

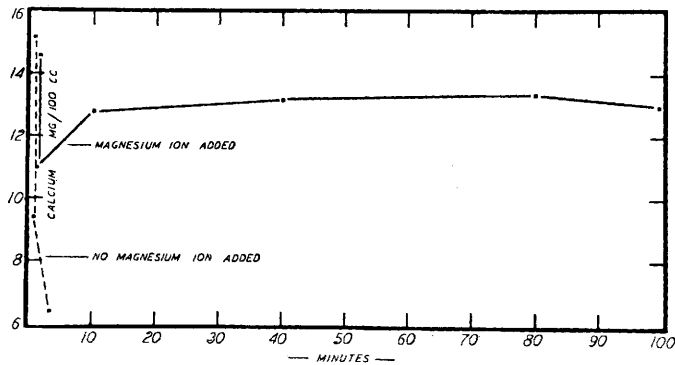
EFFECT OF MAGNESIUM ION ON SERUM CALCIUM
LEVELS OF RABBITS GIVEN E.D.T.A.A.

FIG. 4.

function than hitherto available is presented in Fig. 4. It can be noted that simultaneous administration of magnesium ion with an ordinarily lethal dose of E.D.T.A.A. resulted in a rapid replenishment of the blood calcium levels. This effect was noted whether the magnesium was pre-added to the E.D.T.A.A. solution with formation of the magnesium complex of E.D.T.A.A. prior to injection or if magnesium ion in the form of the sulfate was given simultaneously into the opposite ear vein of the rabbit or was injected directly into the heart simultaneously with the intravenous injection of E.D.T.A.A. in the ear vein. That the result was not due to combination of E.D.T.A.A. with magnesium ion rather than calcium ion has been indicated

by the demonstration that the complex of calcium ion by E.D.T.A.A. as measured by the precipitation of unbound calcium by oxalate did not change in the presence of magnesium ion in the system when tested *in vitro*. Confirmation of this work was reported in a study by physical means of the preferential complex formation of E.D.T.A.A. with various bivalent cations(7). In the physiological pH range E.D.T.A.A. combines with calcium ion preferentially over magnesium ion.

Summary. The ability of ethylenediamine tetra-acetic acid to form undissociated calcium complexes at physiological pH has been utilized as a tool to regulate available serum calcium levels *in vivo*. Further evidence has been presented for the regulatory action of magnesium ion on serum calcium levels.

5. Mendel and Benedict, *Am. J. Physiol.*, 1909, v25, 25

6. Becka, *Z. Ges. Exp. Med.*, 1929, v67, 252.

7. Plumb, Martell and Bersworth, to be published.

Received February 14, 1950. P.S.E.B.M., 1950, v74.

Effect of Physical Factors on Radiosodium Clearance from Subcutaneous
and Intramuscular Sites in Animals.* (17925)

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This experimental study was based upon

the recent work of Kety(1,2,3) and Elkin *et*

* Presented before the American Physiological Society, April 21, 1950.

1. Kety, S. S., *Am. J. M. Sc.*, 1948, v215, 352.

2. Kety, S. S., *Am. Ht. J.*, 1949, v38, 321.

al.(4) in which the rate of clearance of radiosodium from intramuscular injection sites has been used to measure the effective circulation of a tissue. Stone and Miller(5) established that lymphatic drainage does not have a significant role in the intramuscular clearance of Na^{24} , nor does the radioactive material diffuse along intramuscular planes. Since these reports indicate that the clearance of radiosodium may be of investigative and diagnostic aid this study was undertaken to determine: 1. The resting clearance rates of radiosodium from intramuscular and subcutaneous injection sites. 2. The effect of alterations in local temperature, abdominal pressure and hyaluronidase on the clearance rates.

Methods and materials. Radiosodium was prepared in the form of sodium chloride by neutron bombardment in the cyclotron of the Sarah Mellon Scaife Radiation Laboratory of the University of Pittsburgh. Approximately 5.0 microcuries of Na^{24} in physiological saline solution in the volume of 0.25 to 2.0 cc were used for injection into the hamstring muscles of 25 dogs of approximately 12 kg weight under pentobarbital sodium anesthesia. Subcutaneous injections were similarly made into the area on the medial aspect of the thigh in 10 dogs. Using a lead shielded Geiger-Muller tube, coupled to an autoscaler, the decline of activity as expressed in counts per minute was measured by multiple determinations at intervals of 2 minutes. Decline of activity was expressed as a slope or clearance constant after the method of Kety(2) using the formula,

$$K = \frac{\log C_1 - \log C_2}{0.4343 (T_2 - T_1)}$$

K = Clearance constant of Na^{24} .

C_1 = Activity as counts per minute at beginning time or T_1 .

C_2 = Activity as counts per minute at end time or T_2 .

0.4343 = Log e.

3. Wechsler, R. L., Sokoloff, L., and Kety, S. S., *Fed. Proc.*, 1950, v9, 133.

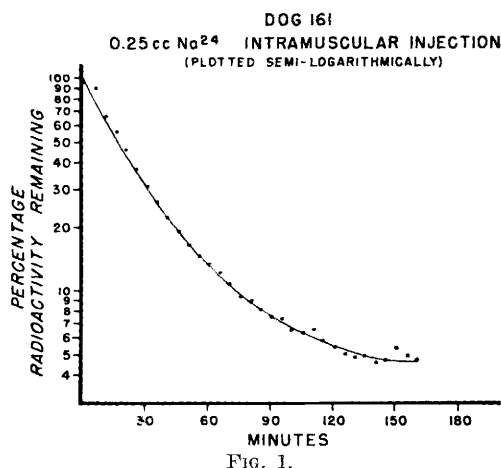
4. Elkin, D. C., Cooper, F. W., Jr., Rohrer, R. H., Miller, W. B., Jr., Shea, P. C., Jr., and Dennis, E. W., *Surg., Gynec. and Obst.*, 1948, v87, 1.

5. Stone, P. W., and Miller, W. B., *Proc. Soc. Exp. Biol. and Med.*, 1949, v71, 529.

The clearance of radiosodium, as the decline in activity measured at the injection site, was also conveniently expressed as the percentage of activity remaining per unit time considering the activity in counts per minute at initial time as 100%.

Following a preliminary 10 minute control clearance, determination of the effect of the application of radiant heat from an infrared source 18 inches from the skin, producing a surface temperature of 55°C , was studied in 6 dogs. The effect of local application of an ice-salt mixture to the site of injection was similarly studied in 8 dogs and the effect of applications of an abdominal cuff inflated to produce interruption of venous return was observed in 5 dogs. The effect of the addition of 250 viscosity units of hyaluronidase to subcutaneous injections was also determined. In a series of 6 rabbits under pentobarbital sodium anesthesia, alterations in clearance rates at intramuscular radiosodium injection sites were studied by separate and simultaneous clamping of the common iliac artery and vein in order to evaluate the comparative role of the artery and vein in altering clearance rates.

Fig. 1 shows the clearance of radiosodium from an intramuscular site observed for a total time of 161 minutes plotted semi-logarithmically. Because of the short period of observation with respect to the 14.8 hour half life of Na^{24} the results are not corrected for the decay of radiosodium over this period



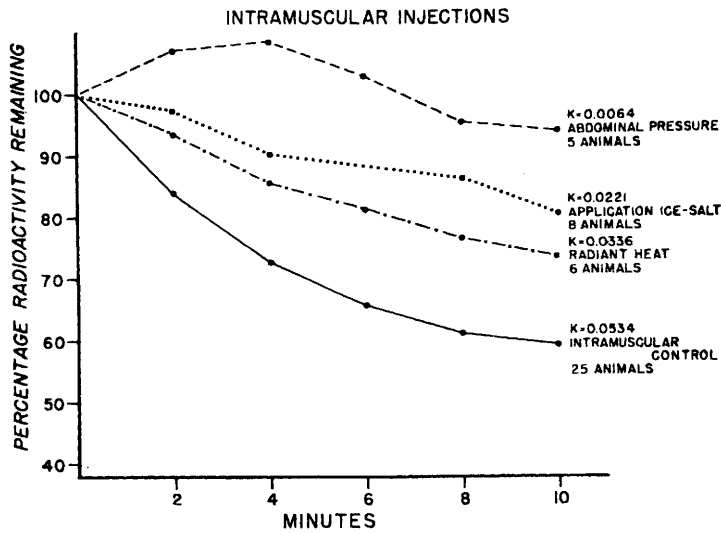


FIG. 2.

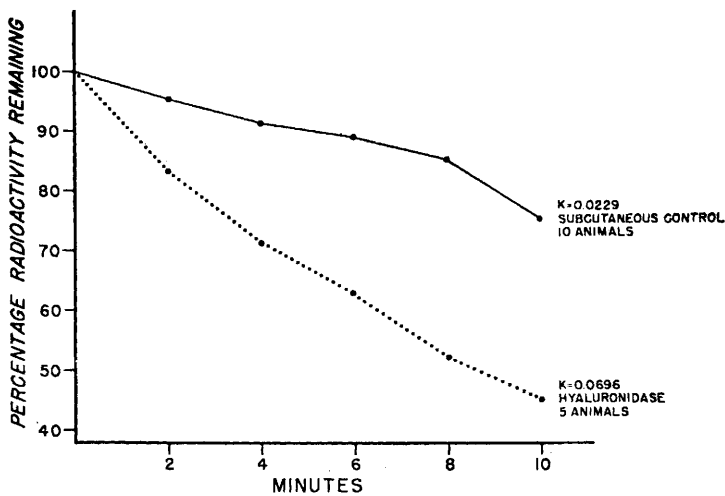


FIG. 3.

but demonstrate that over a relatively long time the rate of intramuscular clearance is not a true straight line function. In none of the intramuscular experiments did the data produce a straight line when plotted semi-logarithmically.

Fig. 2 summarizes the averages of the control and experimental results over a 10 minute observation period chosen for comparison purposes. The average intramuscular control clearance rate in 25 studies for a 10 minute period can be expressed by a clearance constant of 0.0534, or 59.4% activity remaining. Similarly, for 6 studies of radiant heat ap-

plied to the intramuscular injection site the clearance constant was 0.0336 or 73.5% activity remaining. The average clearance constant in 8 animals with the ice-salt application to the intramuscular injection site was 0.0221 or 80.5% activity remaining; with interruption of venous return by the abdominal cuff a clearance constant was obtained of 0.0064 or 94.0% activity remaining. All determinations showed a gradual decline of activity at the site of injection except during the application of pressure in which a preliminary increase in activity at the site of injection was observed.

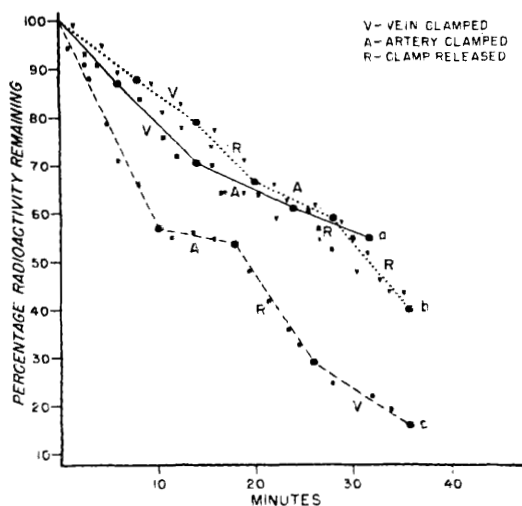


FIG. 4.

Fig. 3 demonstrates that with subcutaneous injection, the average control clearance constant was 0.0229 or 75.8% activity remaining. The addition of 250 viscosity units of hyaluronidase to the isotope solutions produced a marked increase to a clearance constant of 0.0696 or 45.6% activity remaining in 10 minutes.

In Fig. 4 the rate of intramuscular clearance in 3 anesthetized rabbits expressed in per cent activity remaining per unit time demonstrates the comparative effect on the clearance rate by clamping (a) the common iliac vein followed by clamping of the common iliac artery, (b) by clamping the vein followed by release of the vein and then clamping the artery, and (c) clamping the artery followed by release of the artery and then clamping the vein. Decreases in clearance rates in all instances were far more marked with arterial clamping than with clamping of the vein. In Fig. 4a and 4b there is no definite change in clearance rate with the vein clamped.

In these experiments comparison of the control intramuscular clearance rates with body weight, the depth of anesthesia, the volume of the radioactive solutions used, and the total amount of radioactivity per injection showed no discernible correlation.

Discussion. This method provides a technique to determine the gross effective circulation

of a tissue and to quantitate that flow by a standard clearance procedure. The technique is simple and the radiation hazards are minimal. Since the amounts of radioactivity injected are less than $5.0 \mu\text{c}$ a very small quantity of radiation is delivered at the site of injection even when the tissue clearance rate is slow. The total body exposure after the Na^{24} enters into the general circulation is 5% of that used by Smith and Quimby(6). Our results plotted semi-logarithmically (Fig. 1) failed to demonstrate a straight line function for the removal of radiosodium particularly after the first 10 minutes of study. Since the formula suggested by Kety for the calculation of clearance constants was based upon an exponential curve the slope of which should yield a straight line we prefer the simpler calculation of the percentage of activity remaining per unit time. This figure readily serves to indicate the rate of clearance and to quantitate the effective blood flow.

The results for the control intramuscular clearance of radiosodium of 59.4% activity remaining in 10 minutes compared favorably with those of approximately 5% removed per minute reported by others(2,4). The application of freezing mixtures to the site of injection produced a definite decrease in effective blood flow. However, the use of radiant heat to the point of producing visible hyperemia decreased the clearance of radiosodium. This result which is not in agreement with the generally accepted opinion of the effect of local heat, again demonstrates that the status of skin vessel dilation is an inadequate criterion of effective circulation. Abdominal pressure to the point of stopping venous return gave an average of 94% activity remaining after a 10 minute application. The early, small increase in radioactivity at the site of observation may represent a transitory backflow and leakage from vessel wall with the buildup of a pressure head before accessory channels aid in the clearance of the radiosodium.

The subcutaneous clearance rate of 75.8%

6. Smith, B. C., and Quimby, E. H., *Radiology*, 1945, v45, 335.

activity remaining in 10 minutes, showed a marked increase with addition of hyaluronidase to 45.6% activity remaining in 10 minutes. The preliminary study suggests definite advantages to the subcutaneous route over the intramuscular particularly for clinical study of effective blood flow because of the ease of injection, deviation of subsequent clearance rates and the inherent liability of unabsorbed radiation in deep tissue. It is possible that the increased rate of clearance subcutaneously with hyaluronidase represents marked diffusion rather than absorption.

The results of arterial and venous clamping of the common iliac vessels supports the recent view of Cullen *et al.*(7) that there is no benefit in concomitant venous ligation in acute arterial occlusion as was suggested by the early work of Makius(8) and Brooks *et al.*(9).

Summary. A study of the effect of physi-

7. Cullen, M. L., Streppacher, L. G., Greenspan, B., Milliken, H. E., Moore, G. W., and Morris, G. C., *Surg., Gynec. and Obst.*, 1949, v89, 722.

8. Makius, G. H., Bradshaw Lecture, London, 1914.

cal factors on radiosodium clearance in dogs is reported. The average clearance rate in 25 control intramuscular injections was 59.4% activity remaining after 10 minutes. Decreases in intramuscular clearance rates were demonstrated with application of ice-salt mixtures, radiant heat and pressure cuffs. The average subcutaneous clearance rate in 10 control studies was 75.8% activity remaining in 10 minutes. This rate of clearance was increased with the addition of hyaluronidase. The clearance of radiosodium from a part does not appear as a straight line function when plotted and the calculation of per cent activity remaining per unit time is suggested as an expression for determination of clearance rates. The present study entirely supports the view of Kety that this technic is applicable to measure the effective circulation of a tissue and indicates the need for further study to quantitate the procedure for clinical application.

9. Brooks, B., Johnson, G. S., and Kirtley, J. A., Jr., *Surg., Gynec. and Obst.*, 1934, v59, 496.

Received May 2, 1950. P.S.E.B.M., 1950, v74.

Activity of Ethyl-1-Ethanesulfonyl-4-Piperazine Hydrochloride on Traumatic Hemorrhagic Shock on the Dog. (17926)

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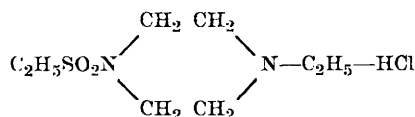
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Researches, in recent years, on traumatic shock therapy, have led us to examine the effects of the following substances on shock induced in the rat by means of the drum technic (Noble and Collip), with polyvinylpyrrolidone(1), some simple aliphatic amides (2), and the protective effect exercised by a new synthetic compound, ethyl-1-ethanesulfonyl-4-piperazine (3885 R.P.)(3).

* Address: Instituto Superiore di Sanita—Roma, (Italy).

1. Bovet, D., Couvoisier, S., and Ducrot, R., C.R., 1947, v224, 70

2. Bovet, D., Couvoisier, S., Ducrot, R., and Jacob, R., C.R., 1947, v224, 496.



In previous experiments we tested the effect of ethyl-1-ethanesulfonyl-4-piperazine (3885 R.P.) on shock produced in the mouse, rat and guinea pig, then decided to carry out further experiments on the dog. We used the technic of Wiggers, Ingraham and Dilles (4) which produced an irreversible shock following a prolonged hemorrhagic hypotension period. The animals were treated as

3. Bovet, D., Couvoisier, S., Ducrot, R., and Jacob, R., C.R., 1947, 227, 1423.