These observations emphasize the necessity of ascertaining further the rôle played by the two chief components of the tumor fraction, namely the lipoids and the nucleoproteins, in the production of tumors.

Summary. By means of a method of differential centrifugation at high speed, a fraction can be separated from normal chick embryo tissue, which, in its main characteristics, resembles the active fraction isolated from chicken tumor extracts by the same method.

The implications of these observations are discussed.

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Constancy of Urea Clearances in Dogs Following Surgical Anesthesias with Cyclopropane, Ether, and Chloroform.*

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Perusal of the literature reveals no extended or correlated study of the effects of the common anesthetics on normal kidney function. Most of the work has been limited to determination of the rate of urine secretion before and after the various anesthetics.[1-4] Studies by Haines, *et al.*,[5] on the effects of ether, morphine, and atropine in various combinations upon dye excretion (phenol red and indigo carmine) before and *during* the period of anesthesia, and of Stehle and Bourne[6] on urine flow, urea, and chloride during and for 3 hours after ether, morphine, or both, have been along similar lines, although with conflicting results. Studies of urea, chloride, phosphate, and water excretion under ether, ethylene, ethylene and amytal, and ethylene and tribromethanol have been carried out by Walton[7] who found no significant changes. Recently Greisheimer, *et al.*,[8] in studies on one dog reported urea clearance elevations of 30%

* Aided in part by a grant from the Wisconsin Alumni Research Foundation.

1 Bonsmann, M. R., *Arch. f. exp. Path. u. Pharm.*, 1930, **156**, 160.

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3 Kemp, R. C., *New York M. J.*, 1899, **70**, 732.

4 Thompson, W. H., *Brit. M. J.*, 1906, 608 and 667.

5 Haines, W. H., and Milliken, L. F., *J. Urol.*, 1927, **17**, 147.

6 Stehle, R. L., and Bourne, W., *Arch. Int. Med.*, 1928, **42**, 248.

7 Walton, R. P., *J. Pharm. and Exp. Therap.*, 1933, **47**, 141.

8 Greisheimer, E. M., Hafkesbring, R., and Magalhaes, H., *Am. J. Physiol.*, 1938, **123**, 85.

on the days following cyclopropane anesthetization, and a return to 7% above normal within 5 to 7 days, with the control level being reached in 3 to 4 weeks.

In the course of other studies being made on cyclopropane anesthesia it was deemed advisable to determine the effect of this agent on kidney function as measured by urea clearance. The effects of ether and chloroform also were determined.

Urea clearance determinations have been made on a group of 5 adult female dogs kept in stock kennels and fed a standard diet of dog biscuits throughout the entire 8 months' test period. The animals have been in our kennels for periods of 10 months to more than 2 years and are all in a very healthy condition. A 4 to 6 weeks' adjustment period to the animal room environment was allowed before any animal was used.

The animals were trained to lie quietly on their backs and submit to catheterization and arterial puncture. The technic of Van Slyke[9] was followed in making the urea clearance determinations, all of which were post-absorptive, maximal, and expressed in cc per meter squared per minute, surface area being determined by the formula of Cowgill and Drabkin.[10] Three or more successive periods of 20 to 30 minute urine collections were made, urine samples of less than 0.4 cc per minute being discarded. The hypobromite method of urea analysis[11] was used routinely, since it simplified the procedures and is considered by Van Slyke[12] to be accurate. Urine simples were tested chemically for albumin and glucose and examined microscopically.

The general plan was first to determine the normal clearance values and then to subject each animal to surgical anesthesia for a period of 60 to 75 minutes. Following this the animals were placed in metabolism cages for checking the return of urine flow to normal and on succeeding days clearance determinations were repeated. Two of the dogs, which had not been subjected to *any* previous anesthetization, were given 3 periods of cyclopropane anesthesia at weekly intervals and a fourth period at the end of 14 weeks. After a rest of 2 months these same dogs were each given 2 chloroform anesthetizations 6 days apart. The other 3 animals were submitted to 2 or 3 etherizations at weekly intervals. These dogs had previously received cyclopropane in the course of other studies, but at least one month had intervened.

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9 Van Slyke, D. D., Hiller, A., and Miller, B. F., *Am. J. Physiol.*, 1935, **113**, 611.

10 Cowgill, G. R., and Drabkin, D. L., *Am. J. Physiol.*, 1927, **81**, 36.

11 Van Slyke, D. D., and Kugel, V., *J. Biol. Chem.*, 1933, **102**, 489.

12 Van Slyke, D. D., *Am. J. Med. Tech.*, 1936, **2**, 42.

TABLE I.

Summary of urea clearances before (control) and after cyclopropane, ether, or chloroform anesthetization. All clearances were maximal and are calculated in cc per square meter per minute. All anesthetizations were at deep surgical level and each period of anesthesia was for an hour or more.

Dog	Anesthetic Agent	No. of Anesthetizations	No. of Clearance Determinations Before Anesthesia	No. of Clearance Determinations After Anesthesia	Maximal Clearances Averages in cc/M²/min. Before Anesthesia	Maximal Clearances Averages in cc/M²/min. After Anesthesia	Coefficient of Variation Before Anesthesia	Coefficient of Variation After Anesthesia
A	Cyclopropane	4	26	14	34.7 ± 6.1	33.0 ± 5.2	17.5	15.7
B	”	4	22	13	32.6 ± 6.7	35.5 ± 5.6	20.6	15.8
	Totals	8	48	27	Avg 33.6	34.2		
C	Ether	3	21	15	34.9 ± 6.1	38.8 ± 4.5	17.5	11.6
D	”	2	5	12	32.7 ± 2.4	27.4 ± 2.2	7.3	8.0
E	”	2	6	12	42.5 ± 3.8	41.3 ± 2.9	8.9	7.0
	Totals	7	32	39	Avg 36.7	35.8		
A	Chloroform	2	5	9	39.7 ± 3.1	34.9 ± 2.3	8.0	6.6
B	”	2	6	8	34.0 ± 3.2	34.5 ± 4.1	9.4	11.9
	Totals	4	11	17	Avg 36.8	34.7		
	Grand totals	19	91	83	Composite avg 34.9 ± 6.1	35.2 ± 5.8	17.6	16.6

Constancy of chloroform and cyclopropane anesthetization was carried out as previously described.[13] Constant depth of ether anesthesia was maintained by connection with an endotracheal tube to an ether bottle, a soda-lime carbon dioxide absorber being interposed. In all the experiments surgical anesthesia was considered present when the eye reflexes were absent and at least partial intercostal paralysis occurred. In most of the anesthesias complete intercostal paralysis prevailed.

As reference to Table I will show, the average of 27 clearances following the 4 periods of cyclopropane anesthesia to each of 2 animals is 34.2 cc/M^2/min. Forty-eight control clearances on the same animals averaged 33.6 cc/M^2/min. For dog A the clearances were 34.7 ± 6.1 before and 33.0 ± 5.2 after cyclopropane anesthesia; for dog B the corresponding values were 32.6 ± 6.7 and 33.5 ± 5.6 cc/M^2/min. The coefficients of variation for A were 17.5 and 15.7 and for B 20.6 and 15.8. In Fig. 1 are plotted all the

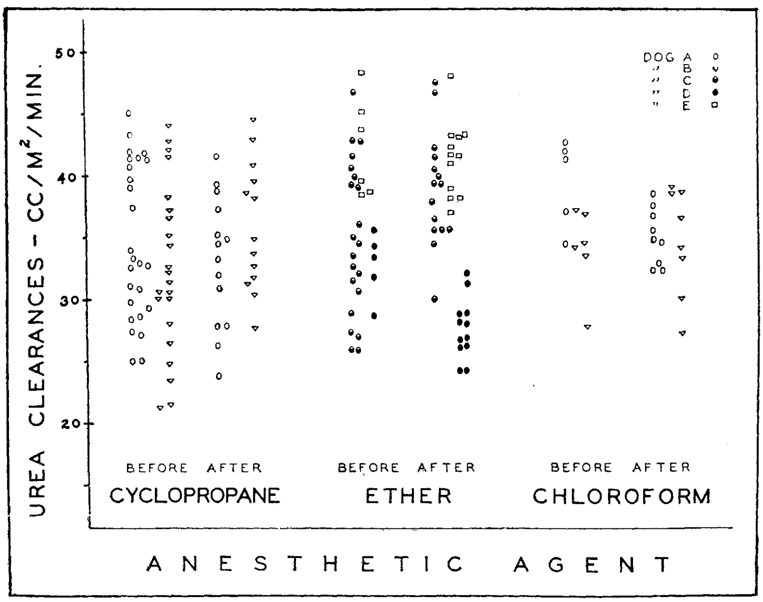

FIG. 1.

Maximal urea clearances in cc/M^2/minute on 5 dogs as determined before (control) and after anesthetization with various agents. Dogs A and B 4 periods each with cyclopropane, and 2 months after the last treatment with this agent 2 periods each with chloroform. Dog C, 3 etherizations and dogs D and E, 2 etherizations each. All anesthetizations were at deep surgical level, and each period of anesthesia was for an hour or more.

[13] Meek, W. J., Hathaway, H. R., and Orth, O. S., *J. Pharm. and Exp. Therap.*, 1937, **61**, 240.

data for the 2 animals, grouped by clearances before and after anesthetization.

Thirty-two control determinations for the 3 animals studied with ether have a mean value of 36.7 cc/M²/min, and 39 clearance determinations after 7 anesthetizations average 35.8 cc/M²/min. The clearances of the 3 animals before etherization were 34.9 ± 6.1, 32.7 ± 2.4, and 42.5 ± 3.8 respectively, the coefficients of variation being 17.5, 7.3, and 8.9. After the anesthetizations the corresponding values were 38.8 ± 4.5, 27.4 ± 2.2, and 41.3 ± 2.9, with coefficients of variation of 11.6, 8.0, and 7.0. These data are plotted in Fig. 1 and summarized in Table I.

Seventeen clearances following the 4 periods of chloroform anesthetization of the same animals used in the cyclopropane study average 34.7 cc/M²/min. The 11 control clearances averaged 36.8 cc/M²/min. For dog A the clearances were 39.7 ± 3.1 before and 34.9 ± 2.3 after chloroform anesthesia, with corresponding coefficients of variation of 8.0 and 6.6. For dog B the corresponding values were 34.0 ± 3.2 and 34.5 ± 4.1 with coefficients of variation of 9.4 and 11.9. Fig. 1 and Table I include these data.

It will be noted that for the 5 animals the average of 91 control clearances was 34.9 ± 6.1 cc/M²/min, with a coefficient of variation of 17.6. For the 83 determinations following a total of 19 anesthesias, given at 10 different times, with cyclopropane, ether, and chloroform, clearances average 35.2 ± 5.8 cc/M²/min, with a coefficient of variation of 16.6.

Repeated collections of 24-hour urine samples following each of the anesthetic agents have shown normal volumes within this period of time. No significant alteration was found upon microscopic examination of the urine, although slight casts, leucocytes, and occasional erythrocytes were present. After ether anesthesia a slight albuminuria was present but cleared within a few days. Cyclopropane and chloroform did not elicit these results. Glucosuria was never present.

The results of these tests seem to indicate that none of the 3 anesthetic agents studied—cyclopropane, ether, or chloroform—interferes with kidney function, as determined by urea clearance. Since urea is the chief product of excretion in the urine and involves activity of both the glomeruli and tubules in filtration and reabsorption it is felt that such determinations give satisfactory indices of the effect of these anesthetic agents on the kidney. While slight fluctuations in clearance values occur, they merely represent the normal variation in function of the kidney, as recently emphasized by Van Slyke.[12]

It is to be noted from Table I that the animals were subjected to much more exposure to any one of the agents than usually occurs clinically. In addition dogs C, D, and E had received 4, 9, and 7 cyclopropane anesthesias in the year previous to the studies under ether, yet their control clearances were in the same range as those of dogs A and B which had been submitted to *no* previous experimentation. This is further proof, though indirect, of the innocuousness of cyclopropane on the kidney.

Direct proof is afforded by dogs A and B (Table I) in which control clearances average 33.6 and clearances after cyclopropane 34.2 cc/M^2/min, a surprisingly close agreement considering the normal functional fluctuations which have been shown to occur in this vital organ. Likewise in Table I is shown strikingly the normal functioning of the kidneys following ether and chloroform anesthetizations, clearances before anesthetization being practically identical with those after either of the agents. Since the same 2 animals that had been tested with cyclopropane showed no kidney injury under chloroform, one feels all the more certain that these agents do not interfere with kidney function, at least as tested by the method of urea clearance.

Summary. On a group of 5 adult female dogs maximal urea clearance determinations following anesthetization with cyclopropane, ether, or chloroform have shown no significant variations over a period of 8 months from control values. Ninety-one control determinations made on the 5 animals before anesthetization gave average clearance values of 34.9 ± 6.1 cc per square meter per minute with a coefficient of variation of 17.6 and 83 determinations made after 19 anesthesias with the three agents averaged 35.2 ± 5.9 cc per square meter per minute with a coefficient of variation of 16.6.†

† We wish to thank Prof. R. C. Herrin for continued advice during the course of this problem, and Dr. M. Digby Leigh of the Anesthesia Department for helpful assistance.