettuce once a week. An excess of food and water was provided at all times, unless otherwise stated. The urethane (ethyl carbamate, C. P., Eimer and Amend) was recrystallized prior to use; its melting point lay between 49.5° and 50°C. It was injected intraperitoneally as a 5 per cent solution in double distilled water. Colchicine (U. S. P., Amend Drug and Chemical Co., Inc.),

was used in isotonic saline at the concentrations mentioned in the protocols, and introduced subcutaneously in the back.

The mice of each group were marked by clipping the ears. Tannenbaum (4) has shown that caloric restriction has a pronounced effect to prevent or retard the appearance of spontaneous adenomas, and for this reason the weights of the animals were followed throughout every test and have been recorded in most of the charts. Any mice which lost considerably were discarded. Also the individuals of each group were matched as to weight in all the experiments except those involving groups of differing ages. Only the results with mice surviving to the end of the test period figure in the charts. The animals were killed by decapitation, and in accordance with general practice the adenomas visible in the gross on the lung surface were counted as an index to their frequency. Their size was checked against a millimeter rule, and none less than ⅛ mm. across was recorded. In the charts they are set down as round because they had the appearance of bulging circular discs. Frequent microscopic sections of the tumors controlled the gross findings,—which were noted down without knowledge of the group from which each mouse came.

Influence of the Age of the Host

Previous investigators have shown that the injection of urethane into pregnant mice is followed by the appearance of adenomas in their offspring (5, 3). So rapidly do the growths form as sometimes to be recognizable within 3 days after birth, all the evidence indicating that their proliferation begins *in utero* (3). During the first weeks of postnatal life mitoses are numerous in the tumors, whereas they are remarkably few in the adenomas of adults, as previous workers agree. Hence it seemed likely that large changes in the response to urethane would take place while young animals were maturing. The experiment which follows proves this to be the case.

Experiment 1.—Five groups were used of 40 male Swiss mice each, 2, 4, 6, 8, and 10 weeks old respectively. The groups were kept separate, 20 to a box during the first 12 days after injection with urethane, and 10 to a box later on. 15 of each 40 animals served as controls; they were kept in the same boxes as the other 25 which were given 1 mg. urethane per gm. mouse, into the peritoneal cavity. All were killed 7 weeks later and the adenomas counted. The findings are set forth in Chart 1A.

Chart 1A shows that every animal of the 2-week-old group developed adenomas, whereas some in the other groups failed to show them, the proportion of negative animals increasing steadily with age until, in those 10 weeks old when injected, about 25 per cent had none. Not only did the incidence markedly lessen, but the number of tumors per animal fell off to an even greater extent.

Many uncontrolled variables entered into the test as reported. Differences in relative weights of the intestinal contents in the various age groups, as affecting the influence of the urethane given, provided an obvious possibility of error. To check upon this, the intestinal contents of several 2-week-old and 10-week-old mice were weighed individually at the same times after feeding; the differences in relation to the total body weight proved negligible. One could not be

Age as Affecting Yield of Adenomas to Urethane

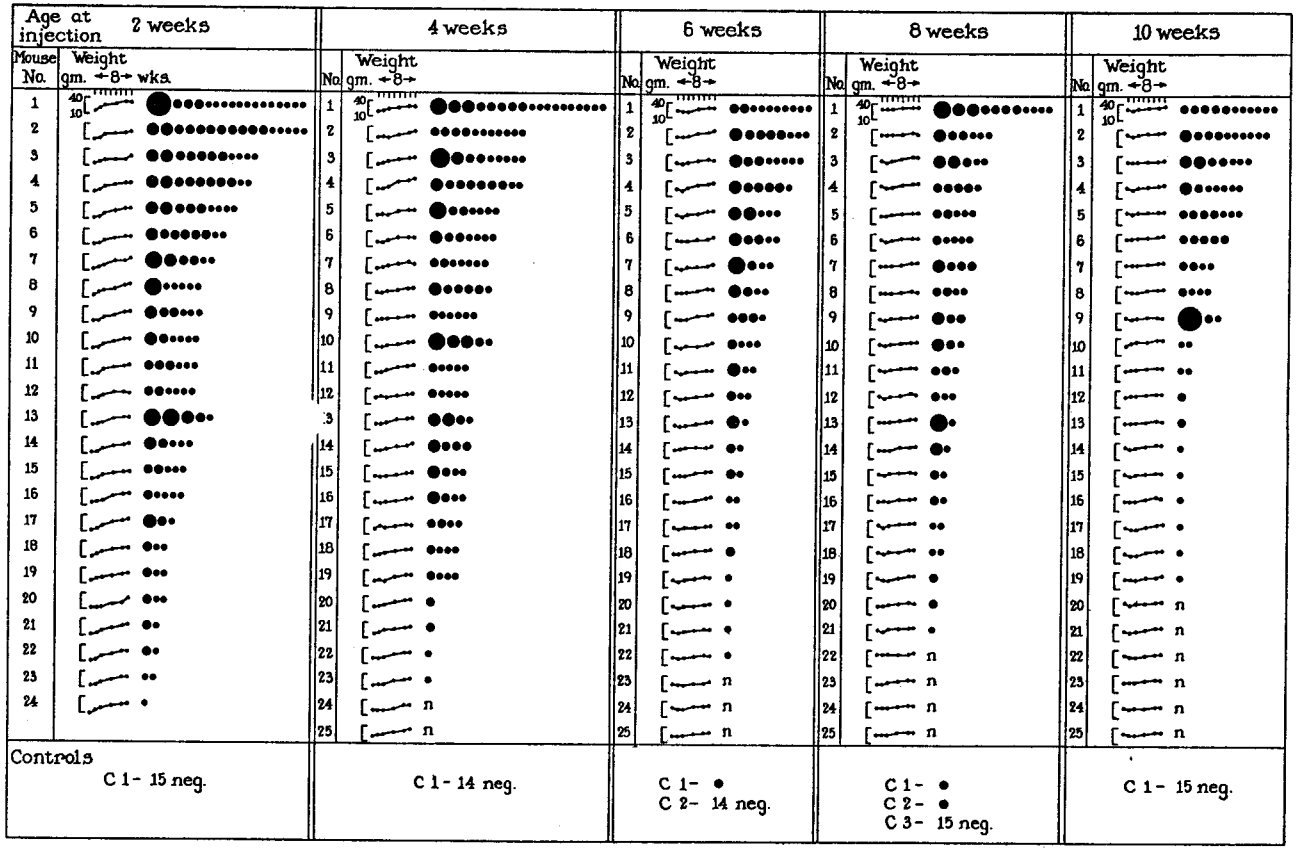


CHART 1A

certain that the peak concentration of urethane was the same in the different age groups or that the substance remained in the blood for the same length of time; but good presumptive evidence in these respects was provided by the depth of anesthesia, which was approximately the same from group to group, both in degree and in the time it lasted.

Smith and Rous (3) found that the adenomas of suckling mice consisted of undifferentiated cells showing many mitoses, and that they grew with great rapidity, whereas those of mature animals contained almost none, and furthermore had in many instances differentiated, coming to look like the cells lining the bronchioles. They concluded from this and other evidence that the natural impulse to grow had far more to do with the activity of the tumors in young sucklings than the unnatural urge consequent on the neoplastic state. One might expect that, if this were the case, the response to urethane, as measured in terms of adenomas reaching a size perceptible in the gross, would fall off in proportion as the rate of growth of the pulmonary tissue slowed while maturation was going on. To learn whether this actually happened the lungs of twenty animals 2, 4, and 6 weeks old, and of fifteen 8 and 10 weeks old respectively were freed of mediastinal structures and weighed, pooled in batches of five. Chart 1B gives the findings in detail. It will be seen that the lung weight increased rapidly between the ages of 2 and 6 weeks, but only a little thereafter. The chart further discloses an almost perfect inverse relationship between the increase in lung weight with age and the total number of adenomas found in the various age groups of Experiment 1.

The next test was devised to learn more of the changes in the responsiveness of growing mice to urethane. Weanlings and young adults were employed in three groups of each, given different amounts of urethane.

Experiment 2.—150 male Swiss mice were used. Groups A, B, and C, of 25 animals each, were 3 weeks old, and groups D, E, and F, 8 weeks old. Groups A and D received a single injection of 0.25 mg. urethane per gm. mouse, groups B and E, 0.5 mg., and groups C and F, 1 mg. The animals were kept in boxes of five, matched as to weight, and in every box there was at least one mouse from each of the groups of its age. All were killed 9 weeks after injection.

Chart 2A corroborates and extends the findings of Chart 1A. Only a few of the adults getting 0.25 mg. urethane per gm. mouse showed any tumors, whereas nearly all the weanlings had them. The differences in the percentage of negative animals after the larger amounts of urethane did not accord with the clear-cut findings of Experiment 1, and were probably accidental. The total number of adenomas developing in the animals 3 weeks old at injection was much greater in all three groups than in those 8 weeks old. The contrast was most striking in those receiving the least urethane.

Again as in Experiment 1 the possibility comes up that differences in the peak concentration of urethane in the mice of differing ages, and in length of

time that the substance remained in the blood stream may have had to do with the results. In tests to be described further on these factors are effectually excluded. The present experiment yielded some evidence in the matter. None of the mice receiving 0.25 mg. of urethane per gm. body weight appeared in

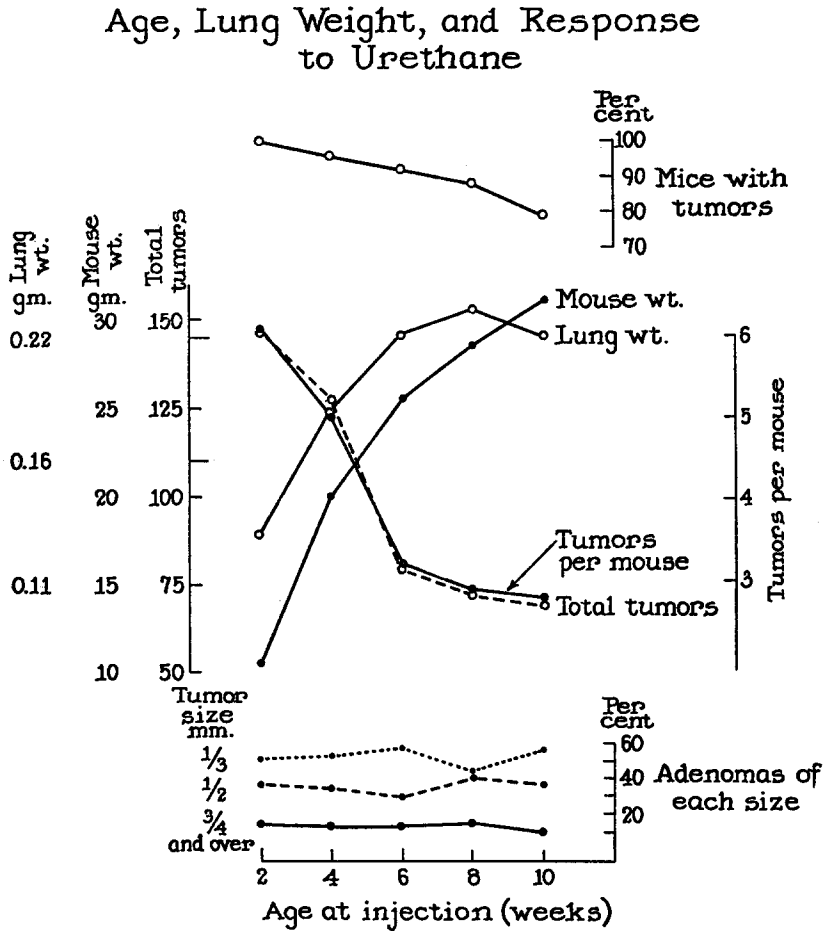


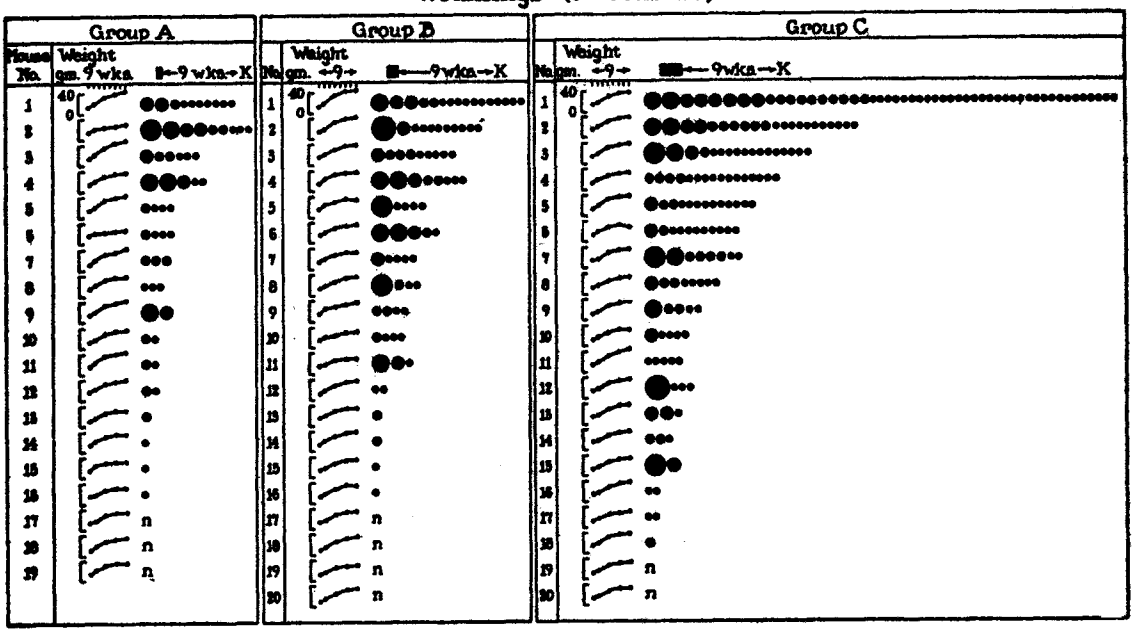
CHART 1B

the least anesthetized. After 0.5 mg. the animals of both ages were groggy for approximately the same length of time, and after 1 mg. the greater proportion of both became unconscious, though some could be roused by tweaking.

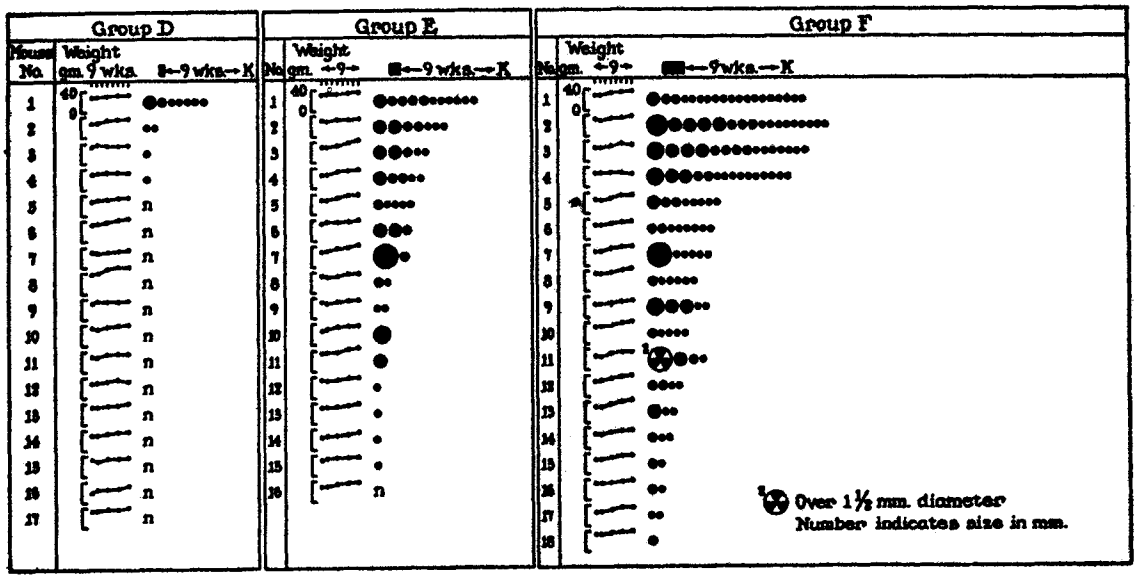
It had been expected that the young animals of Experiments 1 and 2 would have larger adenomas when killed than those which were oldest at time of injection, owing to the greater normal tendency of their cells to proliferate during

Age, Dose, and Response to Urethane

Weanlings (3 weeks old)



Adults (8 weeks old)



▪ = 0.25 mg. urethane per gm. mouse
■ = 0.5 " " " " "
■■ = 1.0 " " " " "

CHART 2A

the weeks subsequent to urethane injection. But no certain differences in this respect could be noted (Charts 1B and 2B). No reliance can be placed on the divergence from the general findings noted in the adults receiving 0.25 mg. (Chart 2B), because of the paucity of tumors.

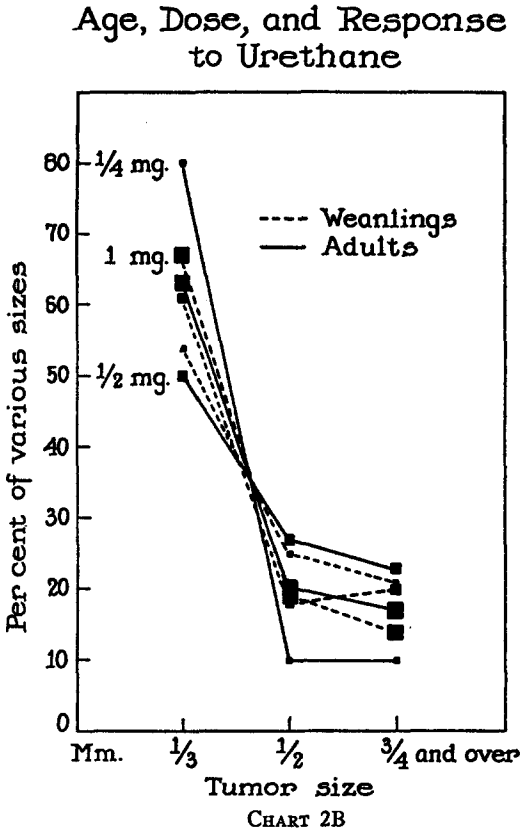


CHART 2B

Influence of Peak Concentration of Urethane and Period in Circulation

The fact is well attested (6) that, no matter how given, by mouth, parenterally, or intraperitoneally, urethane induces adenomas and has furthermore a cumulative effect. Minute quantities taken in the drinking water day after day result in numerous growths, whence it follows that peak concentration cannot be of crucial significance in eliciting them (6).

The next experiments were undertaken to learn whether large immediate differences in this respect, and of time in the blood stream would affect significantly the number and size of the tumors induced.

Experiment 3.—60 male Swiss mice 2 to 3 months old were separated into two equal groups and distributed 15 to a box, with 7 or 8 of each group in each box, matched individually as to weight. Group A received a single intraperitoneal injection of 1 mg. urethane per gm. mouse. Group B received four intraperitoneal injections of 0.25 mg. per gm. mouse, at 4½ hour intervals, the first injection at the same time as the single injection of group A. This last caused the mice to become unconscious for a few hours as usual, whereas the four injections of group B produced no discernible changes in behavior, though the same total quantity was given. The body weights did not alter significantly in either case (Chart 3). After an interval of 9 weeks the mice of both groups were killed and the adenomas recorded as usual.

Chart 3 shows that the same proportion of mice developed tumors in both groups of animals. This was the more remarkable because the peak concentration attained in the animals receiving the urethane at a single injection was much higher, a fact sufficiently shown by their becoming unconscious, whereas the mice receiving the substance on four occasions appeared unaffected. The latter developed more tumors, but this finding was not confirmed in the experiment next to follow. The size of the tumors was the same in both groups.

In Experiment 4 the peak concentration was again varied and the interval between successive injections was extended.

Experiment 4.—75 male Swiss mice 2 to 2½ months old were matched individually as to weight and separated, five to a box, into three groups, with at least one mouse of each group in every box. The mice of group A received a single injection of 1 mg. urethane per gm. mouse. Those of group B, two injections of 0.5 mg. per gm. mouse, the first at the same time as group A and the second 5 hours later. The mice of group C received two 0.5 mg. injections with 24 hours between, the first of these at the same time as group A. After 8 weeks the mice were all killed. The findings are shown in Chart 4.

Again the behavior of the mice testified to wide differences in the peak concentration of urethane. The mice receiving a single injection of 1 mg. per gm. mouse all became unconscious. The first dose, in those getting 0.5 on two occasions, reduced the activity of the animals considerably, though they did not become groggy, and the second, given 5 hours later, caused definite grogginess, enough urethane apparently remaining in the body for it to have had a cumulative effect; but when 24 hours had elapsed before the second dose (group C) this had no more effect upon the activity of the mice than if none had been given previously.

The differences in incidence, number, and size of adenomas recorded in Chart 4, are within the limits of error, and the findings, when considered together with those of Chart 3, make plain that neither the peak concentration nor the length of time over which a given amount of urethane was administered (within a limit of 24 hours) had any significant effect on the outcome in terms of tumors. Data on the effect of a longer interval will be presented further on.

Does Urethane Promote the Growth of Adenomas?

Many agents which bring about tumor formation are not oncogenic in the real sense, that of converting normal cells into tumor cells, but act merely by

The Response to Urethane as Determined by Amount and Peak Concentration

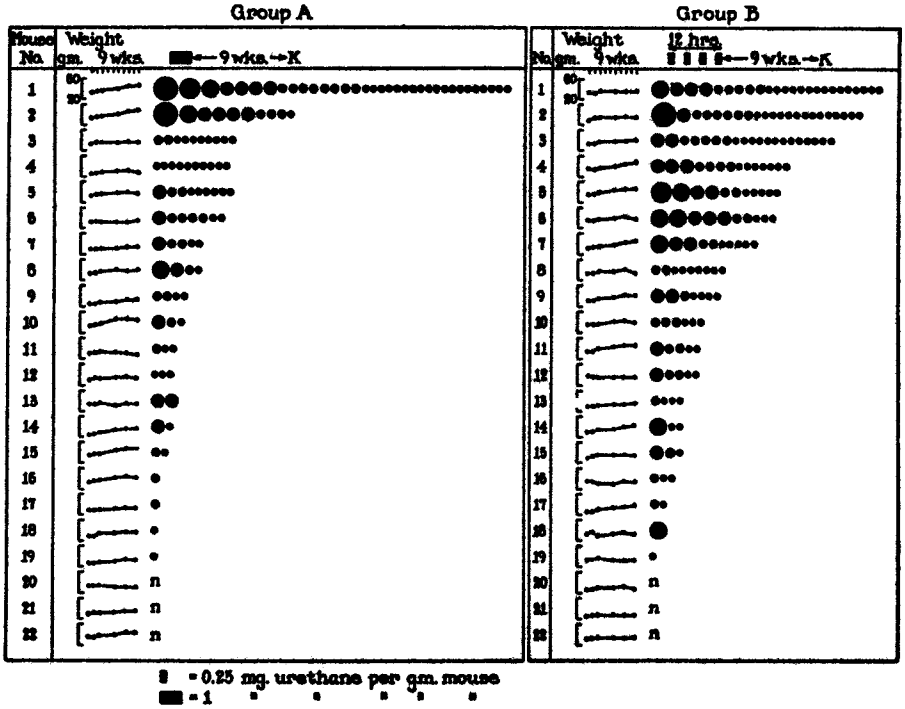


CHART 3

The Response to Urethane as Affected by Amount, Peak Concentration, and Length of Exposure

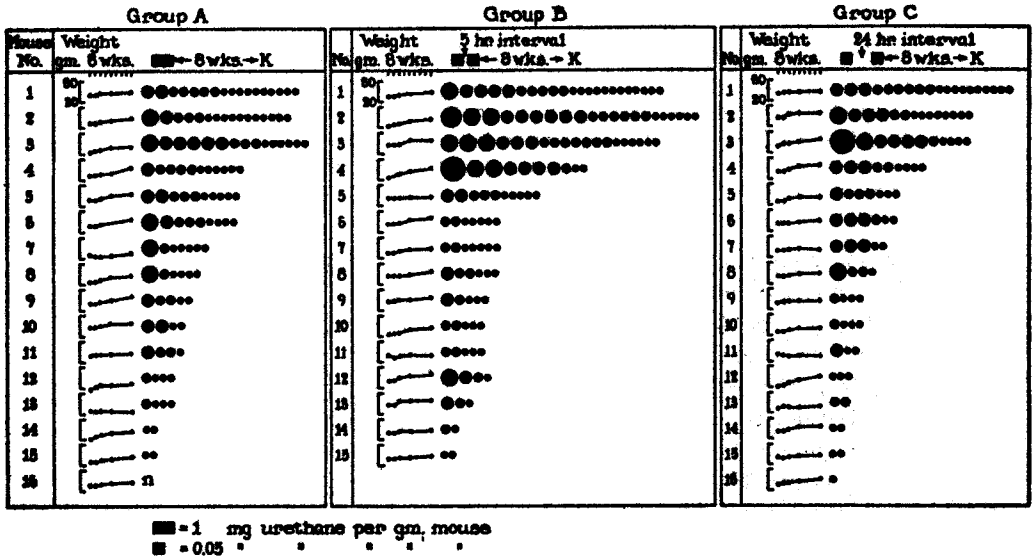


CHART 4

stimulating the proliferation of elements already in the neoplastic state for one reason or another, but nevertheless requiring aid if they are to proliferate into visible masses (7). This had been found to hold true of cutaneous (7), thyroid (8), and mammary growths (9). The influence of urethane might conceivably be a promoting sort, either partially or wholly. If so, one would expect that repeated injections of an adenoma-producing amount of urethane would not only cause more adenomas to become big enough to be visible in the gross, but would result in a generally larger size. The following experiments were done to learn whether this is the case.

Experiment 5.—48 male strain A mice between 8 and 12 weeks of age were matched as to weight and divided into groups, distributed six to a box. Groups A and B received two injections of urethane intraperitoneally (1 mg. per gm. mouse), with 1 week between. 2 weeks after the second injection, the animals of group B received a third, and a fourth after 1 week more. 8 weeks after the last injection of group B the mice were all killed and their adenomas recorded.

Chart 5A tells the result of this test. In group B, which received four injections, every animal had adenomas, large numbers in many instances, yet the maximal size of these was not significantly greater than that of the growths of group A, which had received its two injections on the same days as the first two of group B. True, there were more large adenomas in this latter group, but only proportionately to the total number induced, as calculation showed.

The interval before the third and fourth injections into group B had been so short as to bring up the possibility that a stimulating influence of the earlier injections of urethane might not have worn off when it was again brought to bear, and that it failed to enhance this already existing influence. Hence, in the following experiment the interval was extended to 7 weeks.

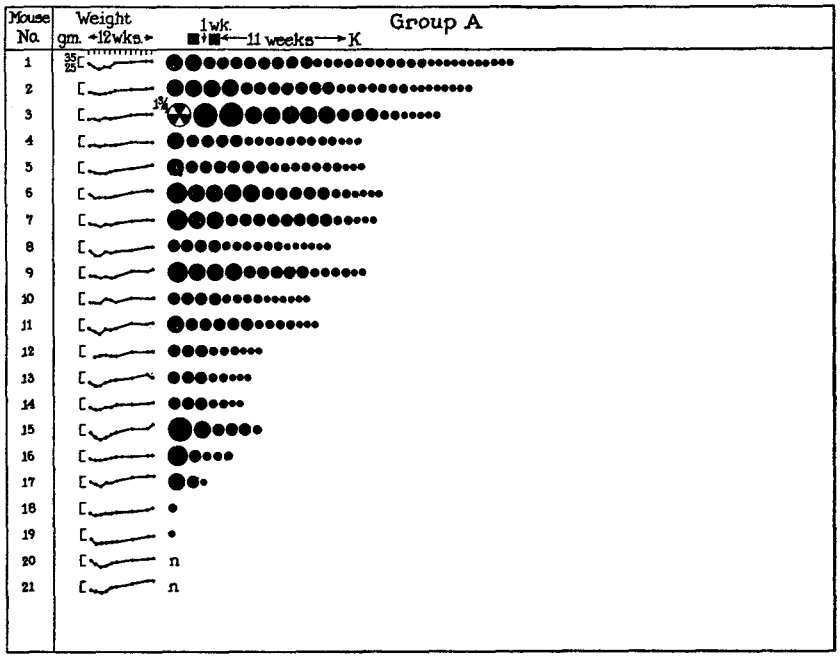
Experiment 6.—54 male strain A mice 9 to 13 weeks old were matched individually as to weight, marked as belonging to two groups, and placed in boxes of five with at least two or three mice from each group in each box. Group A received three injections of urethane (1 mg. per gm. mouse) intraperitoneally with a week interval between. Group B received three similar injections at the same time, and 7 weeks after the last one a second series of three injections. 8 weeks after the final one the lungs of both groups were examined. The results are presented in Charts 6A and 6B.

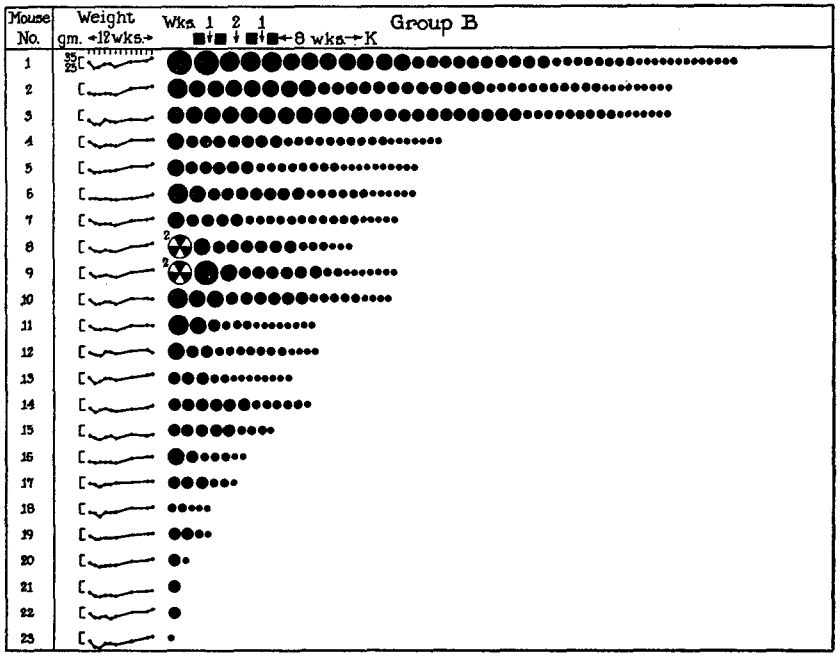
It will be seen that, as in Experiment 5, the second course of injections failed to affect the maximum size attained by the adenomas.

As already remarked, mitoses are notably rare in the large adenomas of adults and often much differentiation has taken place, facts indicating that they are indolent. Conceivably under such circumstances a repetition of the urethane injections which had first called them into being and stimulated their growth might fail to urge them further. Smaller adenomas might well prove more responsive. Hence the figures for all of the growths were analyzed to learn whether they yielded any evidence of stimulation (Charts 5B and 6B).

Does Urethane Promote the Growth of Adenomas ?

I





■ = 1 mg. urethane per gm. mouse

CHART 5A

It will be seen that not only were the adenomas measuring ¾ mm. and 1 mm. or over proportionately no more frequent in the animals getting four or six injections than in those receiving only two or three, but that the percentages of those of smaller size were essentially identical throughout the entire range recorded. Evidently the urethane had no effect whatever to promote the growth of the adenomas it induced.

This was in A mice. To extend the observations a test resembling Experiment 6 was carried out with nearly 200 mice of Swiss strain, 9 to 11 weeks old when first injected. The animals were so many and the induced adenomas were found to be so numerous when they were eventually examined that they had to be killed in batches over the course of 4 successive days instead of all at once;

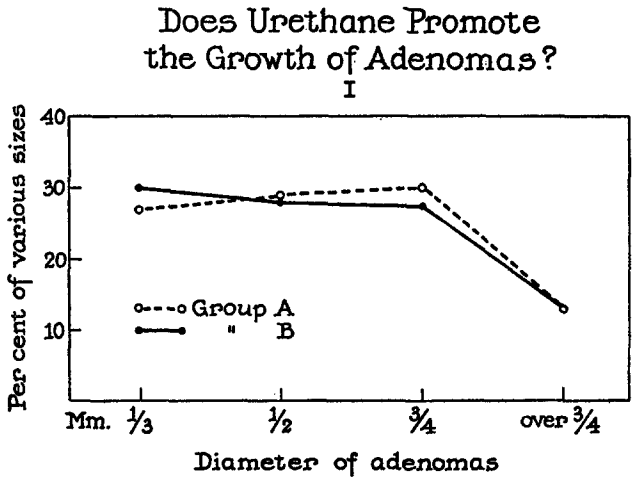


CHART 5B

an approximately equal number from each group were killed on each day. An outline of the experiment and its results are given in Table I.

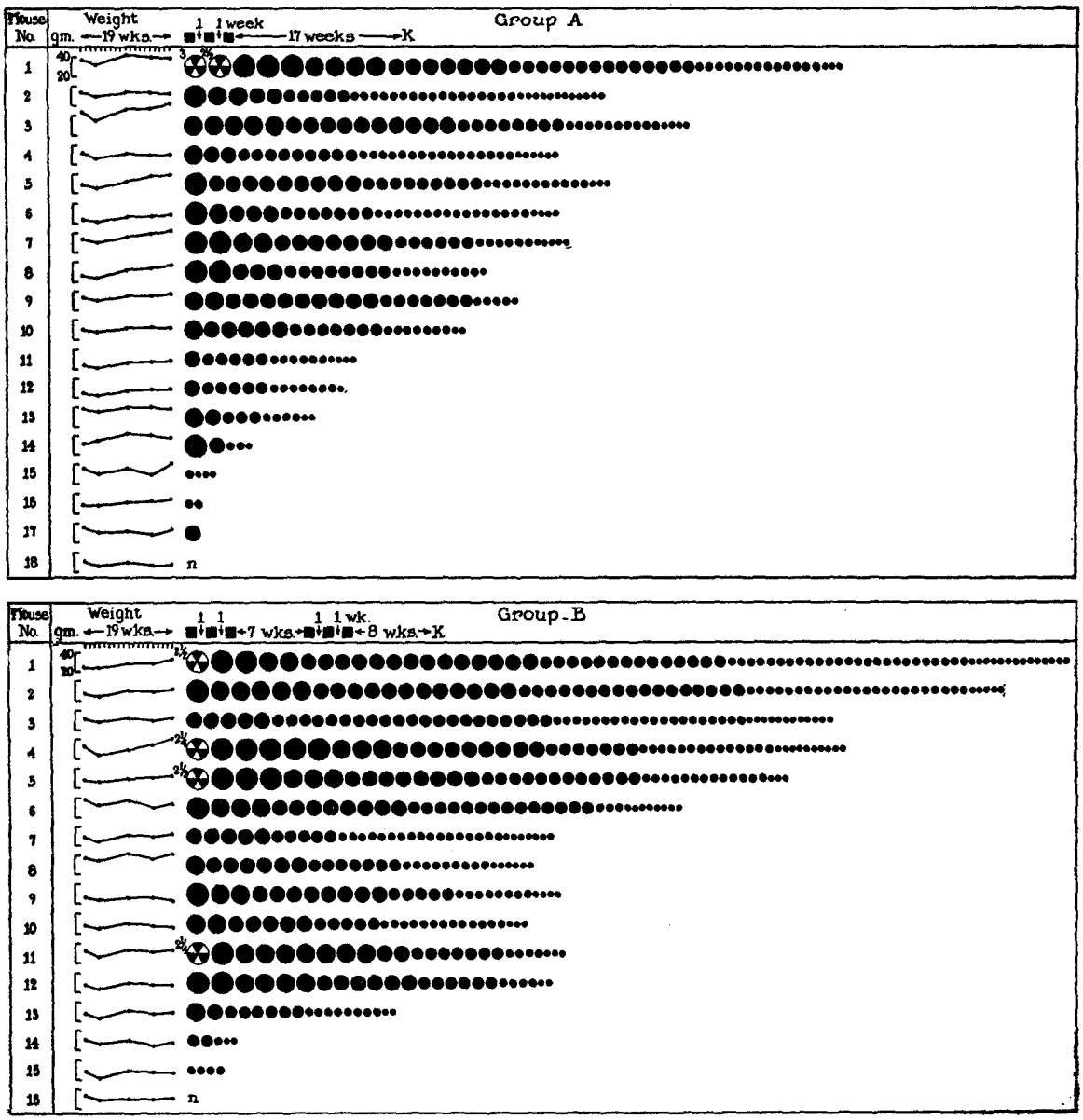
Again it is evident that the urethane had no promoting influence upon the growth of the tumors. This fact is further shown, if in a collateral way, by the findings in Experiment 2, as analyzed in Chart 2B. It will be seen that the larger doses of urethane did not elicit any bigger adenomas.

The Nuclear State and the Response to Urethane

Urethane is well known to be a nuclear poison, producing changes in the cells of intestinal crypts (10), carcinomas (11, 12), bone marrow (13), leukemias (13), and lung (14), as also in cardiac fibroblasts (12), changes which include pathological pycnosis, polyploidy, mitotic inhibition, and karyorrhexis, as well as lesser abnormalities. Loveless and Revell have recently reviewed the

Does Urethane Promote the Growth of Adenomas ?

II



■ = 1 mg. urethane per gm. mouse

CHART 6A

literature on this subject (15). It seemed worth while to learn whether another nuclear poison, colchicine, would induce adenomas, and further, whether the

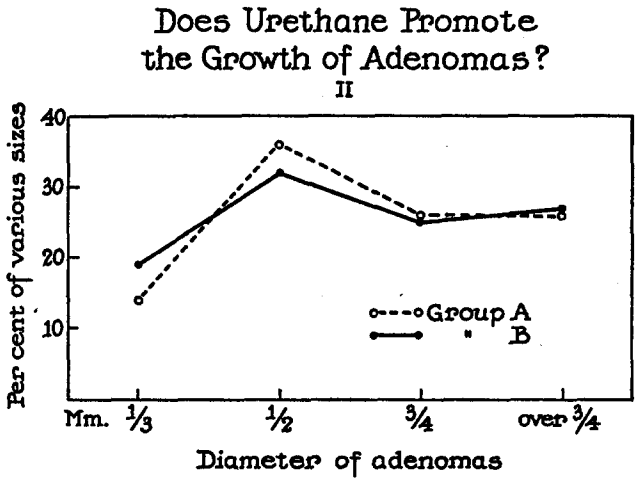


CHART 6B

changes it brings about would influence the response of pulmonary cells to urethane. The following experiment bore on the first of these points.

Experiment 7.—In a preliminary test with a solution containing 0.2 mg. of colchicine per cc. normal saline, 0.004 mg. per gm. mouse had been found to be the maximum tolerated by the majority of young adult Swiss mice. 100 males of this breed, 9 to 12 weeks old, were then matched individually as to weight, distributed five to a box, and marked as in one of two groups. The larger group of 60 were given 0.004 mg. colchicine per gm. mouse on two occa-

TABLE I

Does Urethane Promote the Growth of Adenomas?

III

Group	No. of mice		Adenomas								
			Total	Under 1 mm.	Over 1 mm.	1 mm. and over					
						1	1-1½	1½-2	2-3	3+	
A	95	1 1 17 weeks ■ ■ ■ ←——→ K	4563	3987	576	368	158	33	11	5	
				87%	13%						Per cent of total number
						64%	27%	9%			over 1 mm.
B	92	1 1 7wks. 1 1 8wks. ■ ■ ■ ←→ ■ ■ ■ ←→ K	5850	5199	651	403	201	31	11	5	
				89%	11%						Per cent of total number
						62%	31%	7%			over 1 mm.

■ = 1 mg. urethane per gm. mouse

sions, subcutaneously in the back, with a 1 week interval between them. The remaining 40 mice served as controls. 16 of the injected animals died from the effects of colchicine,—which included bleeding into the bowel, intestinal obstruction, ulceration at the site of injection, with subsequent infection, and kidney lesions with gross hematuria. Stained sections showed that all mitoses in the testis and jejunal crypts of the animals had been blocked in early metaphase. The surviving mice were killed after 6 months and their adenomas counted, but not measured for size.

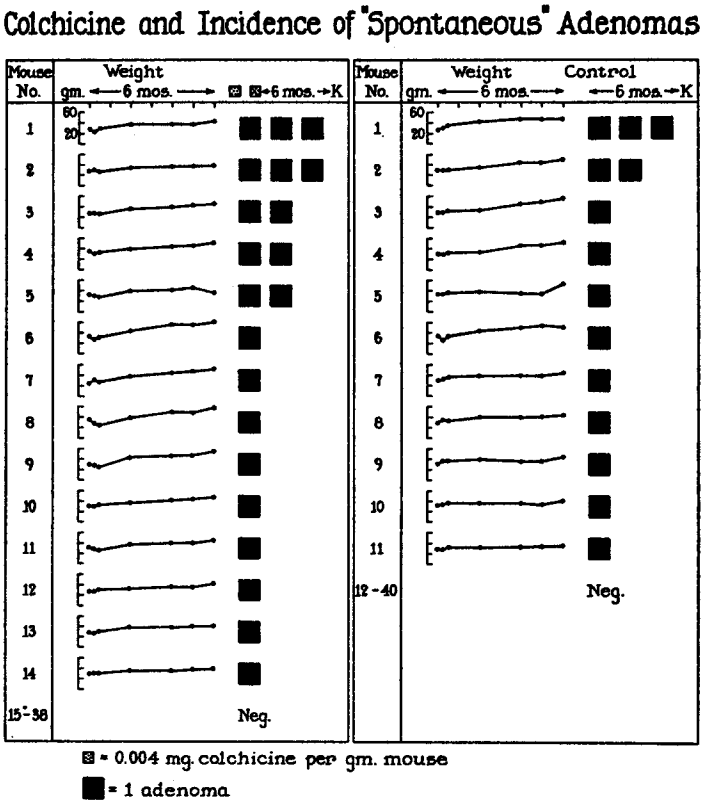


CHART 7

From Chart 7 it is evident that colchicine given in the maximal amount tolerated had no effect upon the occurrence of "spontaneous" adenomas. With this known an experiment was next undertaken to find whether the substance influenced the yield of the tumors to urethane.

Experiment 8.—200 male Swiss mice 9 to 13 weeks old were used. They were divided into four groups matched as to weight, and kept eight to a box, with every box containing some individuals of each group. Group A received colchicine 24 hours prior to 1 mg. of urethane per gm. mouse; group B received only the urethane; group C, urethane 24 hours prior to

colchicine; and group D, colchicine 24 hours before and again 24 hours after urethane. 0.002 mg. colchicine per gm. mouse was injected each time,—less than in Experiment 7 because it had been found out that animals receiving this amount and given urethane 24 hours later were so hard hit as to be sickly for some days. Even 0.002 mg. caused the mice receiving urethane later to become more deeply anesthetized then and to lose more weight. The ulceration occurring in Experiment 7 where the colchicine solution had been introduced was avoided by washing it off the outside of the injecting needle with saline prior to insertion. The substance was given subcutaneously in the back and the urethane intraperitoneally as usual. Two mice died from their combined effects. Each injection was repeated after an interval, as shown in Chart 8. The group alterations in weight were like those in the animals of Chart 9 (*q. v.*), and they were of the same magnitude in the animals receiving colchicine

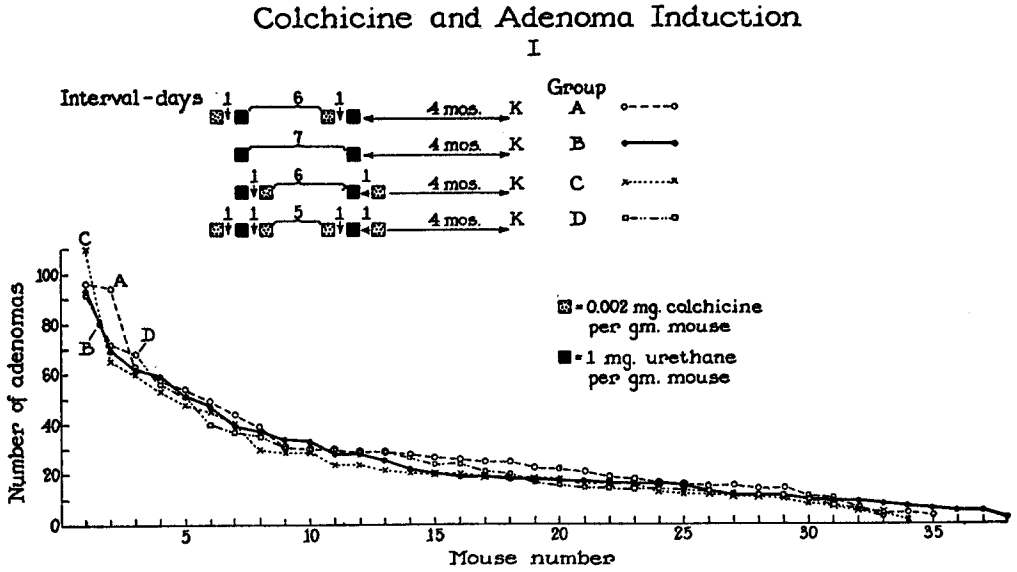


CHART 8

24 hours after urethane and those getting it 24 hours before. Hence the individual changes have been omitted. The mice were all killed after 4 months, and the adenomas charted but not measured, no significant differences obtaining in their size from group to group.

All of the animals developed adenomas and in approximately the same numbers from group to group (Chart 8).

In the next experiment the response to urethane after recovery from the immediate effects of colchicine (16) was determined.

Experiment 9.—90 male A strain mice from 9 to 13 weeks old were matched individually as to weight, and divided into three groups, kept in the same boxes. The same doses of colchicine and urethane were used as in Experiment 8, but only a single injection of each substance was given,—subcutaneously and intraperitoneally as before. Group A received colchicine 1 week prior to urethane; group B, 24 hours before it; while group C got only ure-

thane. The mice receiving both substances were harder hit than those receiving urethane alone, as shown by the changes in weight, and by the fact that more than one-third of them died. None of the animals succumbed as result of urethane given alone. All the groups were killed 8 weeks after the urethane injection.

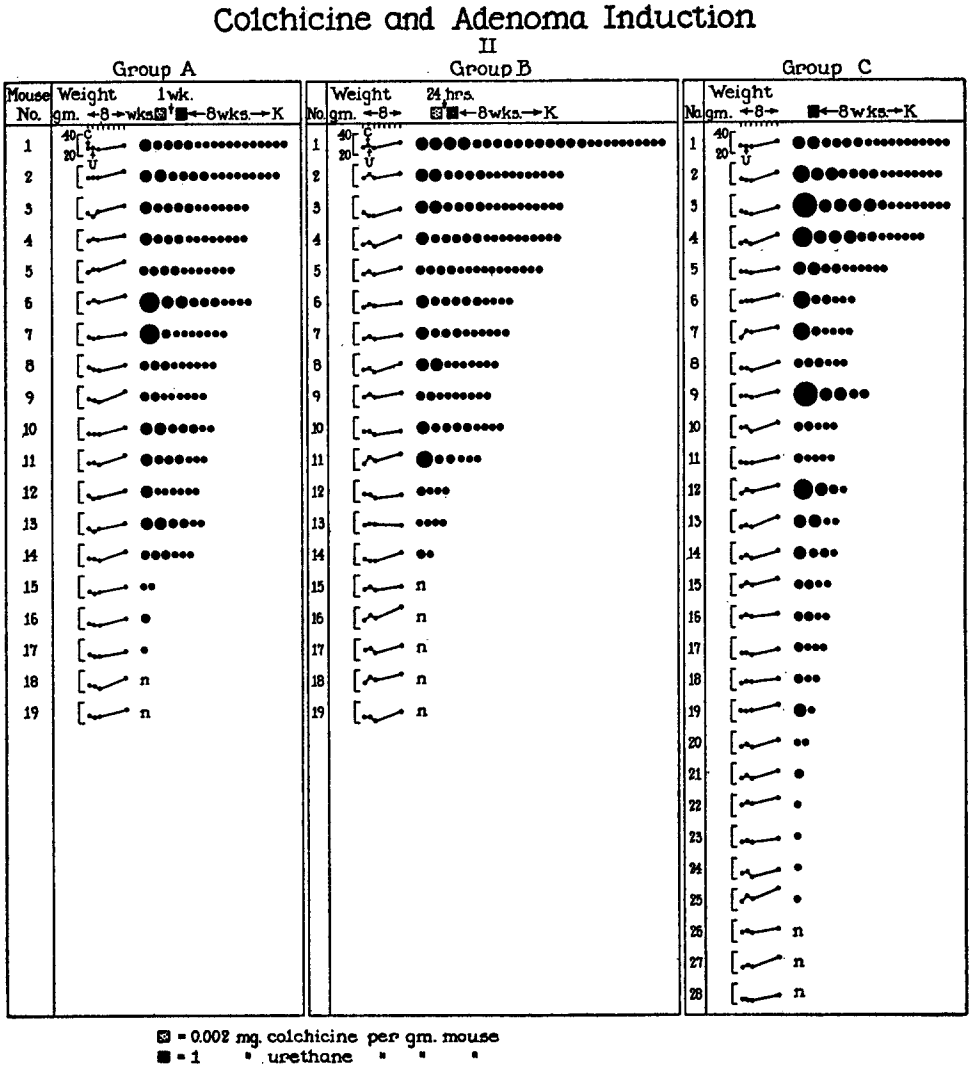


CHART 9

The animals of group A, which got colchicine a week before urethane, had had time to recover from its immediate effects before receiving the latter, whereas those injected with it only 24 hours previously (group B) did not fare so well, a fact indicated by their weight curves (Chart 9). Those of group C,

which received urethane alone, showed little alteration in weight. The chart makes plain that the incidence and yield of adenomas were not significantly different in any of the groups, though the growths tended to be somewhat smaller in group B, as might have been expected from the loss in weight of the hosts during the early development of the growths.

Mitotic Activity and the Response to Urethane

In Experiments 8 and 9 urethane was administered at a time when the colchicine given beforehand should have induced a maximum of nuclear ab-

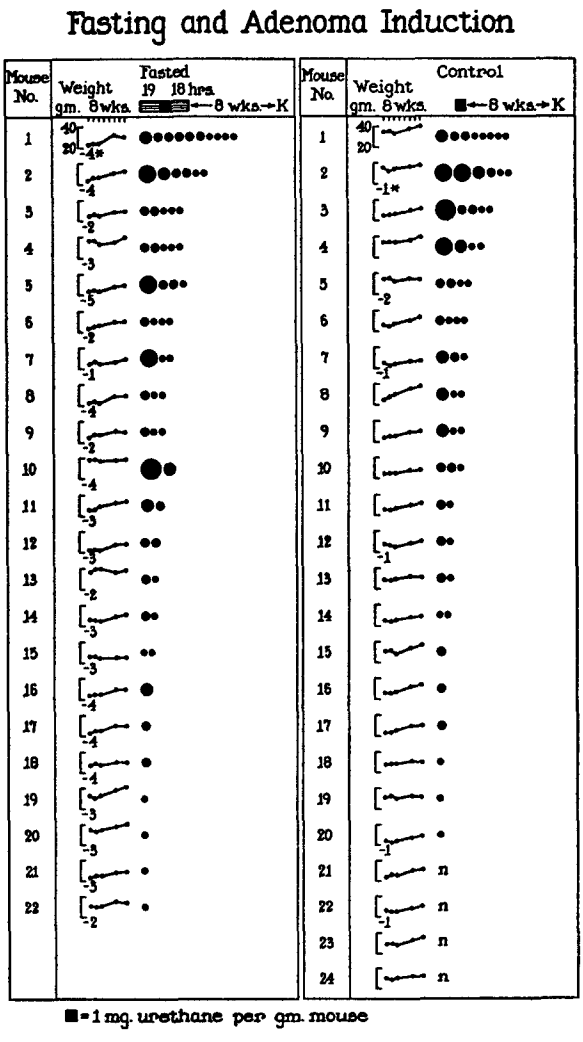


CHART 10

normalities (17), yet the yield of adenomas remained unaffected. To control further the possibility that urethane might exert its effect upon dividing cells, utilization was made of brief fasting, which is known to reduce cell division drastically in every organ thus far examined (18).

Experiment 10.—60 male Swiss mice, 9 to 13 weeks old, five to a box, were matched individually as to weight and separated into two groups, one of which was fasted for 19 hours prior to injection, while the other was kept full fed. 1 mg. of urethane per gm. mouse, as calculated from the weight just prior to injection, was given intraperitoneally to them all. The body weight at the time was 1 to 4 gm. less in the fasted animals, from which it follows that they received less urethane than the controls. The fasting was continued for 18 hours after injection, but at all times the animals had access to water. All were killed 8 weeks later and the adenomas charted.

It will be seen (Chart 10) that fasting failed to affect the yield of tumors.

Since the question at issue in this experiment was whether mitotic activity had any relation to adenomatous change, the conditions were weighted by giving the fasted animals less urethane than they would have received if full fed. Nevertheless they developed as many adenomas as the controls and every animal had them, whereas they were absent from several of the controls. Had urethane been given in the quantity received by the controls, and had the adenomas proved more frequent than in these latter, it could have been urged that in the fasted, underweight animals more urethane had reached the pulmonary cells than in the case of the controls, with result that its influence had more than compensated for the adverse effects of reduction in the number of mitoses.

The experiment was repeated, using fifty C strain mice, with essentially identical results.

DISCUSSION

Experiment 1 showed that the responsiveness of young mice to urethane, as evidenced by the percentage developing adenomas and the number of tumors per animal, fell off greatly between the 2nd and 7th week, and that the alteration continued, though at a much diminished pace, to the tenth week at least. It seems fair to infer that if the smallest amount of urethane given in the corroboratory Experiment 2 had been slightly less, no tumors would have appeared in the 8-week-old animals, while not a few of the 3-week-old mice would still have developed them and in considerable number in some individuals. The weanlings receiving the smallest amount of urethane developed as many adenomas and in nearly as many mice as those 8 weeks old which got twice the quantity. The lessening in the age differences in older animals (Experiment 1, Charts 1A and 1B) was so gradual as to indicate that after 10 weeks of age the changes in responsiveness due to ageing would have been relatively slight. In this relation the failure of mother mice to develop adenomas as large as those induced in the young they were carrying when injected with urethane

(3) is not without significance. The age of the mothers at time of injection was not known, but it is certain that they were considerably more than 10 weeks old, and though they received the urethane directly and the young got it only by way of the placenta their responsiveness was much inferior to that of the latter. In any experiments with urethane involving mice of different ages the influence of age differences as affecting the adenoma yield must be taken into account.

Several factors may conceivably have been concerned in the age differences noted in Experiments 1 and 2. "Spontaneous" adenomas appear early in Swiss mice, and such growths are known to increase in number and get larger as time goes on. A few big enough to be seen in the gross were encountered in the 6- and 8-week-old control animals of Experiment 1 when they were killed after a further 7 weeks, and it follows that some were almost undoubtedly present among the growths found in the corresponding urethanized animals. But they cannot have been more than a few at most, since the possibility that urethane had acted to stimulate their growth, thus bringing more of them to size of record, has been excluded by Experiments 5 and 6. Hence their presence can be written off.

Adenomas of suckling mice from mothers injected with urethane show many mitoses and grow rapidly, owing both to environmental circumstances highly favorable to their multiplication and to the natural proliferative activity of the cells, an activity additional to that due to neoplastic change (3). This state of affairs should make, one would think, for more and larger adenomas in mice injected when young and killed when little more than adult. In this general relation the increase in weight of the lungs as growing mice mature provides telling data, since it can be taken as roughly expressive of the lessening normal activity of the alveolar cells from which adenomas come. It will be seen (Chart 1B) that the curve of lung weight is almost the precise reciprocal of that representing the number of adenomas seen in the gross in the age groups studied. In accordance with these various facts, many more adenomas were visible in the younger animals at death than in those which had already reached maturity when injected. But no difference whatever was visible in the maximum size achieved by the growths in the various age groups nor in the proportionate number reaching a diameter of ⅓, ½, and ¾ mm. or more. Several factors of which little is known might be invoked to explain this finding. Perhaps there was a ceiling to cell multiplication, referable in part to the waning of the natural urge to proliferation as distinct from the neoplastic, and in other part to unfavorable local conditions developing as the adenomas enlarged, expansile compression, for example, of the surrounding alveolar tissue, with what this would mean as concerns blood supply. One must think also of the possibility, if not the probability, that the small number of adenomas appearing in the gross in the older animals were consequent on the proliferation of the most active neo-

plastic cells out of a considerable number brought into being by the urethane, whereas in the younger mice many less well endowed cells also gave rise to visible growths with result in an extensive series of tumors. Other factors might also be invoked. It is conceivable that the alveolar cells of young mice are rendered neoplastic more easily by urethane than are those of adults. On this as on the other possibilities just mentioned nothing definite can now be said.

The occurrence of adenomas as result of a brief exposure to urethane, the fact that it is devoid of oncogenic effect save for the lung, the prompt appearance of the growths, and the observation that the yield varies as does the liability to "spontaneous" adenomas of the mouse strains tested, have together suggested that the substance acts through stimulating, in some special way, the growth of cells already in the adenomatous state. Charts 2B, 5, and 6, and Table I make plain that this is not the case, showing as they do that urethane exerts no influence whatever on the rate of enlargement of adenomas. Some findings of previous workers can be more precisely interpreted in the light of this fact and of the demonstrated influence of age on the yield of the tumors:—

In an experiment involving many animals, Henshaw and Meyer gave one group of 6-week-old mice four consecutive injections of urethane at weekly intervals and another group two such injections followed by two more after a considerable time (6). The results tabulated by the authors are presented graphically in Chart 11. They noted, and the chart shows that the number of tumors was far greater when a considerable interval was allowed to elapse before the third and fourth injections than when the interval was brief. This is the more remarkable because under the former circumstances the time elapsing between the last urethane injection and the final examination of the mice was much less, with result that the adenomas had a considerably shorter period in which to grow. Henshaw and Meyer (6) concluded that the "amount of vulnerable tissue" had probably increased as the animals grew older during the weeks before the third and fourth injections, more pulmonary cells spontaneously becoming capable, during the long interval, of undergoing adenomatous change in response to urethane. But this possibility is negated by Experiments 1 and 2 of the present work wherein it was shown that the older the animal, the fewer tumors does urethane induce. Nor can one assume, in view of Experiments 2, 5, and 6, that the increased "vulnerability" was due to any promoting effect of the second set of injections on the growth of the adenomas resulting from the first. It may well be that the first exposures to urethane started many cells on the way toward becoming tumor cells, and that during the interval before the second set of injections the change progressed so far as to be consummated by them. This possibility is the more worth stress because the reexposure, after a considerable interval, of rabbit or mouse skin to carcinogenic hydrocarbons (tar, methylcholanthrene, benzpyrene) results in a phenomenon precisely simi-

lar to that disclosed by Henshaw and Meyer, namely, a much greater yield of tumors than if the reexposure had been prompt (19). But the hydrocarbons wherewith this has been shown all promote in greater or less degree the proliferation of the tumor cells they bring into being, and the increased yield of tumors might in large part have been due to this. Urethane is strictly initiatory in its effects, the first carcinogen of which this has been found to hold true.

Urethane blocks mitosis and induces other nuclear abnormalities in the alveolar cells of the lung (14), and it is from such elements that the adenomas derive (20, 3). These facts have been taken to suggest that the substance induces

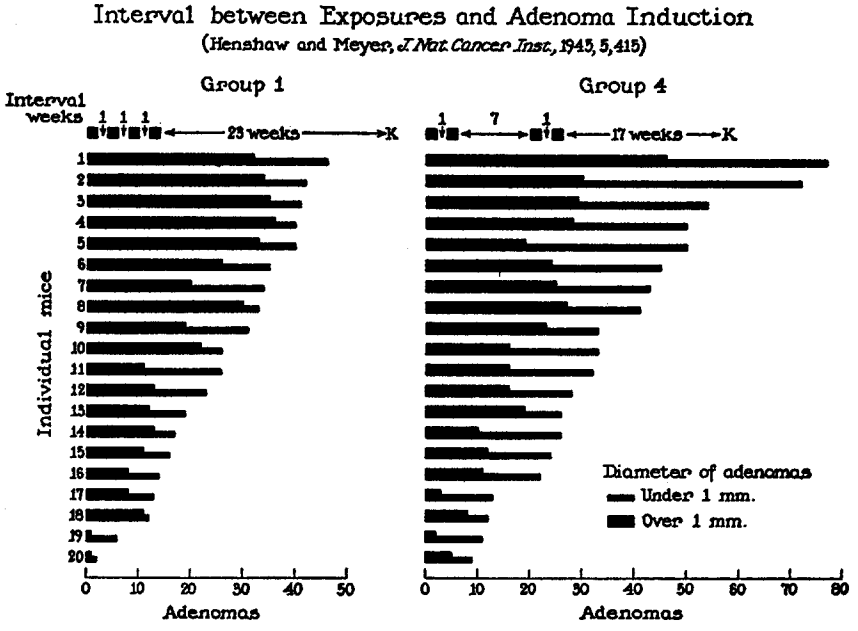


CHART 11

neoplastic change by acting on the nucleus. Were this the case colchicine might very well influence the course of events, since it too is a nuclear poison effective on the alveolar cells (21). That it failed to do so in the least (Charts 8 and 9) and that fasting, to the extent which reduces mitotic activity, was also devoid of effect (Chart 10) are significant findings. It would seem well to look elsewhere than to the nucleus for the changes which result in the adenomatous state.

SUMMARY

Young, rapidly growing mice are greatly more responsive to the adenoma-inducing influence of urethane than are those just arriving at maturity. This is manifest both in the proportion of animals developing the tumors and in their

number per individual. An amount of urethane per gram body weight which suffices to induce adenomas in only an occasional 8-week-old animal will cause them to appear in quantity in more than half the 3-week-old mice injected. There is an almost absolute inverse correlation between the rate of growth of the pulmonary tissue between the ages of 2 and 10 weeks and the response to urethane in terms of adenomas. Hence the conclusion seems justified that the natural proliferative activity of the alveolar cells during youth plays a major part in the formation of the tumors. After the 6th week the age differences become relatively slight, yet there is reason to think that they continue in some degree as life goes on.

Urethane has no effect to promote multiplication of the cells it has rendered neoplastic, its whole role being to initiate neoplastic change.

The abnormalities induced by urethane in the nucleus of normal and neoplastic cells, as observed by previous workers, have suggested that the substance brings about the adenomatous state by acting upon the nucleus. But colchicine, also a karyolytic poison causing pronounced nuclear changes, does not alter in the least the yield of adenomas to urethane when administered concurrently. Nor does fasting influence the yield, though it markedly reduces mitotic activity.

The meaning of these facts is discussed.

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