horse have failed to produce any infection demonstrable by repeated cultures.

Sincere appreciation is expressed for the help of John C. Carey and Corwin L. Smith in carrying out these experiments.

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Passage of the Blue Dye T-1824 from the Blood Stream into the Lymph.*

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(Introduced by W. W. Palmer.)

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The fate of intravenously injected Brilliant Vital Red has been discussed by H. P. Smith with special reference to its use in blood volume determinations.[1, 2] The following observations on the blue dye T-1824 are reported at this time because of the interest in blood volume and shock which has resulted from the present preoccupation with problems of military importance. Our purpose is to record the fact that the blue dye T-1824 passes from the blood into the lymph and to discuss the bearing which this observation has upon estimations of plasma volume by the Blue Dye Method.[3]

Method. Normal dogs were fasted overnight, anesthetized with morphine and nembutal, and the thoracic and cervical lymphatic ducts cannulated following the method of White, Field, and Drinker.[4] Samples of venous blood and of thoracic and cervical lymph were collected to serve as blanks for the subsequent dye determinations. One cc of a 1% solution of the blue dye T-1824 was then injected into the external jugular vein. In each instance solutions of sodium thiocyanate and in 2 instances solutions of horse and rabbit globulins specific for type III pneumococcus were also injected. Observa-

* The writers wish to thank Dr. Magnus I. Gregersen, Department of Physiology, College of Physicians and Surgeons, for his help and the use of his laboratory and equipment in carrying out this study.

1 Smith, H. P., *Bull. Johns Hopkins Hosp.*, 1925, **36**, 325.

2 Smith, H. P., *J. Exp. Med.*, 1930, **51**, 379.

3 Gregersen, M. I., Gibson, J. G., 2d, and Stead, E. A., *Am. J. Physiol.*, 1935, **113**, 54.

4 White, J. C., Field, M. E., and Drinker, C. K., *Am. J. Physiol.*, 1933, **103**, 34.

tions were made of the time at which these materials appeared in the lymph and the distribution which they underwent between the blood and the lymph.

The concentration of blue dye and thiocyanate in the thoracic and cervical lymph and venous plasma was determined with a Koenig-Martins spectrophotometer following the method described by Gregersen and Stewart for blood plasma.[5] The concentration of specific horse and rabbit globulins was estimated grossly by precipitin reactions carried out with samples of pneumococcus type III carbohydrate prepared by Dr. M. Heidelberger.

Results. In each of the 4 dogs studied the blue dye T-1824 made its appearance in the thoracic and cervical lymph sometime during the first hour following its intravenous injection, the exact time and amount seeming to depend somewhat upon the rate at which the lymph was flowing. Fig. 1 illustrates the quantitative relationships developed between the dye concentration in the thoracic and cervical lymph and the blood plasma in one of the dogs in which satisfactory

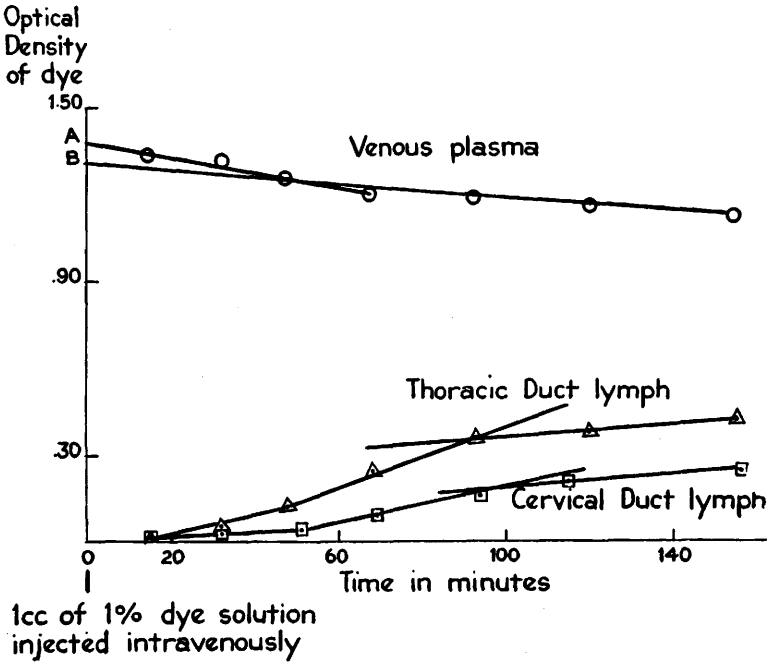

FIG. 1

Optical density of dye in venous plasma, thoracic duct lymph, and cervical duct lymph following the intravenous injection of 1 cc of a 1% solution of the blue dye T-1824.

[5] Gregersen, M. I., and Stewart, J. D., *Am. J. Physiol.*, 1939, **125**, 142.

collections of blood and lymph were possible over a considerable period of time. Similar results were obtained in the other animals.

It was found that the concentration of dye in the thoracic lymph considerably exceeded that in the cervical lymph, the values at 2 hours being of the order of 20 to 40% of the plasma concentration in the case of thoracic lymph and 3 to 15% of the plasma concentration in the case of cervical lymph. The volume of lymph collected also varied greatly but was of the order of 100 to 200 cc of thoracic duct lymph and 10 to 20 cc of cervical duct lymph per hour. In a dog such as that of Fig. 1 with a plasma volume of 600 cc these data indicate that approximately 7.5% of the injected dye† and 15% of the plasma proteins passed through the thoracic duct in the course of 2 hours.‡ How much more dye passed into the lymphatic system and remained there during this period cannot be estimated because the volume of the lymphatic system is not known.

The thoracic and cervical lymph of both animals receiving intravenous injections of horse and rabbit globulins gave strongly positive precipitin reactions for these foreign proteins at the end of 1 hour. A severe serum reaction occurred in one of the animals immediately following the injection of a toxic solution of rabbit globulin and it was interesting to observe that the urticarial wheals which developed on the abdomen were blue in color.

The concentrations of thiocyanate in the cervical and thoracic lymph and the blood plasma were found to be within 2 or 3% of one another at the end of 1 hour.

Discussion. Abell[6] has observed that in rabbits T-1824 is not visible in the extracellular tissue spaces of the ear during the first 2 hours following its intravenous injection.[6] Our experiments with normal dogs indicate that considerable concentrations of dye may be

† Calculations:

Plasma vol. = 600 cc.

Lymph collected = 300 cc.

$$\text{Conc. dye in lymph (mean during 2 hr period)} = \frac{0. + .30}{2} = .15 \text{ plasma conc.}$$

$300 \times .15 = 45$ or amount of dye equivalent to that in 45 cc plasma.

$$\frac{45}{600} = 7.5\% \text{ of total amount of dye injected.}$$

‡ Using, instead of mean concentration of dye, the approximate equilibrium concentration obtained at the end of 2 hours:

$300 \times .30 = 90$ or an amount of protein equivalent to that in 90 cc of plasma.

$$\frac{90}{600} = 15\% \text{ of total plasma protein.}$$

[6] Abell, R. G., *Anat. Rec.*, 1940, **78**, 215.

expected in the thoracic and cervical lymph during this period. The differences encountered are probably explainable on the basis that plasma proteins to which dye is attached circulate through the lymph, particularly through the thoracic duct lymph, even under relatively normal conditions. To what extent the circulation of dye through the lymph may be increased by damage to capillary membranes has not been determined. Maurer's experiments on lymph flow in anoxemia[7] would indicate that the increase may be considerable. As for the general extracellular fluid spaces, the increased escape of dye which follows capillary damage[8] is well illustrated by our dog with blue urticaria.

The simple fact that dye appears in the lymph during the 30 to 60 minutes required for a determination of plasma volume is not of critical significance in evaluating the accuracy of the Blue Dye Method. The important question is to what extent correction can be made for the dye losses by extrapolating the curve of dye disappearance. The fundamental problem here lies in distinguishing the mixing curve from the disappearance curve. Whipple's observations on the mixing time of labelled erythrocytes[9] appear to establish the validity of the assumption that the first 10 minutes of the curve represents mixing time and should be neglected in the extrapolation.[10] Linear extrapolation of the remainder of the curve may be and frequently is arbitrary (Fig. 1, intercept A or B?) but the uncertainty involved rarely exceeds 5%. Moreover, such evidence as is available indicates that estimations obtained by this method under normal conditions agree as well as can be expected[11, 12] with those derived by other technics.[13] Whether equally satisfactory measurements can be made in shock under abnormal conditions of capillary permeability, lymph flow and mixing time remains to be seen. It is obvious, however, that difficulties will be introduced by variations in capillary permeability[14] and pressure and consequent

7 Maurer, F. W., *Am. J. Physiol.*, 1940, **131**, 331.

8 Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

9 Hahn, P. F., Balfour, W. M., Ross, J. F., Bale, W. F., and Whipple, G. H., *Science*, 1941, **93**, 87.

10 Gibson, J. G., 2d, and Evans, W. A., Jr., *J. Clin. Invest.*, 1937, **16**, 301.

11 Smith, H. P., Arnold, H. R., and Whipple, G. H., *Am. J. Physiol.*, 1921, **56**, 336.

12 Fåhraeus, R., *Physiol. Rev.*, 1929, **9**, 241 (esp. 262-266).

13 Bazett, H. C., Sunderman, F. W., Doupe, J., and Scott, J. C., *Am. J. Physiol.*, 1940, **129**, 69.

14 Peters, J. P., *Body Water*, Charles C. Thomas, Baltimore, Maryland, 1935, Summary of literature, p. 58.

fluctuations in plasma volume during the period of measurement.[15] Presumably, significant determinations will be possible only during periods in which constancy of hematocrit and protein concentration indicates a fair probability of equilibrium in the exchange of fluid between the blood and extracellular spaces.

Summary. 1. Blue dye T-1824, used in the determination of blood plasma volume, passes from the blood stream into the thoracic and cervical duct lymph of normal dogs within the first hour following its intravenous injection. 2. Under normal physiological conditions correction for the escape of dye from the vascular bed can probably be approximated by extrapolating the curve of dye disappearance. Under abnormal and varying conditions application of this correction may be difficult.

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Effects of Cyanide and Iodoacetate on Survival Period of Infant Rats.*

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It has been demonstrated that the infant rat and dog (1-12 days of age) exhibit a tremendous tolerance to anoxia, hypoxia, nitrous oxide, carbon dioxide, cyclopropane, and hypoglycemia.[1] Among the factors permitting prolonged survival in the infant are (1) low cerebral metabolic rate, (2) poikilothermia, and (3) some anaerobic source of energy. In the present experiments we wish to present additional evidence on the third of these possibilities. Rats 1 day postpartum (6 g wt) were given 5 mg sodium cyanide subcutaneously and their survival time, as indicated by respiratory efforts, was noted. Adult rats (150 g wt) given the same dose of sodium cyanide were also studied.

Young rats can tolerate for a long period (50 min) an amount of sodium cyanide, 5 mg, which will cause death in the adult animal in about 10 minutes. Since sodium cyanide is known to inactivate

15 Magladery, J. W., Solandt, D. Y., and Best, C. H., *Brit. Med. J.*, 1940, 248.

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1 Himwich, H. E., Alexander, F. A. D., and Fazekas, J. F., in press, *Proc. Am. Physiol. Soc.*, April, 1941.