been shown to inhibit tobacco mosaic virus infectivity by direct combination with the virus(8). It is therefore suggested that polylysine inhibits infectious bronchitis virus by a similar combination of the basic polypeptide with acidic groups on the surface of the virus protein. With egg passage, the virus becomes less antigenic, the surface pattern of the virus protein is modified perhaps in such a manner as to render it less available for combination with polylysine. It is also possible that the resistance to polylysine inhibition with increased egg passage reflects only an increase in the pathogenicity of the virus.

Conclusion. Polylysine was found to exert a marked protective effect in embryonated eggs subsequently infected with recently isolated infectious bronchitis virus. This protective effect was significantly less after several egg passages and with strains of infectious bronchitis which were completely egg adapted. Embryonated eggs infected with Newcastle disease virus were also protected by polylysine.

1. Stahmann, M. A., Graf, L. H., Patterson, E. L., Walker, J. C., and Watson, D. W., *J. Biol. Chem.*, 1951, v189, 45.

2. Rubini, J. R., Rasmussen, A. F., and Stahmann, M. A., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 662.

3. Watson, D. W., and Bloom, W. L., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 29.

4. Becker, R. R., and Stahmann, M. A., *J. Am. Chem. Soc.*, 1952, v74, 38.

5. Green, M., and Stahmann, M. A., *J. Biol. Chem.*, 1952, v197, 771.

6. Groupe, V., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 354.

7. Loomis, L. N., Cunningham, C. H., Gray, M. L., and Thorp, F., Jr., *Am. J. Vet. Res.*, 1950, v11, 245.

8. Burger, W. C., and Stahmann, M. A., *J. Biol. Chem.*, 1951, v193, 13.

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Application of Tissue Clearance Technics in Small Animals.* (20445)

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Careful work in many laboratories has established the fact that the disappearance of an isotope from the site of local injection is an exponential function. In his original analysis of the dynamics of tissue clearance, Kety(1) postulated that such an exponential relationship should obtain, if one could assume that the arterial concentration of the tracer were small enough to be neglected. That this assumption is substantially true in the case of clearance from the tissue of man has been amply confirmed in many laboratories(2-7). A most stringent test of the validity of this assumption is the linearity of the disappearance curve over long periods of time; thus, McGirr(3) and Rapaport *et al.*(4) find no significant deviation from linearity in disappearance curves followed for more than 60 minutes. However, data obtained in similar experiments with small animals frequently yield curves which are not strictly exponential. This is especially evident where the disappearance is followed for periods greater than 20 or 30 minutes. Thus, in short term investigations (8-10), *i.e.*, 5 to 10 minutes, the clearance of radioisotopes from various sites of injection in rats, rabbits and dogs was quite linear. When the period of observation was extended beyond 30 minutes deviations from the simple exponential relationship were always found(9, 10).

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The possible sources of this non-linearity have been considered by Cooper *et al.*(5), Miller *et al.*(6), and Wisham *et al.*(7). These factors include: a) a continually changing blood flow in the tissue; b) the deposition of significant amounts of the tracer in areas where the rate of removal was slower than from the bulk of the injection; and c) an increase in the concentration of the isotope in the arterial blood returning to the original injection site, *i.e.*, a negation of Kety's assumption. In the present study, we have attempted to determine which of these factors is the most significant and to devise methods to compensate for the deviations observed.

Methods. All clearances were measured on pentobarbital anesthetized rabbits. About 10-15 μC of I^{131} as iodide or Na^{24} as sodium chloride were injected in a total volume of 0.5 ml of isotonic saline. All injections were made into the subcutaneous tissue on the medial aspect of the thigh at least 24 hours after shaving the skin. In all cases small short needles were used. An attempt was made to keep the depth of injection, the length of the needle, the amount of solution and the geometry of the counting tubes constant. In addition, environmental factors were kept as nearly identical as possible for each rabbit: room temperature, duration of the experiment and depth of anesthesia were carefully controlled. All of our experiments were continued for at least 30 minutes. A) If the change in clearance reflects a change in blood flow attendant on anesthesia or on the abnormal position of the animal, we assumed that these factors would ultimately achieve a steady state and become relatively constant, or if variations continued, they should become more random and show increases as well as decreases. Therefore, clearances were determined in some animals for a 30 to 60 minute period immediately after induction of anesthesia; in others, clearances of similar duration were determined beginning about an hour after anesthesia was begun; and in a third group clearances were followed continually for a period of more than 3 hours beginning soon after induction of anesthesia. B) Another possible explanation of the deviation from linearity might be the deposition of some radioisotope along the needle track, involving several layers of tissue having different blood-flow rates. This possibility was explored by making subcutaneous injections with a long needle (3″) introduced through the skin at a point remote from the site of injection at the final position of the tip. As an added precaution, the needle was emptied of all isotope solution by passing a small air bubble through just prior to withdrawal. C) Finally, we investigated the possibility that significant quantities of the isotope were being returned via the arterial blood to the area "seen" by the counter over the injection site. To accomplish this, we placed a second counter over a control area on the contralateral limb in a position corresponding to the counter over the injection site. Lead bricks were arranged in such a way as to minimize cross-counting. The activity recorded by the G. M. Counter over the uninjected limb was taken to represent the contribution of all the counts brought by the arterial blood into this area and hence into the corresponding area on the injected leg. The difference between the two counters then should represent

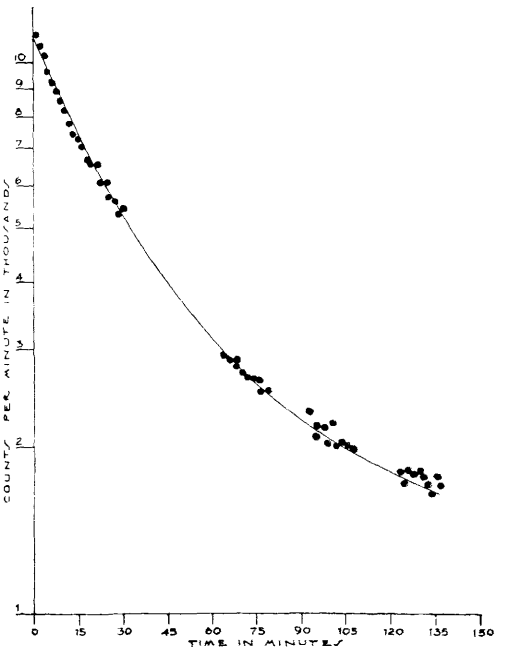

FIG. 1. Clearance of I^{131} from subcut. tissue of rabbit. The animal was anesthetized just prior to injection at zero time and the counting rate over injection site was determined for the following 2½ hr.

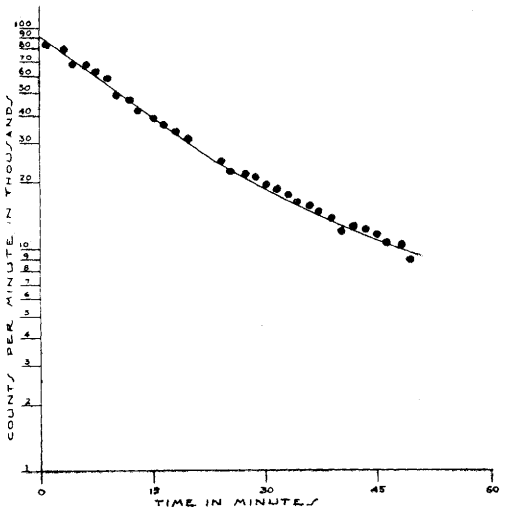

FIG. 2. Clearance of Na^{24} from subcut. tissue of rabbit. Injection of isotope was made through a 3″ long needle, which was flushed through with a small bubble of air before withdrawal.

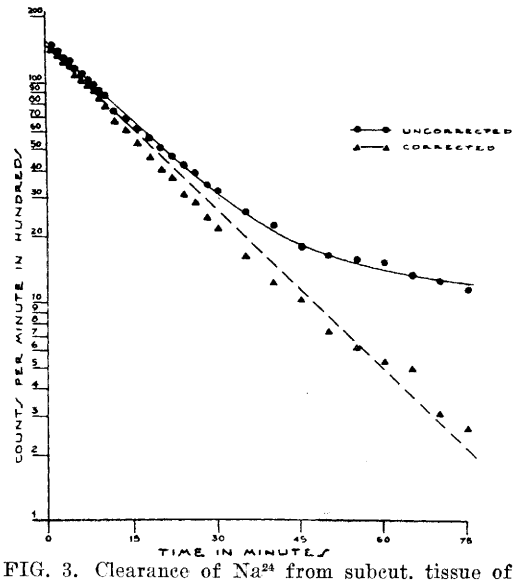

FIG. 3. Clearance of Na^{24} from subcut. tissue of cat. Solid line is the counting rate over the injection site at various times after injection. Broken line shows counts corrected by subtraction of simultaneously measured rate over control area.

the actual amount of injected isotope remaining at the injection site, and this function should show an exponential relation with time.

Results. A) Clearances obtained on animals after various intervals under anesthesia show a continuing decrease in the disappearance rate of I^{131} from the site of injection (Fig. 1). The initial rate is the most rapid with a gradual decrease throughout the period of observation. The curvilinear form of these clearance curves could not be attributed to changing blood flow unless this change continued at a uniform rate over the entire period. However, since the animals in all cases recovered from the anesthesia, and in many cases the depth of anesthesia became less while the clearance continued to fall, this explanation appears to be unlikely.

B) The results of one of the experiments outlined in section B are shown in Fig. 2. It will be noted that in spite of all precautions related to the deposition of isotope in tissue other than the subcutaneous location of the bleb, the results are still non-linear. It may therefore be argued that some other factor is involved in causing the deviation of the curve.

C. Fig. 3 presents the results of a typical experiment in which correction is made for the return of isotope to the area of the injection. It will be noted that the final corrected curve is a close approximation to a straight line, and for all practical purposes can be used as a measure of the true clearance rate.

Discussion. The results of the experiments recounted above strongly suggest that the major factor responsible for the deviation of the clearance curves from a simple exponential relationship is the return of isotope to the area under study. This factor would be of minimal importance where the isotope is highly diluted by a relatively large blood volume or where the isotope after removal from the injection site is immediately withdrawn from the blood and hence would return to the area in negligible concentration.† In humans, the

† Since iodide is rapidly removed from the circulation by the thyroid, the deviation from linearity is less marked in the iodide clearance studies than in the sodium experiments. However, the error in an iodide clearance becomes clear when the experiment is continued for a longer period of time (Fig. 1). In the shorter experiments the deviations are more dramatic when sodium is used, and hence this isotope was employed to illustrate the point in Fig. 2 and 3. It should be noted that the rate at which the thyroid removes iodide is not sufficiently great to eliminate this ion entirely from the arterial blood returning to the periphery, and thus there remains a significant deviation from linearity in these clearances.

dilution of the small amount of injected (and smaller amount of cleared) material by the relatively large extracellular fluid volume results in insignificant concentrations of isotope in the arterial blood. In the case of the small animal, the dilution is not nearly as great and the returning isotope is responsible for a significant and increasing fraction of the total activity seen by the counter over the injection site.

The apparent linearity of short term experiments in animals can be accounted for if one recognizes that the arterial concentration is progressively increasing, and that while after 30 minutes the returned isotope may account for as much as half of the total activity at the injection site, during the first few minutes the correction is but a negligible one. Results based on the clearance during the first few minutes after injection are therefore admissible; the advantage to be obtained from making the correction outlined herein has to do with extending the period of observation. Several workers (*e.g.*, McGirr) have called attention to the variability in clearance during the first 3 to 5 minutes after injection of the isotope. The clearance measurements without correction must therefore be based on a relatively short length of curve beginning after the initial irregularities and ending before the returning isotope is present in sufficient concentration to cause significant error.

Conclusions and summary. We have shown that a major factor responsible for the apparently nonlinear tissue clearances as measured in small animals is the return of isotope to the area of the injection in the arterial blood. By means of a relatively simple procedure it is possible to make correction for this error and to obtain reasonable, exponential clearances. This correction is made by subtracting the simultaneously measured activity over a corresponding area of tissue from the count over the injection site.

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1. Kety, S., *Am. Heart J.*, 1949, v38, 321.

2. a. Elkin, D. C., Cooper, F. W., Jr., Rohrer, R. H., Miller, W. B., Jr., Shea, P. C., Jr., and Dennis, E. W., *Surg., Gynec., and Obst.*, 1948, v87, 1. b. Green, H. D., McFall, C. F., Pollitzer, R., and Moore, M., *Am. J. Med.*, 1950, v9, 399; c. Semple, R., McDonald, L., and Elkins, R. P., *Am. Heart J.*, 1951, v41, 803.

3. McGirr, E. M., *Clin. Sc.*, 1952, v11, 91.

4. Rapaport, S. I., Saul, A., Hyman, C., and Morton, M. E., *Circulation*, 1952, v5, 594.

5. Cooper, F. W., Jr., Elkin, D. C., Shea, P. C., Jr., and Dennis, E. W., *Surg., Gynec., and Obst.*, 1949, v88, 711.

6. Miller, H., and Wilson, G. M., *Brit. Heart J.*, 1951, v13, 227.

7. Wisham, L. H., Yalow, R. S., and Freund, A. J., *Am. Heart J.*, 1951, v41, 810.

8. a. McInally, M., Campbell, J. A., Robertson, D. J., and Douglas, D. M., *Clin. Sc.*, 1952, v11, 183. b. Cullen, M. L., *et al., Surg., Gynec. and Obst.*, 1949, v89, 722; c. Stone, P. W., and Miller, W. B., Jr., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 529.

9. Boatman, J. B., Kendrick, T. R., Franke, F. R., and Moses, C., *J. Lab. and Clin. Med.*, 1950, v36, 456.

10. Franke, F. R., Boatman, J. B., George, R., and Moses, C., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 417.

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Effects of Alpha-estradiol Benzoate and Methyl Testosterone upon the Platyfish, *Xiphophorus maculatus* Skeleton.* (20446)

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It has been shown that estrogenic hormones induce profound modifications of the pelves in pocket gophers and mice through the resorption of cartilage and bone(1,2). Recent morphological studies of 2 species of viviparous fishes indicate that there is a partial resorption of the anterior hemal spines in females(3,4) Fig. 1. In the light of these data, the present experiments were undertaken to evaluate the influence of an estrogen and an