THE DISTRIBUTION, DESTRUCTION AND ELIMINATION OF MUSCLE RELAXANTS

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An excellent review by Foldes on the fate of muscle relaxants has been published recently.1 The present review deals particularly with d-tubocurarine and succinylcholine. Modes of application other than intravenous injection are disregarded. It may be useful to start with some definitions and general remarks.

Definitions

Distribution: The distribution of drugs in the body may be defined as a process of moving drug molecules into various compartments. Typical compartments are body fat, plasma, extracellular water, and total body water. Such compartments cannot easily be described in anatomical terms. Classes of specific proteins, lipids or polysaccharides can also function as compartments which accept drugs but do not provide a continuous medium for drug movement.2 If a drug exerts its action on the surface of a cell, the concentration in extracellular water must be related to the intensity of action. Once a drug is distributed throughout the extracellular water ("the central compartment"), its concentration in this compartment is reduced by any further process of distribution, whether this process be a binding to protein or a dilution in a larger fluid compartment. There is evidence which suggests that distribution processes are important for the termination of action of all relaxants, including succinylcholine.

Excretion: Solubility of drugs in lipids favors their reabsorption from the renal tubules;3 ionization decreases the lipid solubility. Hence, the body frequently eliminates a drug by converting it into an ionized compound. The commonly used skeletal muscle relaxants have two cationic charges and thus need no conversion for their urinary excretion. A substantial renal elimination by glomerular filtration is therefore to be expected for all relaxants, unless they are quickly destroyed. Some quaternary agents other than relaxants become involved in specialized tubular mechanisms.4 Thus tetroethylammonium is eliminated by glomerular filtration and in addition by tubular secretion.4

Biliary excretion appears to be favored by ionization,5 and several relaxants have been demonstrated in bile.6,7 Intestinal reabsorption is generally slow for ionized compounds, but not necessarily negligible, as shown by Waser et al. for C-curarine.6

DeSTRUCTION: Many of the nonspecific enzymes which are capable of destroying drugs are contained in the microsomes.8 It has been suggested that ionized drugs do not have ready access to these enzymes.3 This should restrict the pathways of destruction for the relaxants, or at least tend to slow the rate of metabolic conversion. Rapid rates of destruction are known only for succinylcholine and similar esters which are hydrolyzed in serum where no membranes are to be passed in order to reach the hydrolyzing enzyme.

\[d\text{-tubocurarine}\]

The fate of d-tubocurarine will be reviewed in some detail because this information may help in assessing the distribution of other relaxants. The passing of d-tubocurarine through the body will be described as occurring in three different but overlapping, phases. Although this division is based on hypothetical grounds, it permits a grouping of experimental observations and emphasizes the blank spots in our knowledge.

First Phase: Distribution in Extracellular Fluid and Binding to Plasma Proteins: Plasma levels of d-tubocurarine in man have been determined by Mahfouz,9 Pittinger, Morris and Cullen,10 Marsh,11 Cohen, Paulson and Elert,12 Aladjemoff, Dikstein and Shafrir.13 Typical examples from some of these papers are shown in a semilogarithmic plot (fig. 1).
One observation covers a period of 3 hours; this is the longest test which has been recorded. Aladjemoff et al. used more than double the dose Marsh used but the resulting plasma concentrations which are shown in figure are similar. There is no sign to indicate that this is due to different techniques of investigation. All the test subjects of Cohen et al. received an identical dose but their plasma levels vary as widely as the levels shown in the highest and lowest curves of figure 1. If there is any regularity in these curves, it is not expressed by their levels but by their shapes. All curves show an initial rapid drop of plasma levels which is followed by a gradual decline. The data are compatible with the assumption that the two slopes in each curve are identical for all sets of measurements. The initial steep slope is drawn to indicate a process with a half life of 5.7 minutes (table 1). The further decline is assumed to proceed with a half life of 45 minutes during the first one or two hours.

Curves of a shape similar to those of figure 1 are encountered, for instance, in studies on the distribution of radiosodium. The steep initial slope is explained as the rapid transfer of sodium from plasma to interstitial fluid. Hence the steep initial drop of plasma levels of d-tubocurarine is likely to indicate that the drug enters the interstitial fluid. Volumes of distribution are calculated from the ratio of dose to observed concentrations. At the end of an intravenous injection, the volume of distribution is approximately 5 per cent of body weight, i.e., the plasma volume. After equilibration, numerous substances occupy 20–25 per cent of body weight, namely the extracellular fluid volume. If one analyzes the available data on d-tubocurarine, the results shown in table 1 are obtained.

The interpretation of table 1 should take into account the following sources of error besides the experimental error. It requires at least five circulation times for an intravenously injected substance to be evenly distributed throughout the plasma. Hence, individual determinations of plasma levels within a few minutes after injection do not necessarily result in representative data, but an average of several determinations is not likely to be affected by this error. The dose in mg./kg. is not an ideal measure since it does not take into consideration variations in body fat or body water. Data on the apparent volume of distribution are subject to systematic error from the choice of a fixed slope for the extrapolation. However, while some data would permit the choice of slightly different slopes from the one used here, they all seem to permit the interpretations which will be presented.

In spite of possible errors, the data of table 1 strongly suggest that one to two minutes after injection most of the tubocurarine is still in the circulating plasma. The average initial volume of distribution indicates that only about 20 per cent of the injected amount has left the plasma. This estimate, which is based on a comparison of the first measurement with theory, agrees with the estimate of the half time which is based on the comparison between the first two measurements.

Pittinger et al. and Marsh state that d-tubocurarine started to act before they had begun their first sampling. In other words, the drug action started before it had come into equilibrium in the extracellular fluid. If suitably stained preparations of skeletal muscle are inspected, a close spatial relationship between capillaries and end-plate is apparent. Several tissue sections checked by Dr. S. H. Bensley of the Department of Anatomy, University of Toronto, showed capillaries touching the end-plate, indicating there is a very short distance for diffusion from plasma into the end-
TABLE 1

THE ENTRANCE OF d-TUBOCURARINE INTO THE INTERSTITIAL SPACE AFTER INTRAVENOUS INJECTION INTO 21 PERSONS (DATA ARE BASED ON CHEMICAL DETERMINATIONS OF BLOOD LEVELS REPORTED IN THE LITERATURE)

<table>
<thead>
<tr>
<th>Source of Data</th>
<th>Dose mg./kg.</th>
<th>Initial Volume of Distribution (Observed as Indicated time in Minutes) Per Cent of Body Weight</th>
<th>Apparent Volume of Distribution After Equilibration (Extrapolated Values) Per Cent of Body Weight</th>
<th>Minutes for Reduction to Half Initially Determined Plasma Level</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pittinger et al.\textsuperscript{19} L.c., Table I and Figure 1</td>
<td>0.23</td>
<td>8.2 (2)</td>
<td>32.8</td>
<td>2</td>
<td>Patient 1</td>
</tr>
<tr>
<td></td>
<td>0.21</td>
<td>4.4 (1)</td>
<td>17.4</td>
<td>7</td>
<td>Patient 2</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>4.2 (2)</td>
<td>18.5</td>
<td>7</td>
<td>Patient 3</td>
</tr>
<tr>
<td></td>
<td>0.21</td>
<td>3.4 (2)</td>
<td>6.3</td>
<td>10</td>
<td>Patient 4</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>3.7 (2)</td>
<td>10.0</td>
<td>5.5</td>
<td>Patient 5</td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>3.3 (2)</td>
<td>17.1</td>
<td>4.5</td>
<td>Patient 6</td>
</tr>
<tr>
<td>Marsh\textsuperscript{11} L.c., Figure 4</td>
<td>0.15</td>
<td>4.8 (1)</td>
<td>6.8</td>
<td>5</td>
<td>Healthy subject</td>
</tr>
<tr>
<td>Cohen et al.\textsuperscript{14} L.c., Figure 1</td>
<td>0.22</td>
<td>4.4 (1)</td>
<td>5.2</td>
<td>5</td>
<td>Subject A</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>4.7 (1)</td>
<td>5.5</td>
<td>9</td>
<td>Subject B</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>—</td>
<td>6.9</td>
<td>—</td>
<td>Subject C</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>5.6 (1)</td>
<td>14.0</td>
<td>13</td>
<td>Subject D</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>7.2 (1)</td>
<td>16.7</td>
<td>7</td>
<td>Subject E</td>
</tr>
<tr>
<td>Aladjemoff et al.\textsuperscript{13} L.c., text and Figure 1</td>
<td>0.33</td>
<td>3.5 (2)</td>
<td>6.7</td>
<td>5</td>
<td>Average of two refractory patients</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>9.2 (2)</td>
<td>16.6</td>
<td>4</td>
<td>Average of seven patients</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>6.3</td>
<td>13.7</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

\* Doses in mg./kg. multiplied by 100 and divided by the initially observed plasma level. The average indicates that most of the drug is still in the circulating plasma (5 per cent of body weight).

\dagger Approximately 10 minutes after injection, plasma levels decrease at a slow rate (fig. 1); plasma levels observed during that period of slow decrease were extrapolated to time zero to give a hypothetical level, from which the indicated volumes were calculated as for the previous column. The extrapolation was made graphically on semilogarithmic plots. The average is less than the extracellular fluid volume (22 per cent of body weight), and suggests that a part of the dose is bound to plasma proteins.

\ddagger Calculated from difference between first and second determinations made within 7 minutes after injection.

plate (fig. 2). Couteaux\textsuperscript{18} describes endothelial nuclei of the blood capillaries as one component of the heap of granular nucleated substance at the junction.

The ability of the end-plate to hold relaxant molecules has been recently demonstrated\textsuperscript{19, 20} for C-curarine and decamethonium with the aid of autoradiographs. As many as $8 \times 10^6$ molecules of C-curarine could be bound per end-plate. There is every reason to assume that d-tubocurarine could be bound in a similar manner.

The circulation time in a healthy subject at rest is about one and a half minutes. Thus tubocurarine apparently starts to act in its first passage through the muscle, and the time for diffusion from the capillary to its site of action can only be seconds. In short, the end-plate seems to be bombarded with curare molecules from the plasma without prior equilibration in the interstitial fluid. This process appears to be possible because of the close proximity of capillaries and end-plate and the special affinity of tubocurarine for the end-plate.

As shown by Hanna,\textsuperscript{21} the tertiary amine, dihydro-beta-erythroidine, acts still faster than d-tubocurarine and other quaternary relaxants in cats. In travelling from plasma to the site of action, the relaxant molecule has to penetrate at least the capillary wall and the axonlemma. Hence, in spite of its great speed of action, the tubocurarine molecule seems to be
slightly delayed at these barriers because of its ionization.

Marsh \textsuperscript{11} injected a small dose of d-tubocurarine into a human subject. The effects began 30 seconds after the end of injection and lasted for 4 to 6 minutes. Within these few minutes, the plasma levels fell rapidly, indicating that the injected amount was being distributed in the extracellular fluid. The drug concentration near the end-plate must have decreased during this process: the forces of attraction between drug and receptor were overcome by the tendency of the drug molecules to diffuse away from the end-plate so that the action ceased. As will be explained, this particular process of distribution throughout extracellular water does not seem to terminate the action of high doses.

The relative size of the extracellular space varies from organ to organ.\textsuperscript{16} The extracellular fluid content of skeletal muscle varies somewhat between types of muscle but it is usually 100–150 ml per kilogram of muscle.\textsuperscript{22} Hence, if a drug is evenly distributed throughout the extracellular fluid, the amount per gram of skeletal muscle will not exceed 15 per cent of the amount per milliliter of plasma. The data of Mahfouz\textsuperscript{9} on rabbits sacrificed 10 minutes after the injection of tubocurarine show its concentration in skeletal muscle to be about 10 per cent of the plasma concentration. The distribution of curare throughout the extracellular fluid may still have been incomplete at that time.

In man, this first phase of distribution of tubocurarine between plasma and interstitial fluid must be finished within 10 to 20 minutes. This can be seen from the curves of figure 1. Furthermore, Cohen et al.\textsuperscript{12} compared plasma levels of the drug in arterial and venous blood: arterial were higher than venous levels up to 12 minutes after injection but the levels were alike after 20 minutes.

From all these considerations, one might expect that the volume of distribution after
equilibration between plasma and interstitial fluid should correspond to the volume of extracellular water, i.e., 20–25 per cent of body weight. However, the apparent volume of distribution is, on an average, only 13.7 per cent as shown in table 1. The simplest explanation is that a portion of the injected tubocurarine is bound to plasma proteins. A bound drug is not free to diffuse rapidly into the interstitial space.23 Direct evidence for the occurrence of binding of d-tubocurarine to plasma proteins has been presented by Aladjemoff et al.13 The drug could be bound in man to both albumen and γ-globulin. The binding to albumen was less readily reversible.

Data in the column on the “apparent volume of distribution after equilibration” in table 1 show a peculiar person to person variation. Measurements are either close to plasma volume or close to extracellular fluid volume, with only one or two intermediate values. Thus, tubocurarine appears to be bound to a small extent in some sera and to a large extent in others. Aladjemoff et al.13 have demonstrated this directly: in two of nine subjects, tubocurarine was strongly bound to albumen and plasma levels were therefore high. These two subjects were investigated because they had been refractory to tubocurarine. Thus it was only natural to conclude that the refractoriness was due to excessive protein binding. However, the collection of data in table 1 suggests that other authors have investigated subjects with just as much protein binding without observing refractoriness. There is a problem which needs further investigation.

Mahfouz9 data on plasma levels which were obtained with a bioassay, differ slightly from data10–13 obtained by chemical methods.24, 25 This may indicate that Mahfouz measured that portion of the drug which was not bound to protein while the other investigators determined the total drug concentration. Species differences may account for the report that proteins of beef plasma did not bind tubocurarine.26

A puzzling observation has been reported by Cohen et al.12 An injection of d-tubocurarine was followed after 3 minutes by an injection of edrophonium. This led to a rapid diminution of plasma levels of the relaxant. Six minutes after the injection of edrophonium, the curare concentration started to rise again to reach, after 10 minutes, the level observed in the absence of edrophonium. The figure presented by Cohen et al. indicates that the antagonism to relaxation persisted for more than 20 minutes. Hence, the reviewer assumes that the lowering of plasma levels of curare and the antitcurare action are two different functions of edrophonium. Edrophonium may compete with tubocurarine for the binding sites on plasma proteins, as well as on neuromuscular receptors. A release of tubocurarine from plasma protein binding, a consequent spreading through the extracellular fluid and a return to the plasma proteins after edrophonium has left the plasma, could explain the observed phenomenon. Neostigmine may cause the same sequence of events at a slower rate.

SECOND PHASE: Redistribution and Urinary Excretion: As visible in figure 1, and perhaps even more clearly in some of the original publications, plasma levels of d-tubocurarine decrease at a slow rate after the first rapid equilibration between plasma and interstitial fluid has taken place. If the first distribution phase has been correctly interpreted, and if the binding to plasma protein is reversible, this slow decrease must be due to a diminishing concentration of drug dissolved in extracellular water. There are several observations indicating the possible nature of the processes which cause this slow decline of drug levels.

With the aid of equations developed by Teorell,27 Kalow28 analyzed urinary excretion rates of d-tubocurarine and arrived at a conclusion regarding the rate of change of plasma levels. The slopes of the shallow portion of the smooth curves in figure 1 are the theoretical slopes calculated from urinary excretion rates. There appears to be an agreement between theory and experimental observation. The theory suggests that plasma levels during this phase decrease with a half time of about 45 minutes for two reasons: within a given time, about one-third of the disappearing drug is excreted in the urine, while about two-thirds move into some unknown compartment which was called "tissues." This phase lasts for 2 to 3 hours, when the process of destruction gradually becomes rate-determining.

In regard to renal excretion, this concept is
supported by several observations which show about one third of an injected amount of d-tubocurarine appearing in the urine.\textsuperscript{6, 11, 28} The drug causes a temporary suppression of urine formation\textsuperscript{7, 11} (cf. c-curarine\textsuperscript{6}).

There is also evidence for the occurrence of a redistribution. Only the compartment into which the drug moves cannot be identified with certainty; it is not necessarily a single biochemical or anatomical entity. The data of Marsh\textsuperscript{11} on rats sacrificed 3 hours after the injection of radioactive tubocurarine-dimethyl ether show that the concentration in muscle and brain is two-thirds the plasma concentration. This might suggest that the compartment of redistribution is the total water of these organs.\textsuperscript{18} Such a conclusion would not necessarily be invalidated by the observation that tubocurarine\textsuperscript{9} and its dimethyl ether fail to enter the erythrocytes.\textsuperscript{11} Permeability constants of drugs differ for various types of cells.\textsuperscript{20} Some membranes form a relative, rather than an absolute, barrier for quaternary ions;\textsuperscript{30} usually little, if any, d-tubocurarine permeates the placental barrier\textsuperscript{51, 52} but it does penetrate if the concentration gradients are sufficiently high.\textsuperscript{23, 54} Ebbinghaus and Otte\textsuperscript{7} found by bioassay some d-tubocurarine in the saliva of dogs. Mahfouz\textsuperscript{9} found more d-tubocurarine in the brain of rabbits than can be accounted for by the blood content. It is conceivable that the permeation of membranes by d-tubocurarine is facilitated by the formation of zwitterions.\textsuperscript{35} Furthermore, as will be discussed below, the major portion of injected tubocurarine seems to be destroyed in the body; most enzymes capable of metabolizing drugs are located in microsomes,\textsuperscript{5, 8} which again suggests that tubocurarine might enter some cells. Thus until the contrary is proved, the possibility should not be dismissed that the extracellular concentration of d-tubocurarine is lowered by a process of dilution in the total water of skeletal muscle and/or some other organs. However, some binding to tissue constituents is also likely to occur.

Creese, Taylor and Tilton\textsuperscript{36} prepared iodocholinium, a radioactive relaxant resembling decamethonium. Isolated diaphragms of guinea pigs kept in a solution of this relaxant, gradually accumulated the drug. After a period of 12 hours, the drug content of 1 Gm. of muscle equaled the drug content of 4.5 ml. of surrounding solution. This is proof for a binding of the drug to muscle constituents which may not only be neuromuscular receptors. The presence of d-tubocurarine diminished the uptake of iodocholinium by the muscle, suggesting competitive occupation of binding sites. Hence, muscle proteins may form part of the pool into which d-tubocurarine disappears.

Binding of d-tubocurarine does not only occur with proteins. Paton\textsuperscript{57} mentioned an ability of heparin to combine with d-tubocurarine. Since heparin is an acid mucopolysaccharide like hyaluronic acid and chondroitin sulfate,\textsuperscript{37b} the question arises whether intercellular ground substance acts as an ion exchanger to bind tubocurarine.

As a reservoir for d-tubocurarine, the liver may accumulate some of the drug but not a substantial amount. If the relaxant is injected into rats and cats and the liver excluded from the circulation shortly afterwards, a reopening of the liver vessels causes recirculation;\textsuperscript{38} hence liver proteins cannot rapidly bind the drug. If tubocurarine is injected into a splenic vein, more of the drug reaches the general circulation than after injection into a femoral artery.\textsuperscript{30} Ten minutes after injection into rabbits, the liver contained 3 per cent of the administered amount.\textsuperscript{9} In dogs, tubocurarine was measurable in bile\textsuperscript{7} but only up to 2 hours after injection. Three hours after the injection of d-tubocurarine dimethyl ether into rats, the concentration in liver equaled the concentration in plasma;\textsuperscript{11} conversion products are included in this figure.

In summary, plasma levels of d-tubocurarine during this second phase seem to be lowered by urinary excretion and by a process of redistribution. While one cannot be certain about the nature of this redistribution, it must have a bearing on the termination of action if the duration of this action exceeds 20 minutes. Pelikan \textit{et al.}\textsuperscript{49} found that after 45 minutes a second injection of half the initial dose gave the same effect as the full initial dose. This observation gives quantitative support to the interpretation that 45 minutes after an injection, half the administered amount is still in plasma and interstitial fluid.
Fifth Phase: Destruction: About two thirds of injected d-tubocurarine seem to be destroyed, since only one-third is excreted in the urine, and there is no suggestion of any substantial elimination with the feces. The isomer d-chondocurarine appears only in traces in the feces of animals.

The mathematical analysis of urinary excretion rates suggested that d-tubocurarine is destroyed in that compartment which is reached by redistribution from the extracellular fluid. That is, the destruction seems to start gradually during the second phase, while more and more of the drug reaches the unknown compartment. Three to four hours following the injection, the concentrations in extracellular fluid and in that compartment have nearly reached equilibrium, so that the rate of destruction determines more and more the rate of disappearance of tubocurarine. The rate of the destruction process can be described by a half-life of about 3 1/2 hours. Although the rate of destruction is slow, urinary excretion becomes of lesser importance because the amount of drug in the extracellular fluid is smaller than the amount in the compartment in which it is destroyed. In other words, in spite of a slow rate of destruction, only one third of the drug (as stated previously) is eliminated by urinary excretion.

Although the hypothetical nature of this interpretation must be stressed, it still seems to fit with the few known facts. The hypothesis can explain the very prolonged durations of action of high doses of d-tubocurarine in dogs which were observed by Bovet (cf. figure 3). The postulation of a slow rate of destruction in a compartment which is reached during several hours might also explain why neither the sites of destruction nor the destruction products are known.

Marsh injected into rats radioactive d-chondocurarine, an isomer of d-tubocurarine. He found by paper chromatography that the urine contained besides the drug, four different metabolites. One of the metabolites was probably a demethylation product. Under similar conditions, he found no evidence for the formation of a tertiary amine from d-tubocurarine dimethyl ether. High doses of d-tubocurarine added to powdered chow of rats did not seem to act as a methyl donor because the effects of choline deficiency were not prevented.

Considering the theoretical suggestion that d-tubocurarine diffuses towards the site of destruction at a slow rate, one might expect that studies on homogenized tissues will permit the demonstration of destruction more readily than studies on intact or isolated tissues. Albanese and Pick and Richards have used homogenized ox and rat liver for such experiments and found an inactivation of d-tubocurarine; unfortunately, these studies leave the possibility open that the inactivation was due to protein binding rather than to metabolic conversion of the drug. The numerous contradicting reports in the literature on the inactivating function of liver have been discussed recently by Stead and Andrews; these authors found that dog liver perfusate did not lose its curarizing activity during one hour. The interpretation of such experiments presents an additional difficulty because of species differences; e.g., guinea pigs and rats metabolize d-chondocurarine to a different extent. Nothing is known about the ability of human liver or muscle to inactivate the drug. According to Dundee and Gray liver disease tends to render a person refractory to d-tubocurarine.

Succinylcholine

A very complete review by Brücke appeared in June 1956. This contained several hundred references to succinylcholine. Repeated here are only a few citations which are essential for the present discussion.

In order to understand the fate of succinylcholine in the body it would be necessary to know its rates and pathways of distribution, and when, where, and how fast its destruction by esterase takes place. However, no blood level of the drug has ever been determined—not to speak of drug levels in plasma water. No data on tissue levels are available. It is unknown whether the pseudocholinesterase in tissues like liver and pancreas is able to take part in the inactivation of the drug.

Any interpretation of data as to the fate of succinylcholine in the body has to take into account the following observations: (1) Normal esterase action on succinylcholine is fast and ultimately so complete that on an average not more than 2 per cent of an injected dose
appears in the urine.40 (2) The serum cholinesterase can only act while the drug is in
the serum. The drug has to leave the plasma and thereby the circulating cholinesterase in
order to reach its site of action at the neuromuscular junction (cf. fig. 2).

Before the influence of esterase action on the fate of succinylcholine is discussed, two possible pitfalls in the interpretation of experimental data will be pointed out. First, an in vitro test for esterase activity may indicate a well-functioning esterase, while the same enzyme in vivo is actually inhibited.50,51 The reason is that all routine tests for esterase activity are performed with diluted serum. The serum is usually diluted 1:50 or 1:100 to reduce the esterase activity in order to make it measurable. The dilution diminishes the concentration of any inhibitor which is in the serum; thereby a reversible inhibitor is “diluted out.” Many drugs used for preanesthetic medication, atropine, morphine, meperidine, chlorpromazine, have a complex influence upon pseudocholinesterase and are potential inhibitors.52 Thus, some inhibitors of cholinesterase are simultaneously substrates and may be destroyed while the blood sample is being taken or prepared for the test. This could happen, for instance, with procaine and other local anesthetic esters which are strong inhibitors53 with a relatively uncomplicated mode of action.52 Foldes et al.54 have shown experimentally that the presence of procaine can prolong the effect of succinylcholine. In short, it may be very hard to prove that the esterase is, or was, acting effectively during a surgical procedure.

Second, a lowering of esterase levels occurs in liver disease,55 malnutrition and cachexia.56 This lowering is probably a sign of a generally reduced protein synthesis, since it is correlated with reduced levels of other enzymes57 and of albumen.58 If a prolonged duration of action of succinylcholine occurs in a patient with a failure of protein synthesis, the prolongation of action may be a consequence of decreased esterase activity plus, for example, electrolyte disturbance.

The hydrolysis of succinylcholine by serum cholinesterase (pseudocholinesterase) leads first, and with relatively high speed, to the formation of succinylmonocholine and cho-
line;48,61 the breakdown of succinylmonocholine into choline and succinic acid follows at a lower speed.62,63,64 The liver contains an esterase which is apparently specific for succinylmonocholine59 so that its hydrolysis probably does not depend on cholinesterase alone. Human acetylcholinesterase (true cholinesterase of erythrocytes) cannot hydrolyse succinylcholine.61 The nonenzymatic, alkaline hydrolysis of succinylcholine can be impressive if investigated at high concentrations in vitro. However, in contrast to the enzymatic hydrolysis, the alkaline hydrolysis is a first order reaction;64,66 that is, the hydrolysis rate is proportional to the concentration of succinylcholine. If available data48,61,66,67 are assessed on this basis, one estimates that less than 5 per cent of that succinylcholine which is in the mammalian body at any given time will be destroyed per hour by alkaline hydrolysis. A fixed ratio of enzymatic and alkaline hydrolysis does not exist.

Since succinylmonocholine has much less pharmacological activity than succinylcholine,61,68,69 the first step of enzymatic hydrolysis in serum must be regarded as a prominent process of inactivation. Unless otherwise stated, the following discussion pertains to the conversion of succinylcholine to succinylmonocholine.

It has been recognized recently70,71 that there are two types of serum cholinesterase in man, which behave differently towards succinylcholine.73 Both types are inherited71,72 and must be regarded as normal variants, although they do not occur with equal frequency. The rarer form has been called atypical esterase. In a Canadian population about 96.2 per cent of investigated persons had the normal esterase, 3.8 per cent had a mixture of both types of esterase in their serum, while probably 1 in 2,800 persons possess only the atypical esterase.72 Figure 3 shows the rates of hydrolysis of various concentrations of succinylcholine by purified normal and by purified atypical esterase. It can be seen that both types of esterase are capable of hydrolyzing succinylcholine at a fast rate. The shape of the concentration-velocity curve of the normal type of esterase is peculiar and grossly deviates from the indicated sigmoid curve but this is
of no concern for the following discussion.* It is important to note that both enzymes increase their activity with rising concentrations of succinylcholine; vice versa if the drug concentrations fall, the enzyme activity slows down until it becomes negligible. However, the normal esterase is very active at drug concentrations which are too low to induce an activity of the atypical esterase.

Let us suppose that 100 mg. of succinylcholine chloride are injected rapidly into the vein of a normal adult with a plasma volume of 3.5 l. Let us further make the not quite valid assumption that the drug is evenly distributed in plasma. Then the drug concentration would be 100 mg. per 3.5 l., i.e., slightly less than 30 mg./l. According to figure 3, the action of atypical esterase at that concentration would be negligible, while the normal esterase contained in 3.5 l. of plasma would hydrolyse roughly 70 mg. of the drug per minute. Since the investigations shown in figure 3 were performed at 30 C. and a pH of 7.0, about double the indicated reaction rates can be expected in vivo at 37 C. and pH 7.38. This rapid action would lower the drug concentration within a few seconds and thereby automatically decrease the esterase activity. Simultaneously, some part of the drug must be expected to leave the plasma and to reach the neuromuscular junction. Thus the drug concentration in plasma should drop substantially in a number of minutes. According to figure 3, this would cause the esterase action to become very slow; how slow it becomes at any given time cannot be stated, since actual plasma levels of the drug are unknown. In other words, very soon after the injection the concentration of succinylcholine becomes too low for an effective functioning of the normal esterase; the concentration of succinylcholine is even initially too low for an effective functioning of the atypical esterase.

Since there are no data on blood or tissue levels of succinylcholine, it is not possible to prove directly, or to disprove, the validity of these conclusions. However, these concepts are supported by the following observations:

1. The action of succinylcholine lasts much longer in a person with atypical esterase than in a person with normal esterase. In both

* The deviation of the data from the sigmoid curve suggests that the normal enzyme protein carries numerous active centers whereby the attachment of a drug molecule to one center impedes an attachment to the next center; there is no evidence for a similar interrelation between the active centers on the atypical esterase. These concepts agree with observations made when succinylcholine and decamethonium were used as inhibitors of pseudocholinesterase. 55

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**TABLE 2**

<table>
<thead>
<tr>
<th>Dose in Mg.</th>
<th>Minutes of Apnoea, Average and Usual Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Persons with Normal Esterase</td>
</tr>
<tr>
<td>10</td>
<td>0 (0–1.3)</td>
</tr>
<tr>
<td>40</td>
<td>2.2 (1.6–3.2)</td>
</tr>
<tr>
<td>100</td>
<td>4.2 (3.0–5.9)</td>
</tr>
<tr>
<td>400</td>
<td>10.0 (7.1–14.1)</td>
</tr>
</tbody>
</table>

For persons with normal esterase, the usual range indicates the response of 95 of 100 individuals. For persons with atypical esterase, the normal range is calculated from the variation of response to small doses. It may take up to three times the indicated durations of complete apnoea until respiration is adequate.
cases, the usual duration of action is predictable (table 2). The long-lasting paralysis in a person with atypical esterase must be due to a lack of enzymatic hydrolysis of succinylcholine in clinical concentrations, since these persons react normally to decamethonium. It should be stressed that the long duration of action in the presence of atypical esterase is a regular and predictable phenomenon, although rare and therefore usually unexpected. In the reviewer's experience, these cases account for roughly 10 per cent of "prolonged apnoea after succinylcholine." The duration of action in a person with a mixture of normal and atypical esterase is rarely clinically disturbing.

(2) The injection of cholase (purified, concentrated human serum cholinesterase) into persons with a low esterase activity shortened the duration of action of succinylcholine if given prior to the relaxant but had no effect if given during the relaxation. Also, a blood transfusion did not more than slightly modify an existing apnoea due to succinylcholine. This is understandable only if the main action of the esterase takes place immediately after the injection of the drug.

(3) In numerous studies (Brücke for more than 30 references), the administration of inhibitors of cholinesterase, including neostigmine, has been found to prolong the action of succinylcholine. Since the esterase inhibition develops slowly after the injection of many inhibitors, the inhibitors were usually injected prior to succinylcholine. Thus, the esterase was more or less blocked while the relaxant was administered. By contrast, neostigmine has frequently been found to terminate a prolonged action of succinylcholine, that is, the effects of neostigmine at the neuromuscular junction had become more important than the blocking effect on pseudocholinesterase.

Argent et al. have previously pointed out that serum cholinesterase activity could only be of importance for the short period in which succinylcholine circulates in the blood stream after intravenous injection. Brücke criticized this conclusion by stating that even when succinylcholine is attached to its specific receptors at the end-plates, there will always be an equilibrium between bound and free succinylcholine which will be disturbed if the latter is hydrolyzed by serum cholinesterase. Brücke's statement is undoubtedly correct but it does not constitute an objection against the basic idea of Argent, Dinick and Hobbiger. In the study of enzyme kinetics it is a very general observation that a high enzyme activity requires a suitably high substrate concentration. This is the key to the present problem. No back diffusion of succinylcholine from the neuromuscular junction into the plasma can possibly result in plasma levels comparable to those which exist immediately after injection. The back diffusion might maintain an esterase activity of a slow but often negligible rate, as long as succinylcholine is in the body.

Thus one can conclude that the action of esterase regulates predominantly the amount of drug which reaches the end-plate in the first phase of distribution. In analogy to the fate of d-tubocurarine, the termination of action of succinylcholine seems to be caused by a process of dilution, i.e., diffusion away from the end-plate into the interstitial fluid. Argent, Dinick and Hobbiger wrote: "We found that the recovery of neuromuscular transmission from block after a single small injection of decamethonium and succinylcholine was identical, regardless of whether the blood supply to the muscle was undisturbed or the muscle rendered completely ischemic at the peak of paralysis." The assumption of a termination of action by a diffusion process is also comparable with an observation which is otherwise hard to explain. The logarithm of the duration of action is proportional to the logarithm of the dose of succinylcholine in the cat and in man. At least in the absence of pathological conditions, the slopes of logarithmic dose-duration curves show hardly any person-to-person variation; even extreme variations of esterase activity cause merely parallel shifts of these curves.

If succinylmonocholine is hydrolyzed only by serum cholinesterase, an accumulation of it during continuous infusion of succinylcholine is bound to occur. Since it can be hydrolyzed also by succinylmonocholine esterase in liver, the likelihood of an accumulation cannot be assessed at present. Serum cholinesterase readily hydrolyzes succinylmonocholine in high concentrations. However, the affinity between succinylmonocholine and serum cholinesterase seems to be so small that its hydrolysis in the serum of a living person is probably
very slow. Succinylmonocholine-esterase in vitro hydrolyzes approximately 20 mg. per minute per kilogram of liver; the accessibility of this enzyme in vivo is unknown. The first products of hydrolysis, succinic acid and choline, are normal constituents of the body but the appearance of an excess of choline may influence the duration of the neuromuscular block.82

**Other Relaxants**

*d-Tubocurarine dimethylether is excreted in the urine of animals to a larger extent than d-tubocurarine.83 In man, 55 per cent of an injected dose was recovered in urine.11 The action of Laudesium methylsulfate (Laudolisin) commences later and lasts longer than that of *d*-tubocurarine; detailed information on the fate of this agent is not available. Calabash-curarine in the cat has been found to accumulate in the liver; 25–30 per cent of the drug was diminished by urinary excretion. Dihydro-beta-erythroidine combines with plasma proteins. Blood levels of the drug show a rapid initial fall,20 as *d*-tubocurarine does.

Several synthetic relaxants are apparently not metabolized in the mammalian body. It was possible to recover from urine 80 to 90 per cent of dexamethonium,86 up to 100 per cent of gallamine,87 and about 80 per cent of benzoquinonium.88 Nevertheless, the usual termination of action of these agents must depend on some process of redistribution.80,87,88 The ability of gallamine to pass the placental barrier has been stressed.91

Suxamethonium, the diethyl derivative of succinylcholine, is hydrolyzed by pseudocholinesterase. The reaction rate is faster than that observed with succinylcholine.92

**SUMMARY**

There is a close anatomical relationship between capillaries and end-plate; this, and a special affinity of relaxants for receptor sites at the neuromuscular junction, account for the observation that the paralysis of skeletal muscle may begin during the first circulation of a relaxant.

The passage of *d*-tubocurarine through the body can be divided into three distinct phases. Depending on the dose of the drug and the sensitivity of the end-plate, the termination of action can be brought about by the different processes which dominate each phase. During the first phase, plasma levels drop with a half time of 5–6 minutes due to a distribution in the extracellular fluid; there is probably a dynamic equilibrium between drug in solution and drug bound to plasma proteins. During the second phase, the drug disappears from the extracellular fluid with a half time of about 45 minutes. The cause is probably urinary excretion but mainly a redistribution, perhaps into some cells. The third phase seems to consist of destruction in that reservoir which is reached during the second phase. The destruction is postulated to proceed with a half time of approximately 3½ hours.

The actions of normal and atypical cholinesterase of human serum on succinylcholine were compared and found to be correlated with the duration of apnea in persons possessing either the one or the other type of esterase. These studies led to the conclusion that the main action of pseudocholinesterase on succinylcholine is confined to the period immediately following the injection of the relaxant. The termination of action of succinylcholine depends probably on some process of diffusion.

The fate of relaxants other than *d*-tubocurarine and succinylcholine, is reviewed briefly.

**REFERENCES**

17. Ham, A. W.: Histology, ed. 3. Montreal, J. B. Lippincott & Co., 1957, Fig. 266, p. 404.


