TABLE II.
Hydrolysis of Phenyl Acetates in the Presence of Various Cholinesterase Preparations.

Source of ChE	Type	Q_{ACh}	Substrate	Q	Q substrate + 0.2 mM eserine
Venom of *Bungarus fasciatus*†	e	25,000	Phenyl acetate, .002 M	16,200	0
Hemolyzed erythrocytes‡	e	11,750	” ” .002 M	7,250	0
Plasma*	s	6,500	” ” .002 M	117,000	102,000
Purified erythrocyte ChE§	e	12,000	Acetylsalicylic acid, .06 M	160	0
Venom of *Naia melanoleuca*‖	e	30,800	” ” .08 M	650	0
Purified erythrocyte ChE¶	e	5,550	*p*-Nitrophenyl acetate, .003 M	2,680	

Total volume in all cases = 1 ml.
* 0.002 ml of heparinized human plasma.
† 0.02 mg of dry venom.
‡ 0.1 ml of washed and hemolyzed erythrocytes; washed 3 times with saline solution and hemolyzed with 3 volumes of distilled water. This suspension was diluted with 9 volumes of "Ringer-30."
§ 0.3 ml (*cf.* "Methods").
‖ 1 mg of dry venom.
¶ 0.025 ml.

choline is the physiologic substrate of the e-ChE.

Summary. e-Cholinesterase ("true" cholinesterase) from human erythrocytes and snake venoms is capable of catalyzing the hydrolysis of ethoxyethanol acetate, desoxycorticosterone acetate, ketopropanol acetate, phenyl acetate, p-nitrophenol acetate and acetylsalicylic acid. The first mentioned ester is attacked more effectively by e-cholinesterase than by s-cholinesterase ("pseudo"-cholinesterase).

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17245. Mobilization of Radioactive Sodium from the Gastrocnemius Muscle of the Dog.*

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The experimental study reported here was based on the clinical application of intramuscular injection of radioactive sodium 24 in studies of peripheral vascular disease by Elkin *et al.*[1] This technic and the inferences drawn from the results of those studies are predicated upon the disappearance of the radioactive sodium by way of the blood stream. However, the possibility existed that a portion of the sodium was being removed by the lymphatic system. A further consideration was that the isotope might diffuse along intramuscular planes, thereby removing itself from the range of the Geiger-Mueller counter, in which event it would erroneously appear that the sodium had been removed by the circulating blood. The study reported here was undertaken, therefore, to determine:

(*1*) The role of the lymphatics in the mobilization and removal of intramuscularly injected radioactive sodium.

(*2*) A means of quantitative estimation of

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1 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, **87**, 1; Cooper, F. W., Jr., Elkin, D. C., Shea, P. C., Jr., and Dennis, E. W., *ibid.*, 1949, **88**, 711.

TABLE I.
Analysis of Chyle for Radioactive Sodium.
Chyle collected from thoracic duct fistulas after injection of isotope into the gastrocnemius muscle.

Dog	Wt, kg	Age of fistula	Chyle collected, cc	Time of collection, hr	Sodium injected, microcuries	% return
1*	11.3	1 day	30	1.5	10	<1
2*	20.0	Immediate postoperative	15	2.5	5†	<1
3	20.0	8 days	30	3	10	<1
4	8.1	1 day	12	3	10	<1
5	10.4	1 day	42	3	15	1.1
6	11.3	1 day post thoracic duct cannulization	16	3.25	13	0.75
7	10.0	1 day	12	1.5	19	<1

* Anesthetized.
† Injection in left hind and forelegs.

radioactive sodium in the blood draining from the injection site.

Role of Lymphatics. The route of egress of any material injected into muscle must be either lymphatic or vascular. Therefore, this portion of the experiment was undertaken to determine if appreciable quantities of the radioactive material could be found in lymph collected from the injected extremity. Although thoracic duct lymph contains lymph from both lower extremities, abdominal viscera, and the left upper extremity, the additional volume and added metabolites found in thoracic duct lymph would not invalidate the accuracy of the counting methods as the presence of the radioactive material could be detected regardless of its dilution. Accordingly, 7 dogs were subjected to operation: thoracic duct fistulas were made in 6, using a modified Biedl technic;[2,3] one additional animal was subjected to thoracic duct cannulation.

Procedure. A longitudinal incision was made along the course of the external jugular vein with ligation and division of all tributaries. The thoracic duct was identified as it entered the external jugular vein approximately at its junction with the left subclavian vein. Identification of the duct was facilitated by feeding the dog one pint of milk and cream 30 minutes prior to operation.

The subclavian and innominate veins were tied and divided below the point of entrance of the thoracic duct, leaving a segment of the external jugular to be used as a conduit to the outside. The vein segment was sutured to the skin, care being taken not to angulate it. A soft rubber catheter was introduced into the fistula and sutured in place; usually the catheter fell away on the first postoperative day.

As it was desirable to collect the chyle from the unanesthetized, ambulatory animal, the following method was devised for this purpose: At the conclusion of the operative procedure, a finger cot, with the end cut off, was sutured around the mouth of the fistula. On the following day, with the animal up and about, another cot was attached by means of Michel clips to the rim of the previously sutured finger cot. This provided a sac into which chyle could drain and which could be changed at periodic intervals.

In one additional dog a polythene cannula was placed in the duct. To protect the cannula, a plaster jacket was placed about the animal's upper thorax, and windows were cut for the cannula and front legs.

Following the animal's recovery from anesthesia, intravenous pentobarbital sodium (Nembutal, Abbott), a 0.1 to 0.2 cc solution of sodium chloride containing 5 to 20 microcuries of radioactive sodium 24 was injected into one of the gastrocnemius muscles. The animal was then encouraged to move about the laboratory, and the thoracic duct lymph was collected for a variable period of time, ranging from 1½ to 3¼ hours. The relative

[2] Biedl and DeCastello, V., *Pfluegers Arch.*, 1901, **86**, 259, cited by Lee, F. C., *Bull. Johns Hopkins Hosp.*, 1922, **33**, 21.

[3] Markowitz, J., Textbook of experimental surgery, William Wood and Co., Baltimore, 1937.

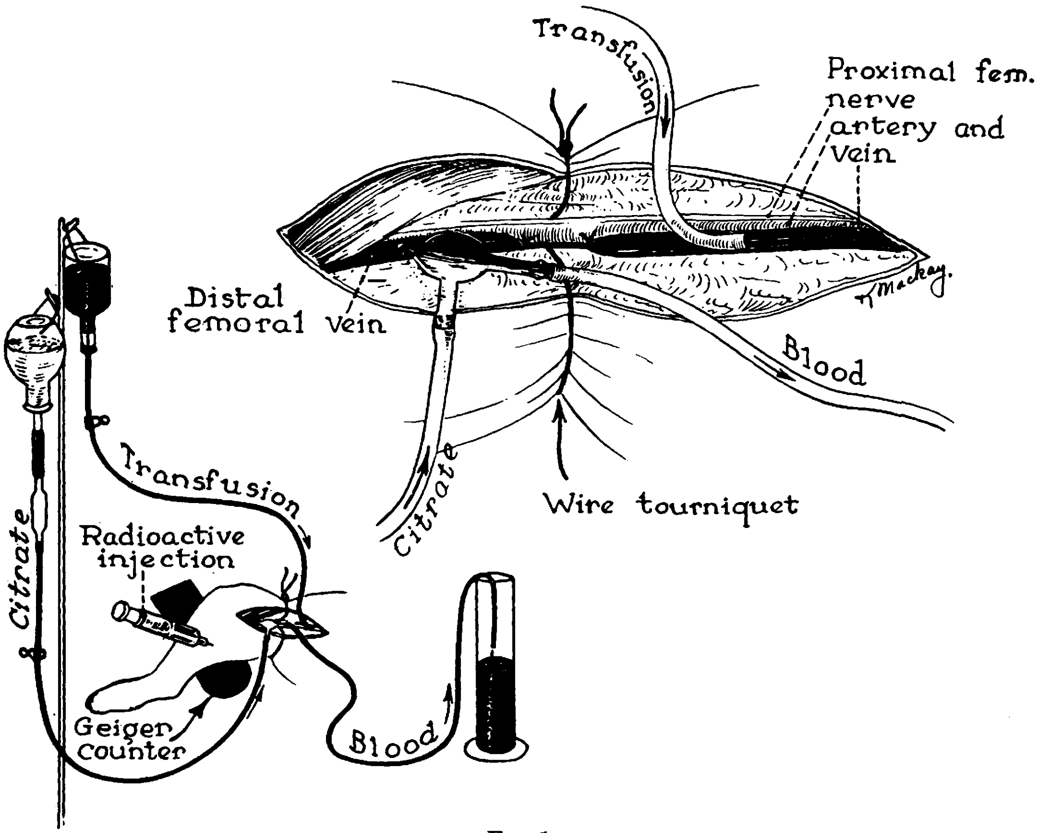

FIG. 1.
a. Geiger-Mueller counter in position to record disappearance of radioactive sodium from site of injection. The femoral nerve, artery and vein are lying above the tightened wire which has compressed and occluded the venous collateral vessels.
b. Relationship of transfusion and collecting cannulas in the femoral vein.

amount of radioactive sodium present in the collected lymph was determined.

As a control, an amount of radioactive sodium equal to that injected was added to a volume of water equal to the volume of chyle collected, and the relative amount of radioactive sodium determined. Correction was made for background radiation, and the relative amounts of sodium in the collected and in the control specimens were compared and expressed in percentage form.

The results of these experiments are recorded in Table I. The 2 anesthetized dogs are included for the purpose of comparing volume of flow in the anesthetized and unanesthetized animals.

Role of the Capillary and Venous Systems. The radioactive sodium content of blood returning from the gastrocnemius muscle was studied in 9 dogs. In 2, the procedure was repeated in the opposite leg after an interval of at least 3 days. Therefore, a total of 11 studies was made.

Procedure. With the animal under intravenous pentobarbital sodium anesthesia, a longitudinal incision was made over the femoral nerve, artery and vein. These structures were isolated, and a malleable copper wire placed beneath them and brought around the thigh so as to completely encircle it. On tightening the wire, all vessels of the leg, with the exception of the femoral, were compressed; therefore, any sodium being mobilized at the capillary loop and returning to the general circulation had to pass cephalad via the femoral vein.

A Geiger-Mueller counter connected to a

recording mechanism§ was placed under the leg, just beneath the gastrocnemius muscle. A Jackson cannula was introduced into the distal segment of the femoral vein, and sodium citrate, 2.5%, was allowed to flow in one arm of the cannula and mix with the blood as it left the vein. Attached to the third arm of the cannula was a rubber tube which ran to a collecting flask outside the operative field. To obviate shock, a condition incompatible with normal sodium mobilization, blood from healthy mongrel dogs was given to maintain blood volume. The transfusion technic was to introduce a glass cannula into the proximal end of the femoral vein and replace the blood by way of this cannula. The transfusion was regulated so that blood was replaced at the same rate it was withdrawn. At the moment the collecting cannula was introduced, a saline solution containing 7 to17 microcuries of radioactive sodium was injected into the gastrocnemius muscle directly over the Geiger-Mueller counter. Fig. 1 illustrates the equipment and methods.

The blood was collected for periods ranging from 10 to 20 minutes. During this time the amount of radioactive sodium present at the

§ Wire recorder.[4] To simplify the procedure at the time of the experiment and to minimize the factor of human error inherent in recording the counting rate by the usual method, an electromechanical system was used in which the impulses from the Geiger-Mueller counter were recorded as they occurred, and analyzed at a later time under more favorable conditions (*i.e.*, in the isotope laboratory rather than in the animal operating room).

A portable wire recorder was used, into which the output of the Geiger-Mueller counter was fed after being passed through an appropriate amplifier. The result was a complete record of the counts as they occurred at the site of injection for the entire period of the phlebotomy.

The wire recording was then fed through an amplifier and conventional scaler, and thence to a translator which drove a mechanical chart recorder. The chart produced was a complete record of the impulses in their order and time of occurrence, from which the counting rate for each minute throughout the run could be computed and plotted.

[4] Kety, S. S., *Am. J. Med. Sciences*, 1948, **215**, 352.

TABLE II.

Analysis of Femoral Vein Blood for Radioactive Sodium. Isotope injected into the gastrocnemius muscle and wire tourniquet tightened.

Dog	Wt, kg	Estimated* blood volume, cc	Blood collected, cc	Duration of experiment, min.	Sodium injected, microcuries	% removed (muscle)	% collected (blood)	"K" value (muscle)	"K" value (blood)	% deviation
8	15.9	1462	1092	15	11.5	54	49	0.051	0.045	— 9
9	15.9	1462	750	15	16.5	47	43	0.042	0.038	— 9
10	8.1	745	250	15	11.4	42	17	0.036	0.012	—60
11	7.7	708	640	20	7.6	52	51	0.037	0.036	— 2
12	14.0	1288	750	13	16.5	45	46	0.046	0.047	+ 2
13	12.7	1168	490	18	10.5	80	78	0.089	0.083	— 1
14	14.5	1334								
Right leg			615	15	9.4	47	46	0.042	0.041	— 2
Left leg			385	15	12.6	60	67	0.061	0.073	+12
15	7.2	662								
Right leg			425	16	8.0	60	65	0.057	0.065	+ 8
Left leg			400	15	12.9	62	61	0.064	0.063	— 2
16	10.0	920	260	15	12.8	50	48	0.046	0.043	— 4

* Blood volume was estimated on the basis of 92 cc/kg of body weight.

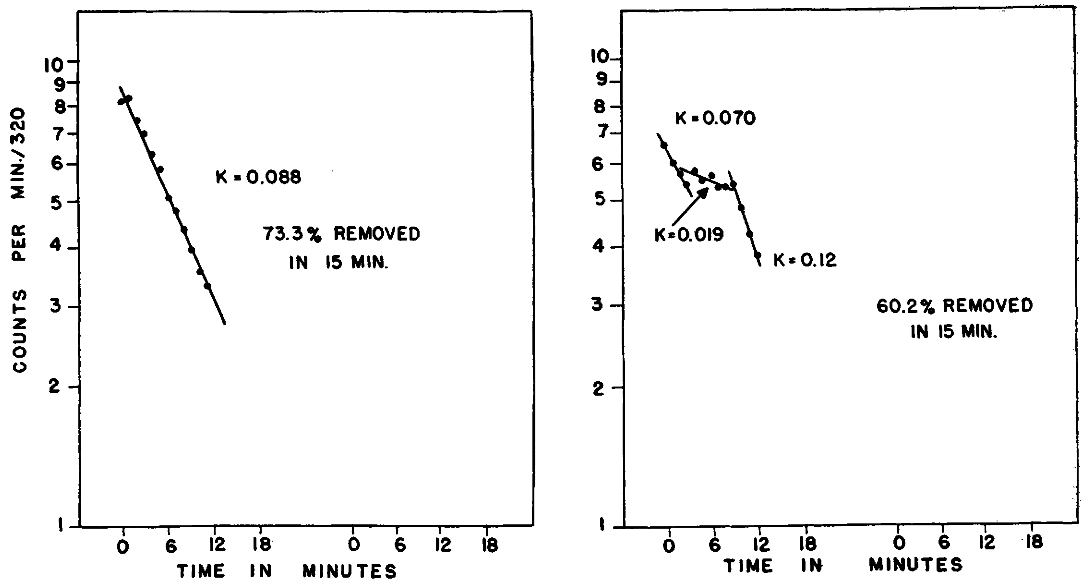

FIG. 2.

Graphs showing rates of mobilization of radioactive sodium from the gastrocnemius muscle of dogs; *a*, uniform rate; *b*, fluctuating rate. The line representing the rate of removal is obtained by plotting the counting rate for each minute against time.

site of injection was recorded continuously.

The radioactive content of the collected blood was determined by the same method used for the thoracic duct lymph. The relative amount of radioactive sodium in the collected blood was compared with a control sample also prepared by the same method used for lymph control. The amount of radio active sodium collected was expressed as a percentage of the amount injected, or, more simply, percentage return in the blood.

The rate of mobilization of the radioactive material from the muscle was determined from the collected blood by the equation:[4]

$$K_1 = \frac{2 - \log(100 - P)}{.4343\ T}$$

K_1 = Tissue clearance constant as determined from the collected blood.
T = Time elapsed or duration of phlebotomy.
P = Per cent of sodium recovered in the collected blood.

The rate of mobilization was also determined directly at the site of injection by plotting against time on semilogarithmic paper the number of counts for each minute throughout the phlebotomy, as detected by the Geiger-Mueller counter at the injection site. Mathematically, this rate may be expressed by the equation:

$$K_2 = \frac{\log C_2 - \log C_1}{.4343\ (T_2 - T_1)}$$

K_2 = Tissue clearance constant as determined by measurement directly at the site of injection.
C_1 = Number of counts at beginning of phlebotomy or Time T_1.
C_2 = Number of counts at end of phlebotomy or time T_2.

The results of 11 procedures are shown in Table II. Comparison can be made of the rates of removal (K values), percentage removed (as measured at the muscle) and percentage returned (as measured from the blood) along with their percentile deviation.

In some instances there was variation in the rate of sodium removal from the site of injection, necessitating multiple determinations of K value and percentage removed at this site (muscle). Fig. 2 illustrates both a constant and sporadic or irregular rate of mobilization.

Results. The lymphatic system of the dog's leg did not play a significant role in the mobilization and removal of the intramuscularly injected radioactive sodium. In no instance was the quantity of radioactive sodium recovered in the thoracic duct lymph greater than 1.1% of the amount injected into the gastrocnemius muscle.

The data obtained on the group of dogs in which the collateral venous system of the thigh was occluded by a wire tourniquet (Table II) shows good correlation between the percentage known to have been mobilized and the percentage actually recovered in the blood draining the limb.||

Failure to recover 60% of the isotope in the case of dog No. 10 may be attributed to one or both of the following factors: It is possible that the wire was not tightened sufficiently to occlude completely the collateral channels; it is possible that a thrombus might have partially occluded the lumen of the cannula. A thrombus would prevent a free flow of blood, resulting in stasis, dilatation of the femoral vein and tributaries, and increased venous pressure. The volume of blood collected in this dog was low, a finding which tended to substantiate the latter hypothesis.

The remaining 10 analyses in this series showed percentage deviations within 12%. Control studies showed that a 12% error was possible with this technic.¶ Therefore, it was concluded that radioactive sodium could be successfully recovered from the blood draining the injection site and that the mobilization of this material was solely a function of the capillary loop. In view of the fact that the isotope was not found in the thoracic duct lymph, and that it was recovered quantitatively in the blood, the factors of lymphatic removal and diffusion beyond the range of the Geiger-Mueller counter appeared to be insignificant. The assumption that removal of sodium 24 was a function of the hemovascular system, and consequently, was dependent upon the state of the circulation at the site of injection, was apparently valid.

Summary. A study of sodium mobilization from the gastrocnemius muscle of dogs by the use of radioactive sodium 24 is reported.

The sodium content of thoracic duct lymph following injection of sodium 24 into the gastrocnemius muscle was measured in 7 dogs to determine the role of the lymphatic system in the mobilization of the sodium.

The capillary mobilization and venous transport of sodium 24 from the gastrocnemius muscle was studied in 11 extremities. The radioactive sodium content of the blood draining from the gastrocnemius muscle was determined by two methods: (*1*) directly, by actual measurement of the sodium 24 content of collected blood, and (*2*) indirectly, by means of a Geiger-Mueller counter placed beneath the site of injection of the radioactive sodium into the muscle.

Conclusions. The lymphatic system of the dog's hind extremity does not play a significant role in the mobilization of intramuscularly injected sodium, nor does the radioactive material diffuse along intramuscular planes beyond the range of the Geiger-Mueller counter.

Intramuscularly injected radioactive sodium can be quantitatively recovered from femoral vein blood if the collateral venous system of the upper thigh is occluded.

The rate of removal of sodium 24 measured external to the site of injection is the same as that determined from the blood actually draining from the muscle.

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|| The radioactive sodium content of femoral vein blood in the absence of collateral venous occlusion was variable. Ten dogs were subjected to femoral phlebotomy without the application of a wire tourniquet, and the radioactive sodium content of the femoral vein blood determined after injection of the sodium into the gastrocnemius muscle. It was possible to recover the total amount known to have been removed from the muscle in only 5 animals; in the remaining 5, a portion of the sodium mobilized, ranging from 15 to 60%, was shunted through venous collateral channels.

¶ To determine the percentage of error inherent in the counting technic used in this experiment, equal quantities of sodium 24 in the form of sodium chloride were placed in equal volumes of water and blood, and counting rates determined. It was found that the percentage of error in counting rates between the two fluids varied from 2 to 12%. Accordingly, the results have been interpreted in the light of a possible 12% deviation.