tains the medullary centers as well as the carotid reflex areas. Gasps were recorded manually on an electric kymograph traveling one centimeter in 5.540 seconds. Ninety-five mice from the ages of 1 to 19 days and 49 mice older than 20 days were used. In no case did any gasping occur beyond the first (aerobic) series in mice over 19 days of age. This observation holds true for many other mice used in other experiments and not included herein.

Table I and Fig. 1 show the results of this study. It can be seen that the aerobic first series of gasping remains quite constant throughout the life of the mouse indicating that the availability of the energy material remains little changed. The second or anaerobic series of gasps diminishes until about the 19th day when it is lost forever, thus indicating that the anaerobic energy of the newborn mouse persists for only 19 days.

Conclusions. The survival of the gasping mechanism of decapitated mice, like rats, is inversely related to age. Mice up to 19 days of age show 2 series of gasps, the early or aerobic series remaining quite constant throughout life, the second or anaerobic series gradually diminishing from the maximum in the newborn until it disappears completely at about 19 days of age.

15680

Effects of Urethane (Ethyl Carbamate) on Mitosis.*

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The authors[1] showed that transplanted rat carcinoma (Flexner-Jobling carcinoma) was far more susceptible to the lethal effects of X-rays when growing on rats which had been treated with colchicine than when growing on untreated controls. Unable because of war conditions to obtain colchicine to continue their experiments, they resorted to urethane (ethyl carbamate, $C_2H_5OCONH_2$) which was reputed to have much the same effects on mitosis. They found that while urethane does affect mitosis, the result was not the same as that following colchicine. The present study deals with their experiments and observations on this point.

The treatments were standardized to intraperitoneal injections of 1 cc of a 10% aqueous solution of urethane per 100 g of body weight of the young adult rats being treated. This was an anaesthetizing dose which left the animals inert for several hours. Of the 42 animals treated, 21 were male and 21 female. No significant sexual differences were found. A series of 14 mice were also injected and studied for comparison.

Since the purpose of our study was to determine the effect of urethane on mitosis it was important to select a tissue favorable for study. After some exploration we settled upon the corneal epithelium, recommended by Buschke, Friedenwald and Fleischmann[2] for mitosis counts and for differentiation of the various phases of mitosis. While the number of division figures vary greatly from the periphery to the center of the cornea, they are approximately the same in all meridians. A band 2 mm wide was cut out along the greatest meridian of the cornea and carefully separated from adherent tissues. After rinsing in 80% alcohol it was placed for an hour each in 95% alcohol, and absolute alcohol, and then stored in cedar oil. After at least an hour in cedar

* Aided by support from McArdle Memorial Laboratory, the Wisconsin Alumni Research Foundation, and the Brittingham Trust Fund.

1 Guyer, M. F., and Claus, P. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 565.

2 Buschke, W., Friedenwald, J. S., and Fleischmann, W., *Bull. Johns Hopkins Hosp.*, 1943, **72-73**, 144.

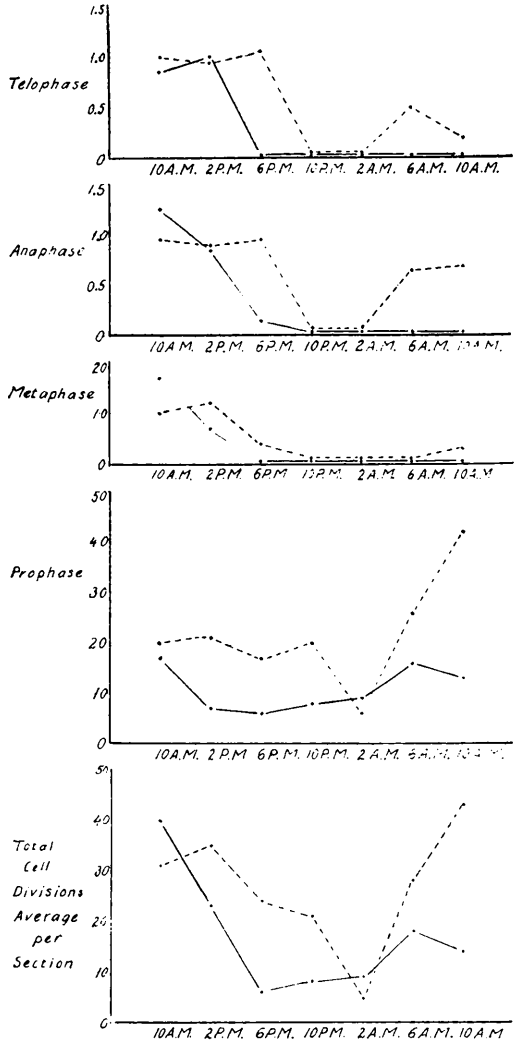

FIG. 1.

Graphs based on counts from the corneal cells of 42 female rats, showing the relative frequencies of the several mitotic stages in individuals killed at 4-hour intervals from 10 a. m. to 10 a. m. The continuous lines indicate mitotic phases in the 21 urethane-treated animals; the broken lines, the phases in the 21 controls. Counts for each phase in both experimental and control animals were made on 3 different individuals. The lowermost series represents the general average for all stages together.

oil the strips were passed successively through absolute alcohol, 95% alcohol, 70% alcohol, and distilled water, remaining for at least 15 minutes in each. They were then stained for 20 minutes in a 1:5 dilution of Harris' modification of Delafield's hematoxylin, rinsed in distilled water, differentiated in 1% HCl until deep red, and then left in ammonia water (0.1%) until blue. No counterstain was used. The strips were then dehydrated in the usual manner and cleared in cedar oil.

For sectioning, the tissues were mounted in paraffin and cut 6 μ thick meridionally of the cornea. According to the study of Buschke, Friedenwald and Fleischmann already cited, one in every 250 cells of the 2 basal layers should, on the average, be found in mitosis. While the range from eye to eye of the same individual is small the variation from animal to animal may be considerable.

The action of urethane on cell-division does not resemble closely that of colchicine. Although delaying mitosis for from 15 to 25 hours, colchicine permits accumulation and persistence of the metaphase stage, with actual continuance of chromosome-divisions. The interference appears to be mainly with

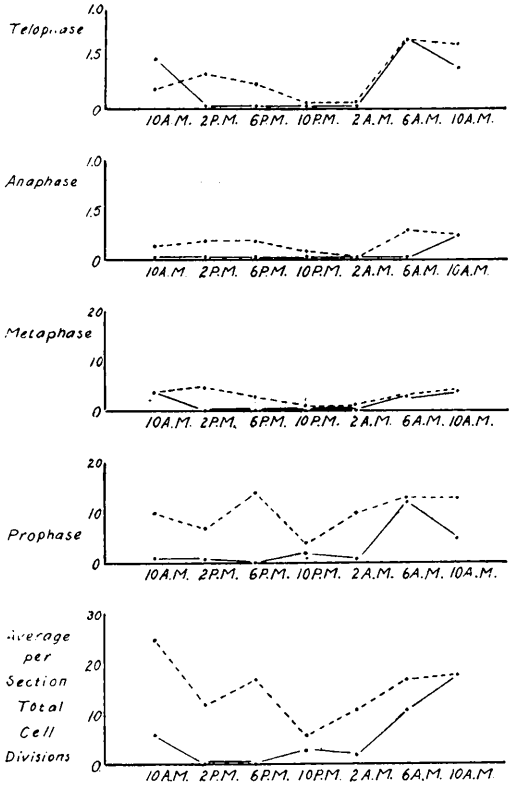

FIG. 2.

Graphs for 28 mice (14 urethane-treated, 14 controls), mixed sexes; counts for each phase made on 2 individuals, otherwise same as Fig. 1.

the distributional mechanism. With urethane, on the contrary, all mitotic stages seem to be largely done away with. Fig. 1 and 2 show how nearly mitotic activities were brought to a standstill.

The graphs are based on corneal sections in which mitotic figures of all phases were counted in every fifth section, with 36 sections inspected for each cornea. Forty-two rats were injected at 9:30 a. m. after which 6 injected and 6 control animals (3 of each sex) were killed at intervals of 4 hours from 10 a. m. one morning till 10 a. m. the next—a sequence of 7 sets in all. For purposes of comparison similar counts were also made on 28 mice.

Fig. 1 represents a series of graphs for the average abundance of each stage, based on sections from the corneas the 21 urethane-treated female rats compared with 21 corneal mitotic counts from untreated controls. The lowermost graph summarizes the general average for all stages taken together. The continuous line of each graph represents the urethane-treated animals, the dotted line, the controls. Horizontal readings indicate the time of day the animals were killed, the upright column shows the average number of division-figures per section in 108 sections (3×36) of the 3 females killed at any one time. The averages for anaphases and telophase (the 2 uppermost graphs) are based upon a 0 to 1.0 scale in contrast with the others in which the scale ranges from 0 to 50. This is because so many fewer division figures are found in anaphase and telophase than in prophase and metaphase.

The controls, indicated by dotted lines, show a daily rise and fall of the mitotic activities of all stages, with the smallest number of division figures appearing at or a little before 2 a. m., after which it ordinarily rises fairly rapidly till at least 6 a. m. In the treated animals, indicated by the solid lines in the graphs, the disappearance of the several stages is more rapid and it reaches its lowest point several hours earlier than the normal—by approximately 6 p. m. After remaining stationary or markedly retarded for from 8 to 12 hours, prophases begin to increase in number again. A series of counts on the corneas of the 21 injected and 21 control male rats was also made but inasmuch as it was not essentially different it has not been pictured. Fig. 2 represents a series of graphs made from the corneal cells of 14 urethane-treated and 14 control mice. The stages and designations are the same as for the rats (Fig. 1). It is obvious that response through decline in the number of mitotic figures, and persistence of the inhibition were even more pronounced than in the rat.

It is evident that instead of arresting mitosis in metaphase and holding it there as colchicine does, urethane almost wholly does away with all stages of it for a time. In this respect its effects seem more to resemble those described as following ether, cocaine or ephedrin.

In subsequent experiments with urethane we were surprised to find that it not only affects mitosis but causes pulmonary tumors† in well over half (75 out of 86) of our experimental animals. The effect is evidently the same as that reported by Nettleship and Henshaw[3] for this drug on mice.

Summary. Intraperitoneal injections of urethane (1 cc of a 10% aqueous solution per 100 g of body weight) almost wholly does away with all stages of mitosis for a period of 8 to 12 hours, judging from counts of mitotic figures in sections of the corneal epithelium from rats and mice. In a later experiment, lung tumors of adenomatous type appeared in 75 of 86 animals treated with urethane, but what, if any, connection there is between the interference of this drug with mitosis and its causation of lung tumors is not evident.

† Report in press, *Cancer Research.*

[3] Nettleship, A., and Henshaw, P. S., *J. Nat. Canc. Inst.*, 1943, **4**, 309.