Kinetics of Distribution of Radioactive Labeled Muscle Relaxants:

III. Investigations with ^{14}C-succinyldicholine and ^{14}C-succinylmonocholine during Controlled Conditions

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A trace dose of ^{14}C-succinylcholine was administered intravenously to dogs under pentobarbital anesthesia and controlled ventilation with air. Five minutes later, 80 per cent of the radioactive material had disappeared from the plasma and 10 per cent was found in the urine. Seven hours later 1.4 per cent of the radioactivity was still present in the plasma, 70 per cent had been eliminated in the urine and 28.6 per cent was untraced. No radioactivity was detected in cerebrospinal fluid.

The presence in urine of succinyl*mono*choline and choline as degradation products was confirmed by radiochromatographic determinations.

Pretreatment with hexafluorenium (Mylaxen®) slowed the disappearance rate of ^{14}C-succinyl*di*-choline and its metabolites, and increased urinary elimination.

The plasma of the dogs was shown to bind succinyl*di*choline. The bound fraction gradually increased over five hours to 84 per cent of the total radioactivity (bound and unbound) present in the plasma. Succinyl*mono*choline also binds to plasma, but to a lesser extent. This correlates well with its more active urinary elimination.

IN PREVIOUS STUDIES,[1, 2] findings regarding the distribution, metabolism, and elimination of ^{14}C-labeled dimenthyl-*d*-tubocurarine in dogs under controlled conditions, as well as following hypoxia, hypercapnia, hemorrhagic shock, arterial hypotension, hypothermia and ligation of renal vessels, were reported. The present study was designed to elucidate the kinetics of distribution and the transport in plasma of succinylcholine and to determine whether distribution and protein binding of succinylcholine, in addition to rapid metabolism, might be responsible for the short-lasting muscle-paralyzing effects. The investigation was carried out in dogs, employing radiocarbon (^{14}C)-labeled succinyl*di*choline and succinyl*mono*choline.

Procedure

Twenty-eight adult mongrel dogs, weighing between 13 and 18 kg., were fasted for 12 hours, unpremedicated, and were anesthetized with an initial dose of 20 mg./kg. of sodium pentobarbital (Nembutal®) intravenously. Artificial ventilation with air was maintained through a cuffed endotracheal tube with a respirator set at a rate of 12 respirations per minute and a tidal volume of 300–400 ml. When necessary, anesthesia was maintained with repeated doses of pentobarbital in half the initial dose. Arterial blood pressure, lead II of the ECG, the EEG, as well as arterial *p*H, P_{O_2} and P_{CO_2} were recorded simultaneously and continuously. Physiologic saline solution was infused through an 18-gauge intravenous needle (8–10 drops/minute) to maintain blood volume, extravascular fluid, and constant urinary output for the duration of the experiments. Blood and plasma volumes were calculated, employing the ^{131}I-serum albumin method.[21] Both ureters were cannulated and urine samples collected at intervals. An indwelling 20-gauge spinal needle was inserted into the cisterna magna. A few minutes were allowed to establish control values; then an average dose of 50 microcuries of ^{14}C-succinyl*di*choline, specific activity 6.32

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mC./m*M*, purity controlled with radiochromatography, and labeled at the ammonium methyl groups, was administered rapidly, intravenously. Samples (average number, seven) of the following were taken during the seven hours after injection: a) 1.5–2.5 ml. arterial blood, b) 0.5 ml. cerebrospinal fluid, and c) a few ml. of urine. A parallel study was conducted in dogs to which 50 microcuries of ^{14}C-succinyl*mono*choline, specific activity 4.87 mC./m*M*, had been administered intravenously.

A group of 11 dogs, maintained under the same conditions as the control animals, was pretreated with hexafluorenium (Mylaxen®) intravenously (1.5 mg./kg.), in nine animals, as a single dose, and in two in an intravenous infusion (average 18 drops per minute). Radiolabeled succinyl*di*choline was then administered intravenously in the same dosage as above.

Plasma of clinically normal adults was incubated *in vitro* with known trace amounts of ^{14}C-succinyl*di*choline, and equilibrium dialysis[8] was performed to study the *in vitro* binding to plasma protein. Two ml. of plasma were dialyzed * for 24 hours at room temperature against 4 ml. of 0.9 per cent saline solution. Plasma of blood taken from dogs at various intervals subsequent to the administration of ^{14}C-succinyl*di*choline and ^{14}C-succinyl*mono*choline, was also dialyzed. In order to determine that fraction of plasma involved in binding succinyl*di*choline and succinyl*mono*choline, Krebs-Ringer phosphate solutions of human serum albumin and of gamma-globulin † in physiologic concentrations were prepared and the equilibrium dialysis method applied.

The radioactivity of ^{14}C was determined with a liquid scintillation counting system according to a method previously described.[3]

Scanning of unidirectional ascending paper chromatograms of samples of urine was performed with an Actigraph II apparatus. Buta-

* A Visking Najax casing, 3/8 in. flat width, was employed.

† Kindly provided by Cutter Laboratories.

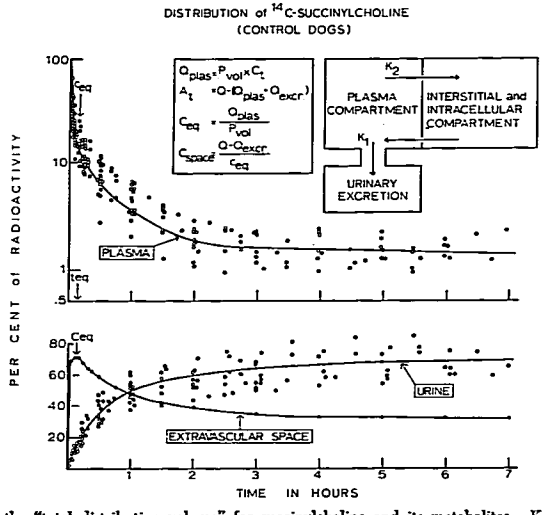

FIG. 1. Disappearance from plasma, cumulative urinary elimination, and passage into the extravascular space of radiocarbon (^{14}C) following intravenous administration of a trace dose of ^{14}C-succinyl*di*choline to control dogs. Q_{plas} is the amount of ^{14}C in plasma at any time following the administration, P_{vol} is the plasma volume in ml., Ct is the content of ^{14}C in 1 ml. plasma at time t from administration, A_t is the amount of ^{14}C in the extravascular space at any time t from injection, Q is the amount of radioactivity administered, Q_{excr} is the amount of ^{14}C excreted in urine at time t, c_{eq} is the amount of ^{14}C in 1 ml. plasma when plasma and extravascular space, having both attained a transient equilibrium point at time t_{eq}, both contain equal total amounts (C_{eq}) of radioactivity, C_{space} is the "total distribution volume" for succinylcholine and its metabolites. K_1 represents the transfer coefficient governing renal excretion, and K_2 the coefficient related to the passage into the extravascular compartment.

nol in acetic acid and water (100:30:85—V/V/V) was used as a solvent.

Results

Kinetics Studies Under Controlled Conditions

The radioactivity of a *trace* amount (*i.e.* having no detectable pharmacologic effects) of ^{14}C-succinyl*di*choline, equivalent to 4×10^5 counts/minute, administered to 28 dogs, disappeared rapidly from plasma. Five minutes after administration 80 per cent of the radioactive material was no longer present in plasma, having been distributed into the extravascular space. Conversely, only 10 per cent of the administered amount had been eliminated in the urine (fig. 1). The initial fast component of the plasma curve was followed by a second, much slower component of two hours' duration. Coincident with this, urinary excretion became significant, amounting to a total of 60 per cent of the administered dose. From the second hour on, the concentration of the radiocarbon in plasma remained close to 1.5 per cent. Seven hours following administration, an average of 1.4 per cent of radiolabeled material was still present in the plasma, 70 per cent had been eliminated in the urine, and 28.6 per cent was untraced (fig. 1).

From the slopes of the three components of the curve of disappearance of ^{14}C-succinyl*di*choline from plasma, K_1, the transfer coefficient governing renal excretion of the labeled drug and of its metabolites, and K_2, the coefficient relating to the passage into the extravascular compartment, were calculated (fig. 1).

The curve of passage into the extravascular compartment of the labeled material (succinyl*di*choline and its metabolites) presents: 1) a steep ascending slope which mirrors the phase of rapid disappearance of radioactivity from plasma; 2) an equilibrium point (C_{eq}); and 3), a descending slope. These two slopes may reflect the rapid rate at which succinyl*di*choline and its metabolites diffuse to and from the site of action during the period of full paralyzing effect.

Radioactivity was not detected in the cerebrospinal fluid. However, when labeled succinylcholine was injected into the cisterna magna of the dog, a significant amount of radioactive material was shown to pass into plasma and urine (fig. 2).

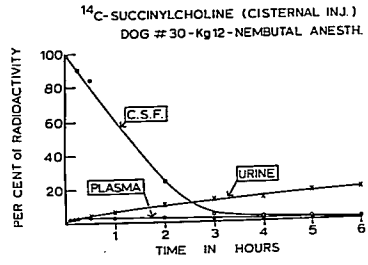

Fig. 2. Cisternal injection of a *trace* dose of ^{14}C-succinyl*di*choline in a dog. Rate of disappearance of ^{14}C from cerebrospinal fluid and of appearance in plasma, cumulative urinary elimination.

Subsequent to the intravenous administration of ^{14}C-labeled succinyl*mono*choline to the control dogs, a significantly higher urinary elimination of radioactive material was found, as compared with ^{14}C-succinyl*di*choline. In addition the passage into the extravascular space was significantly less (fig. 3).

Mylaxen-treated Dogs

An intravenous *trace* dose of ^{14}C-succinyl*di*choline was given to 11 dogs pretreated with hexafluorenium. Hexafluorenium, a potent inhibitor of plasma cholinesterase, is used clinically to reduce the dosage of succinylcholine and to extend the duration of its effect.[4-7] As shown in figure 4, under hexafluorenium treatment, a significantly slower disappearance rate of the radioactivity from plasma and a significantly increased rate of urinary elimination occurred.

Metabolic Studies

The distribution and urinary elimination in dogs of single intravenous trace doses of ^{14}C-succinyl*di*choline and succinyl*mono*choline so far has been expressed in terms of radioactivity, no reference being made to the original compounds. This was done in consideration of the fact that both compounds, following intravenous administration, are metabolized.[9-14] In order to establish how much of the radioactivity resides in the molecules of

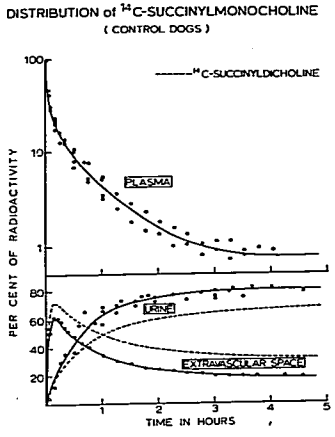

FIG. 3. Disappearance from plasma, cumulative urinary elimination, and passage into the extravascular space of radiocarbon (^{14}C), subsequent to the intravenous administration of ^{14}C-succinyl*mono*choline to control dogs.

the metabolites, and how much in the parent compound, the radiochromatographic technique was employed.

Scanning of paper radiochromatograms of urine after intravenous administration of ^{14}C-succinyl*di*choline, showed peaks located at a distance from the origin, quite different from the administered radiolabeled compound (fig. 5). The original ^{14}C-succinyl*di*choline was shown to be eliminated in part as such, in part as succinyl*mono*choline, and perhaps in part as choline. The identification of a urinary radioactive metabolite with succinyl *mono*choline was proven by administering the latter in the labeled form to control dogs, and by comparing the Rf ratio * of the urinary chromatogram (fig. 6).

* The *Rf ratio* is obtained by dividing the distance a compound has moved from the original point of application (origin) by the distance the solvent front has traveled from that point.[20] With the Rf ratio, it is possible to specify numerically the position of a substance on a chromatogram. The average value for ^{14}C-succinyl*di*choline was 0.16, and for ^{14}C-succinyl*mono*choline 0.45.

Protein Binding and Plasma Transport

Equilibrium dialysis was employed to study the binding capacity of plasma for ^{14}C-succinyl*di*choline *in vivo* and *in vitro*. According to this method, a cellulose sausage casing impermeable to protein molecules, but fully permeable to smaller ions, is filled with plasma in which ^{14}C-succinyl*di*choline (and/or its metabolites) is present, then immersed for 24 hours in a 0.9 per cent NaCl solution in a test tube. If the plasma binds some of the ^{14}C-succinyl*di*choline ions (or its metabolites), then at equilibrium, when the concentration of the unbound fractions on both sides of the membrane is equal, any increment of ^{14}C in the plasma compartment represents bound ^{14}C-succinyl*di*choline (and/or its metabolites).[8]

Experiments "in vivo." Subsequent to the intravenous administration of a *trace* dose of ^{14}C-succinyl*di*choline, plasma was shown to bind radiolabeled material (fig. 7). The bound fraction gradually increased in a five-hour period to 84 per cent of the total radioactivity (bound and unbound) present in plasma. It is likely that part of this fraction was made up of the metabolite ^{14}C-succinyl*mono*choline. In fact, we were able to demonstrate that the latter compound also binds to plasma, although at a slower rate (fig. 8) than succinyl*di*choline. The lesser binding of ^{14}C-succinyl*mono*choline correlates well with a simultaneous, more active urinary elimination of the original compound and its metabolites (fig. 3). Furthermore, during the first 45 minutes subsequent to administration, the binding to plasma of ^{14}C-succinyl*di*choline and its metabolites was negligible, although a high concentration of labeled materials was present. If this also holds true *in vivo*, then the equilibrium of protein binding would not occur until plasma concentrations of succinyl*di*choline and its metabolites had declined to negligible levels.

Experiments "in vitro." Incubation of 150 samples of plasma from clinically normal patients with known trace amounts of ^{14}C-labeled succinyl*di*choline and successive equilibrium dialysis showed that: a) equilibrium was reached in an average of three hours of dialysis; b) temperatures as low as 2° C. prolonged the time necessary to reach equilibrium to about five hours; c) at equilibrium, an

average of 30 per cent of the original radioactivity was bound to plasma, and this amount remained unchanged for as long as 48 hours. Similar results were obtained with ^{14}C-succinyl*mono*choline.

Subsequent to incubation of physiologic concentrations of human serum albumin and gamma-globulin with known trace amounts of ^{14}C-succinyl*di*choline, the following data were obtained: a) equilibrium was reached in four hours with serum albumin, and in eight hours with gamma-globulin; b) temperatures as low as 2° C. prolonged equilibrium time to seven hours for serum albumin; c) at equilibrium, an average of 25 per cent of the original radioactivity was bound to serum albumin, and 40 per cent to gamma-globulin. These percentages remained unchanged for as long as 24 hours.

Discussion

As a result of the degradation of (intravenously administered succinyl*di*choline,[9-14] it is obvious that the radioactivity of the radiolabeled drug traces the fate of the unmetabolized, as well as the metabolized, fractions of the administered compound. For this reason, the kinetics of distribution of ^{14}C-succinyl*di*choline have been expressed in terms of radioactive carbon present in the various compartments (plasma, extravascular space, urine, cerebrospinal fluid). The interdependency of the curves of distribution of the radiolabeled compounds in those compartments as obtained in the animals under controlled conditions may explain why the neuromuscular block induced by a single intravenous dose of succinyl*di*choline usually lasts 4–5 minutes. As shown in figure 1, the short duration of the paralyzing effects coincides remarkably well with the phase of rapid disappearance from plasma of the labeled material and with fast passage into the extravascular compartment. Conversely, the low urinary elimination (less than 10 per cent) cannot affect the kinetics of distribution significantly. The two slopes of the curve of passage into the extravascular space, the peak of which is reached during the same interval of five minutes, might reflect the rate at which the drug diffuses to and from its site of action during the period of full paralyzing effect. Then rapid distribution, more than metabolism

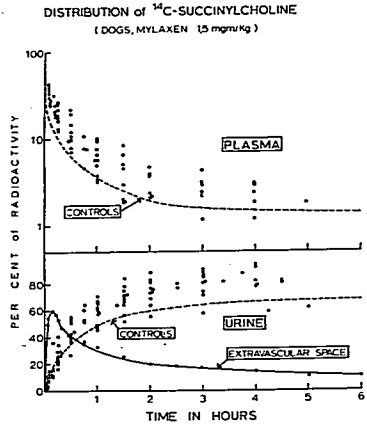

FIG. 4. Disappearance rate from plasma, urinary elimination, and passage into the extravascular space of ^{14}C of a *trace* dose of ^{14}C-succinyl*di*choline intravenously administered to dogs pretreated with hexafluorenium 1.5 mg/kg.

and urinary elimination, plays a fundamental role in the short-lived neuromuscular effect of succinyl*di*choline. The more important role of distribution as compared with that of metabolism is suggested strongly by the fact that the principal metabolite of succinyl*di*choline, succinyl*mono*choline, is also a muscle-paralyzing agent, and is metabolized very slowly.[14] The paralyzing effect of succinyl*di*choline cannot, therefore, be terminated by its metabolism only.

That no radioactivity was detected in the cerebrospinal fluid suggests that succinylcholine and its metabolites do not cross the "blood-to-cerebrospinal fluid barrier" and, possibly, do not exert central effects. However, the finding that ^{14}C-succinyl*di*choline, when injected directly into the subarachnoid space, disappeared from the cerebrospinal fluid coincident with a significant passage into plasma and with active urinary elimination proves that curarizing agents, similar to other quaternary ammonium compounds, may leave the cerebrospinal fluid through the arachnoid villi and/or across the choroid plexuses.[15-17] Furthermore, as we shall point out, a fraction of

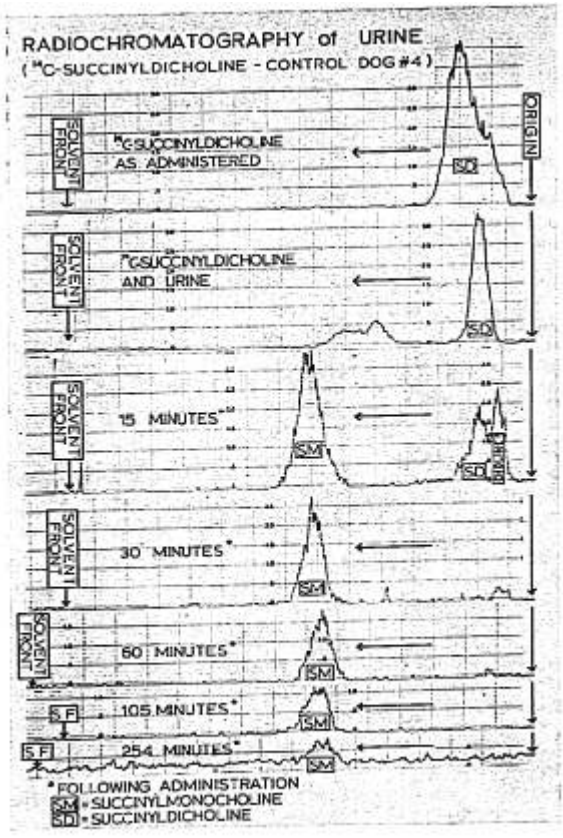

FIG. 5. Paper radiochromatograms of ^{14}C-succinyl*di*choline as administered, ^{14}C-succinyl*di*choline added to a sample of normal urine, and samples of urine subsequent to intravenous administration of ^{14}C-succinyl*di*choline.

succinyl*di*choline and its metabolite, succinyl*mono*choline, binds to plasma protein. In consequence of the formation of a plasma-succinyl*di*choline and -succinyl*mono*choline complex, it may be difficult to anticipate exchanges of succinyl*di*choline between a protein-rich compartment (plasma) and a relatively protein-free compartment (cerebrospinal fluid).

Seven hours after the administration of ^{14}C-succinyl*di*choline, a total of 70 per cent of the labeled material was recovered in urine, while 1.4 per cent was still in plasma: this left 28.6 per cent untraced. The question arises whether this fraction was bound to protein and/or stored in tissue cells. Recently, attention has been drawn to the importance of protein binding as a factor affecting absorption, transport, metabolism, and excretion of anesthetics, as well as the actual mechanisms by which these substances produce anesthesia.[18, 19]

Our data show that subsequent to intravenous administration plasma binds a fraction of succinyl*di*choline and its metabolite, succinyl-

*mono*choline. In this regard, it is known that plasma binding results in the formation of a pharmacologically inactive complex, with no access to sites of action, excretion and metabolism. The protein-drug linkage is usually a labile one. As a result of this lability, the drug (and/or its metabolites) can be liberated easily in the free form. Furthermore, special factors such as variations in blood *p*H, protein content, body temperature, drug concentration, and the dissociation constant of the drug-protein complex may all affect the ratio of bound/unbound drug.[8] One or more of these factors may at times assume importance during anesthesia. It might be anticipated also that interactions of free succinyl*di*choline are not limited to plasma proteins but, as is well documented for a multitude of other drugs, may occur with proteins of all cells of the organism, and in particular with the "specialized functional protein," *receptors*. One wonders, therefore, about the possibility that the drug, especially when

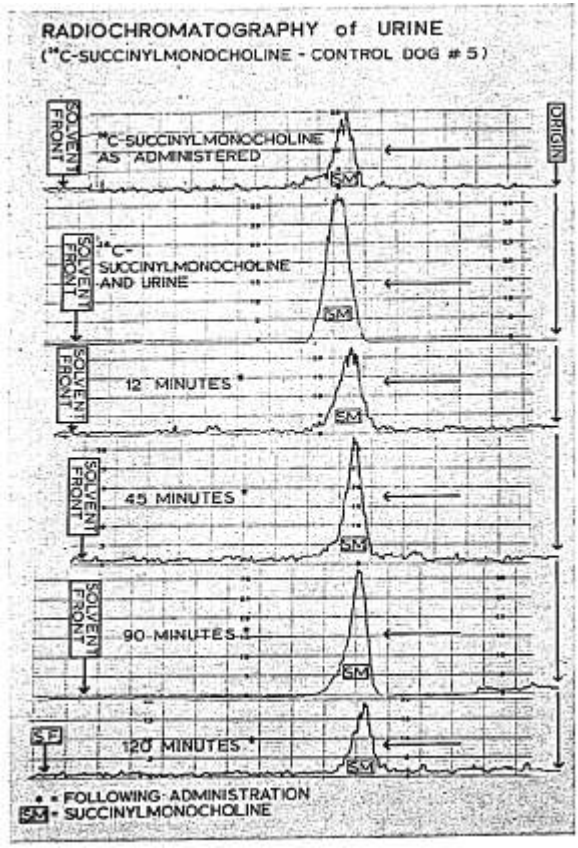

FIG. 6. Paper radiochromatograms of ^{14}C-succinyl*mono*choline as administered, ^{14}C-succinyl*mono*choline added to a sample of normal urine, and samples of urine subsequent to intravenous administration of ^{14}C-succinyl*mono*choline.

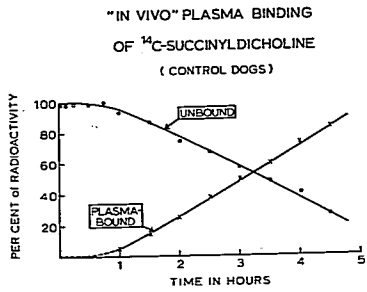

FIG. 7. Equilibrium dialysis of samples of plasma from control dogs, subsequent to the intravenous administration of ^{14}C-succinyl*di*choline. Each value is the average of five experiments. Plasma proteins gradually bind ^{14}C-succinylcholine and its metabolites. The binding, however, is negligible during the first 45 minutes after injection, even though a high concentration of labeled materials is present in plasma during this interval. Five hours after injection, 84 per cent of the total plasma radioactivity is bound to plasma.

administered in high and/or continuous doses, is released from its labile combinations and causes delayed muscle-paralyzing effects. Finally, considering the variety of agents and drugs simultaneously administered during anesthesia and surgery, the possibility also exists that multiple interactions with plasma and other proteins result in competition for protein-binding sites and, as a consequence, increase the concentration of unbound (active) succinyl*di*choline and succinyl*mono*choline.

A schematic representation of the reversible interactions affecting succinyl*di*choline, subsequent to its intravenous administration in a single dose, is shown in figure 9. The complexity of these interactions, and the importance of factors other than a rapid metabolism of the drug, are evident.

Summary and Conclusions

The kinetics of distribution of succinylcholine was studied in dogs by intravenous administration of a single trace dose of radiocarbon (^{14}C)-labeled succinyl*di*choline and succinyl*mono*choline.

It was shown that rapid distribution, and possibly degradation, are responsible for the short-lived neuromuscular effect of succinyl*di*choline, whereas urinary elimination seems to play a far less important role.

No labeled material was found in the cerebrospinal fluid after intravenous administration of ^{14}C-succinyl*di*choline, suggesting that under physiologic conditions succinyl*di*choline and its metabolites do not cross the "blood-to-cerebrospinal fluid barrier," and possibly do not penetrate into the central nervous system.

Seven hours after intravenous administration of ^{14}C-succinyl*di*choline, a total of 70 per cent of the labeled material had been eliminated in the urine, 1.4 per cent was still in plasma, and 28.6 per cent was untraced.

Radiochromatographic scannings of urine confirmed the presence of succinyl*mono*choline and perhaps choline as degradation products.

Treatment with hexafluorenium slowed the disappearance rate of ^{14}C-succinylcholine and its metabolites from plasma and significantly increased their urinary elimination.

Plasma proteins bind succinyl*di*choline and succinyl*mono*choline *in vivo* and *in vitro*. Consequences and characteristics of this binding are: 1) the formation of a pharmacologically inactive complex; 2) the lability of this binding and, therefore, the possibility that active succinylcholine may be released easily; 3) the importance of blood *p*H, body temperature, blood protein content, and the dissociation constant of the succinylcholine-protein com-

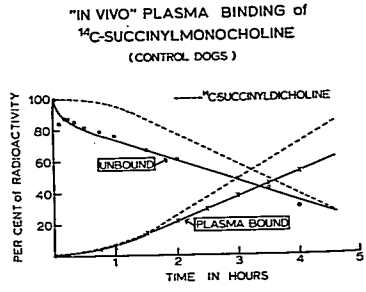

FIG. 8. Equilibrium dialysis of samples of plasma from control dogs, subsequent to the intravenous administration of ^{14}C-succinyl*mono*choline. Each value is the average of values for four experiments.

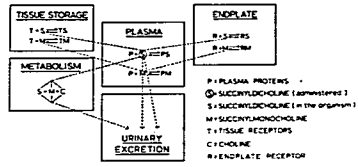

FIG. 9. Reversible interactions upon the molecule of succinyl*di*choline, subsequent to intravenous administration. It is postulated that succinyl*di*choline and its metabolite, succinyl*mono*choline, interact not only with plasma protein, but also with tissue and endplate receptors. The remaining fraction is metabolized and excreted.

plex in affecting binding; 4) the possible competition for protein-binding sites by other drugs simultaneously present in plasma, and the consequent liberation of active succinylcholine.

Human plasma, as well as human plasma albumin and gamma-globulin, *in vitro* bind succinyl*di*choline and succinyl*mono*choline.

The technical assistance of Miss Nancy Freeman is very much appreciated.

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