THE INFLUENCE OF CURARE ON THE UPTAKE AND RELEASE OF A NEUROMUSCULAR BLOCKING AGENT LABELED WITH RADIOACTIVE IODINE

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In studying the mode of action of a drug it is desirable to know the components of its kinetics of action which can be accounted for by diffusion. The problem has been approached experimentally from two quite different standpoints. Either the pharmacological action of the drug may be used to estimate its concentration around the receptors and hence the diffusion gradient, or the drug may be labeled with a suitable radioactive tracer so that its movements may be followed directly. The diffusion studies on the action of curare by Holmes *et al.* (1951) and on epinephrine by Bevan (1960) are examples of the first method while the work described in the present paper is an example of the second.

In the case of curare the rate of paralysis and of recovery is limited by the rate at which the drug can diffuse to and from its site of action (Holmes *et al.*, 1951). The kinetics of the action of decamethonium, on the other hand, are more complex, quite different, and occur in two phases (Jenden *et al.*, 1951, 1954). It has not been possible so far to estimate the diffusion component of its kinetics of action by any method. Moreover, it is anomalous in that, although decamethonium diffuses faster than curare through solution, it takes much longer to reach a condition of steady partial block. Also, curare antagonizes phase I of the action of decamethonium while it adds to phase II.

With the object of studying these and other problems and of comparing the drug movements with studies already made on other substances such as the potassium ion (Creese, 1954), a compound closely related structurally to decamethonium and containing covalently bound radioactive iodine was used to study rates of uptake and release and the effects of curare on these. A preliminary partial report has been published (Creese *et al.*, 1957a,b).

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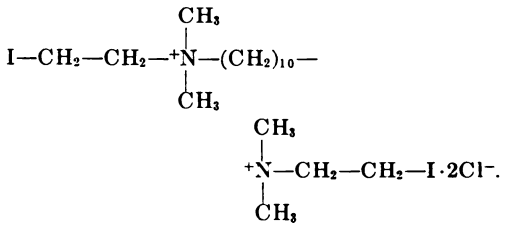
METHODS. *Compound.* The labeled compound used in these investigations was prepared from decamethylene-1,10-bis(2 chloroethyl dimethylammonium)chloride (H. D. Baldridge, 1956, unpublished), called chlorocholinium for short, by the replacement of some of the organically bound chlorine by iodine containing radioactive tracer to give labeled iodocholinium,



To effect the exchange, each 100 mg of dry chlorocholinium chloride was refluxed with 50 ml of anhydrous acetone and 100 mg of carefully dried sodium iodide (NaI^{131}) for 4 to 5 days (S. Friess, personal communication, 1956). The acetone was evaporated under a stream of nitrogen at room temperature and the residue taken up in a small quantity of warm water. This solution was then passed through a small column of Dowex-2 in the hydroxide form, and the effluent immediately neutralized with 6 N HCl. All the unreacted inorganic iodine was retained in the top portion of the column. This exchange procedure was carried out by Isotope Specialties of Burbank, California, and the product sent to the laboratory as a clear, colorless, aqueous solution containing some sodium chloride.

Examination of labeled compound. The product after labeling contains unchanged chlorocholinium, iodocholinium, radioactive iodocholinium and sodium chloride. Each batch upon arrival was examined by a series of tests in order to show pharmacological activity and obtain some idea of composition.

Neuromuscular block of the isolated guinea-pig diaphragm was used to check pharmacological activity and after block the muscle was examined for radioactivity to confirm uptake.

Total halogen ion was precipitated with silver nitrate and the precipitate examined for radio-

activity to detect inorganic iodide ion. Total solids were determined by evaporation and weighing and sodium was estimated by flame photometry. Microkjeldahl nitrogen and total iodine determinations completed the tests.

Of the batches received the average yield of drug was 180 mg with about 4 mc radioactivity and associated with approximately 240 mg of sodium chloride. The activity was standardized by a 0.02-μc sample of I^{131} from the Department of Radiation Biology. It was calculated that 1 μc would give 1.05×10^6 counts per minute with the scintillation counter used.

Two batches out of five were rejected because of low uptake of radioactivity by the muscle. The silver nitrate test showed that in these two batches the majority of the iodine was present as inorganic iodide ions. It was later established that after coming from the ion exchange column both of these batches had been evaporated to dryness on a steam bath resulting in decomposition.

Tissues studied. Diaphragm sections taken from guinea pigs and rats weighing not more than 150 g, as well as isolated rabbit lumbrical muscles (Jenden *et al.*, 1954), accompanying tendon, and strips of urinary bladder from 2 kg rabbits were used. Procedure for studying uptake consisted of suspending the tissues on glass holders with platinum hooks for variable periods of time in Krebs solution to which radioactive drug has been added. Temperature was maintained at 38°C, the bathing solution being renewed frequently and gassed with 95% oxygen–5% carbon dioxide.

Uptake measurement. After removal from the bath, tissues were put into weighed glass tubes of known background. These were reweighed and placed in a scintillation well counter for measurement of radioactivity. The uptake could then be calculated in counts per minute per gram (cpm/g). After measuring the activity of the bathing solution (cpm/ml) tissue uptake could be further expressed as ml of external solution concentrated per g of tissue (ml/g).

Outflow studies. After a variable period of soaking in oxygenated Krebs fluid containing the labeled drug, the diaphragm slips were removed, the ribs and tendon cut away and the tissue transferred to a simple holder made from a glass rod and platinum hooks. The rabbit lumbrical muscles were treated similarly except that it was unnecessary to cut the preparations in any way.

The holder and its muscle were then put into a tube containing about 6 ml of oxygenated Krebs fluid at 38°C, the solution being renewed continuously at about 20 ml/min. This tube was situated in the well of a scintillation counter. Since the fluid flow removes the drug as it leaves the muscle, the count is a measure of remaining agent. Under these circumstances radioactivity is lost mainly in two phases, a rapid initial and a slow later phase, which are considered to represent, respectively, the loss from the intercellular spaces and secondly, the loss from the muscle fibers. Suction through a fine plastic tube from an aspirator kept the fluid in the containing tube at a constant level.

Saline solution going to the tube in the scintillation counter was kept at 38°C by passing it through a spiral glass heat exchanger. The solution in the counter was gassed with a continuous supply of 95% oxygen–5% carbon dioxide.

The rapid phase of outward movement was studied in a series of six guinea-pig diaphragm slips by soaking them for 10 minutes at a radioactive drug concentration of 150 μg/ml. This allowed little of the active drug to be taken up by the cells so that most of the active drug in the muscles was confined to the interfiber spaces. By recording the outward movement of labeled drug it is possible to measure its diffusion rate through the interspaces and also the volume of interfiber space in the muscle.

The slower phase of outward movement was studied in a series of 22 guinea-pig diaphragm slips, 10 rabbit lumbrical muscles, and one piece of rabbit tendon. The initial soaking period varied between 4 and 14 hours. The concentration of radioactive compound also varied between a dose found to be subparalytic after 12 hours (approximately 3 μg/ml) and a concentration some 4 times higher for the shorter period. The higher concentration was adopted to enable outward movement to be observed for a long time without first subjecting the muscle to prolonged soaking.

Influence of curare. The influence of *d*-tubocurarine on outward movement of labeled depolarizer was also determined. In earlier studies outflow from paired muscles was compared, curare being present in one. Later, the wash solution was alternated to a fluid containing curare and then back to curare-free solution again. A separate heat exchanger and reservoir were maintained to supply the curare-containing solution. A curare concentration of 5 μg/ml was used in most of the experiments, as this is known to diminish markedly the uptake of iodocholinium.

Counting. The duration of counting varied between ½ minute for the rapid phase of outflow to 4 minutes in the case of some muscles where radioactivity had diminished to low values and which were in the slow phase of exchange. The intervals between the beginnings of consecutive counts were similarly varied between 1 minute and 10 minutes. All counts were corrected for background, resolving time of the apparatus and decay; and the counts per minute (or the appropriate logarithm)

were plotted at the center of the duration used for counting.

Background counts were made with the muscle holder in position. Contamination was reduced in some cases by a preliminary soaking of the holder in saturated sodium chloride solution. The activity of 1 ml of the solution used for the initial loading of the muscle was also determined.

The thickness of the diaphragm was measured and in some cases the potassium and water content of the tissue were determined (Creese, 1954).

RESULTS. UPTAKE STUDIES. *Guinea-pig diaphragm.* Pharmacological effects of the radioactive mixture of iodocholinium and chlorocholinium were studied with the guinea-pig diaphragm preparation (Jenden, 1955). The response with a small dose (about 3 μg/ml) was a neuromuscular block which slowly progressed until equilibrium was reached at nearly total paralysis after 12 hours (fig. 1) and as in the case of C-10 (Jenden *et al.*, 1954) there was depression of response to both direct and indirect stimulation. Each of five lots of drug used was tested in this manner. With larger, 10-fold doses, a 2-phase block developed on seven separate occasions. Phase II block could be antagonized by potassium, neostigmine, and fall in temperature to 27°C. Figure 2 shows a typical result in which the phase I block was antagonized by preliminary treatment with *d*-tubocurarine (DTC) 0.025 μg/ml. This curare

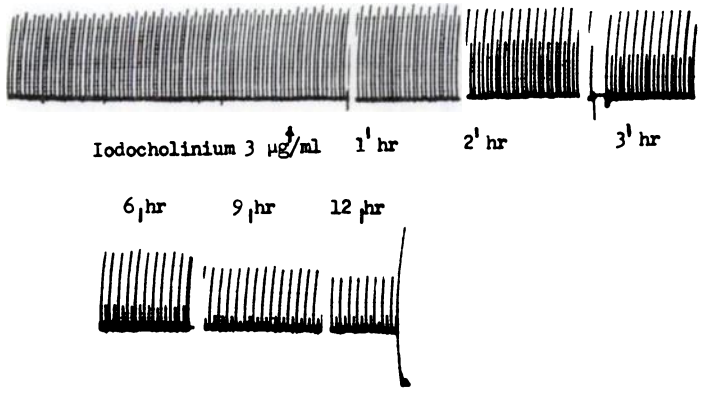


FIG. 1. Isolated guinea-pig diaphragm preparation in Krebs solution at 38°C, 95% oxygen, 5% carbon dioxide.
Strain gauge recording of slow neuromuscular block. Calibration at end: 5 g = 32 mm, resting tension = 13 mm. Stimulation, 6/min.

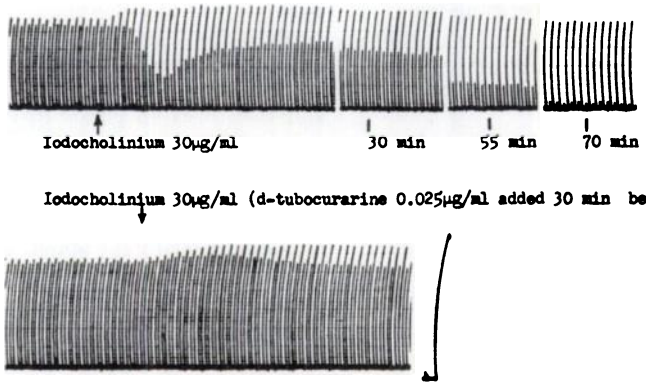


FIG. 2. Two isolated diaphragm preparations from 100-g guinea pigs, stimulated alternately through nerve and tendon at 6/min.
Upper tracing shows effect of iodocholinium (30 μg/ml). Lower tracing shows effect of iodocholinium in the presence of a subparalytic dose of tubocurarine. The initial effect of iodocholinium is absent. Complete block occurred in 60 min. Strain gauge calibration: 5 g = 32 mm, resting tension = 3 mm.

effect was demonstrated on three pairs of preparations. Block was reversed by washing out the drug.

In studying uptake of the radioactive iodocholinium (3 μg/ml) on this muscle, specimens were removed after 10 minutes, 1, 2, 3, 6, 9, and 12 hours of soaking. Radioactivity of the muscle increased continuously during the 12-hour period. This paralleled the slowly increasing block during this period. Uptake was surprisingly high and corresponded at 10 hours to 2.5 μg/g of muscle and concentrated the amount of drug contained in some 4.5 ml of external solution (fig. 3). The volume of extracellular space is less than 0.3 ml/g and cannot account for such results.

d-Tubocurarine (DTC) in a paralytic dose of 5 μg/ml markedly inhibited the uptake of the labeled compound. This was shown in every case of the 17 pairs of diaphragm so tested. It became apparent after 1 hour, and after 12 hours the uptake with curare in the bath was less than half that found in the controls. (Each point in fig. 3 represents the average of two to four separate experiments.) A concentration of curare of 0.1 μg/ml shows a detectable inhibition of iodocholinium uptake at the end of 2 hours and produces a barely detectable paralysis. It takes about 1.0 μg/ml to produce a 95% block under these conditions. Abolition of phase I block due to low doses of depolarizers can be inhibited by curare at concentrations less than those which produce detectable paralyses. Inhibition of iodocholinium uptake can be detected for all curare concentrations over the range which produce partial paralyses. The concentration of curare, however, above which no further effect on iodocholinium uptake is seen, is several times higher than that required to produce a complete paralysis.

In order to compare the results with inorganic radioactive iodine, similar uptake studies were done on the guinea-pig diaphragm using NaI^{131}, 0.01 μc/ml. Uptakes of both control and curarized muscles were comparatively low and showed little difference at any time period. At 1 hour an average of 0.4 ml of bathing fluid had been cleared of radioactivity per gram of muscle. At 12 hours this had increased to only 0.7 ml/g (fig. 3). Curare had no effect on the uptake of inorganic iodide.

Other skeletal muscle. In the rabbit lumbrical, the uptake was smaller but again DTC inhibited uptake in each pair of muscles soaked for 12 hours. Mean value for control uptake of radioactivity was 2.28 ml/g ± 0.14 (S.E. of 6) compared with 1.60 ml/g ± 0.04 (S.E. of 6) with DTC.

Smooth muscle. Pairs of strips from the rabbit urinary bladder were soaked for 2, 4 and 6 hours.

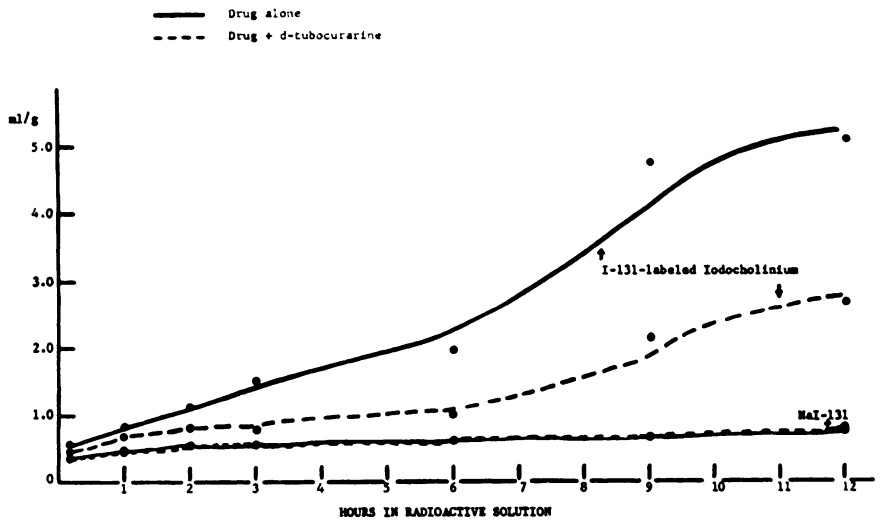


FIG. 3. Uptake of labeled iodocholinium by guinea-pig diaphragm.
The uptake is expressed in ml/g given by (activity per g tissue)/(activity per ml external saline). Solid lines = uptake of drug alone (3 μg/ml). Dotted lines = uptake of drug (3 μg/ml) in presence of tubocurarine (5 μg/ml). Each point gives mean of 2 to 4 muscles. Uptake of NaI^{131} is also shown.

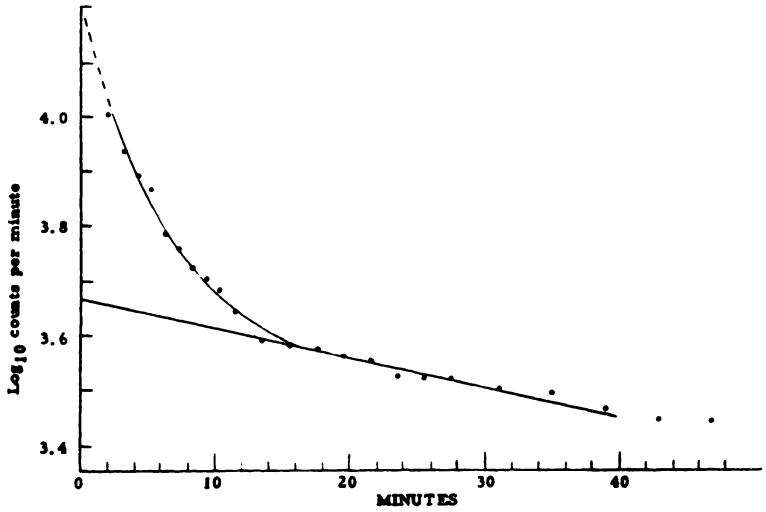


FIG. 4. Short time study of outward movement of labeled iodocholinium.
Guinea-pig diaphragm slip (0.0983 g) soaked in iodocholinium 150 μg/ml for 10 minutes prior to washout.

Uptakes were comparatively low and there was no appreciable change in uptake between 2 and 6 hours. Mean uptakes were 0.82, 0.82 and 0.90 ml/g for controls and similarly 0.88, 0.88 and 0.90 ml/g for the curarized solution.

Nonmuscle tissue. Rabbit tendon accompanying the lumbrical muscles had low uptakes with very little difference of radioactive iodocholinium uptake alone and in the presence of DTC. Mean value for controls was 0.99 ± 0.02 and 0.96 ± 0.06 ml/g with curare after 12 hours.

OUTFLOW STUDIES. *Rapid initial outflow.* Figure 4, where log of the count per minute is plotted against time, is typical of the six guinea-pig diaphragm muscles in this group which had previously been soaked for a short time in solution containing a relatively high concentration of labeled drug as described above.

The curve clearly consists of two parts, a very rapid initial loss which is virtually complete after 20 minutes, and a further slower secondary fall.

Slower delayed outflow. Guinea-pig diaphragm: Figure 5 is typical of washout curves for the 21 guinea-pig muscles studied over a more extended period of time. These had been immersed in solutions containing 7 μg/ml of iodocholinium before the washout.

Curves of outward movement from 14 diaphragm muscles without curare and from three muscles with curare (5 μg/ml) were determined and the half-times calculated from the slopes of the curves. The mean half-time for the 14 con-

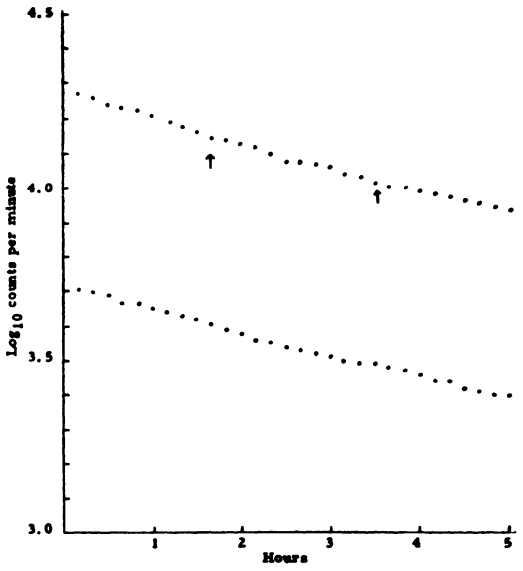


FIG. 5. Longer time studies of outward movement of iodocholinium.
Upper curve from guinea-pig diaphragm slip washed with curare solution between arrows. Lower points from a similar tissue with less initial radioactivity and washed without curare. No counts were recorded for the first 10 minutes.

trol muscles was 226 minutes; the values ranged from 52 to 528 minutes. The mean half-time for washout in the presence of curare was 233 minutes with a range of 34 to 430.

Because of this wide variation in half-times, the influence of curare on the washout curves

from four diaphragm muscles were studied in a different way. Washout was begun with curare-free solution to establish a control curve. After 90 minutes the wash was changed to a solution containing curare for 120 minutes and then back to the original solution again. The upper curve of figure 5 is typical of the results obtained in this way. The mean half-time for washout in these four cases was 269 minutes with a range of 116 to 356. Although there was considerable variation in the washout half-times, curare had no action on outflow in any of the experiments nor did the addition of curare displace any radioactive drug from the muscles.

Rabbit lumbrical: The preparation of slips of diaphragm inevitably involves damage to the muscle at the cut edge. Moreover, in preparation of the muscle for counting during drug outflow the muscle has to be removed from the rib, and this damages the fibers. The rabbit lumbrical can be dissected intact and it was thought advisable to repeat the experiments with this muscle.

Four lumbrical muscles were loaded with 3 μg/ml of iodocholinium for 14 hours. In two muscles untreated with curare the half-times for outward flow were 32 and 83 minutes, while two muscles in which outflow was measured with curare had half-times of 98 and 99 minutes. In some of these curves a sudden increase in outflow was seen after 1.5 to 2 hours unrelated to any known experimental variable.

Six of the muscles were loaded with 12 μg/ml of iodocholinium for 4 hours, and all exposed to curare for a period during outflow (fig. 6). None of these showed inflections and the net flows were all slower than those from the muscles loaded for the longer periods. The half-times had a mean of 466 and varied from 142 to 692. Again, as in the case of the diaphragm slips, curare had no effect on rate of outflow nor did it displace any of the labeled drug.

Rabbit tendon: A slip of rabbit tendon soaked in 12 μg/ml of iodocholinium solution showed a smooth washout curve with a half-time of 105 minutes. Curare was not used.

Potassium and outward movement. In a single guinea-pig diaphragm study, a high (6.3 mM) potassium solution was alternated with a low (1.3 mM) potassium solution during washout. The muscle had been soaked in 8 μg/ml of iodocholinium for 4 hours. The half-time for outward movement was 470 minutes, the curve was smooth and the change in potassium content had no influence on the slope.

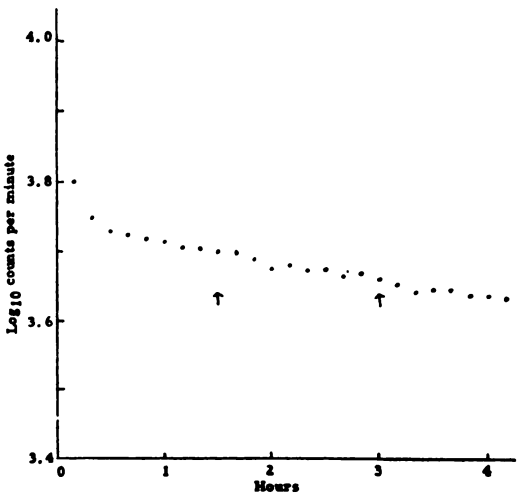


FIG. 6. Loss of iodocholinium from rabbit lumbrical, which was soaked in iodocholinium solution, 12 μg/ml for 4 hours.
Curare was present in wash solution between arrows.

Potassium contents for six pairs of the rabbit lumbricals were also determined. The mean value of those subjected to 4-hour loading and 4-hour washout was 51.7 ± 7.6 (S.E.) mEq of potassium/kg of wet tissue. Untreated controls were taken from the opposite hind foot of the rabbit. Potassium content for these averaged 59.9 ± 4.7 (S.E.) mEq/kg. The mean values for water content were 83.5 ± 1 (S.E.) % for the treated and 80.4 ± 0.5 (S.E.) % for the controls. The potassium content of fresh diaphragm muscle of the guinea pig was 90.6 ± 2.5 mEq/kg (S.E. of 4). The low potassium content of lumbrical muscle as compared with diaphragm may be attributed to a relatively large amount of fibrous tissue in the former.

INTERPRETATION OF RESULTS. *Analysis of washout curves.* Rapid exit component: The data from the rapid initial outflow experiments on the diaphragm slips were analyzed by a method similar to that used for the study of the outward movement of labeled sodium from rat diaphragm (Creese, 1954). In figures 4 and 7 it was assumed that the loss of activity from the intercellular spaces followed diffusion kinetics while that from the fibers occurred exponentially.

A diffusion curve for flat muscle, such as the diaphragm, is exponential after the first 40% of

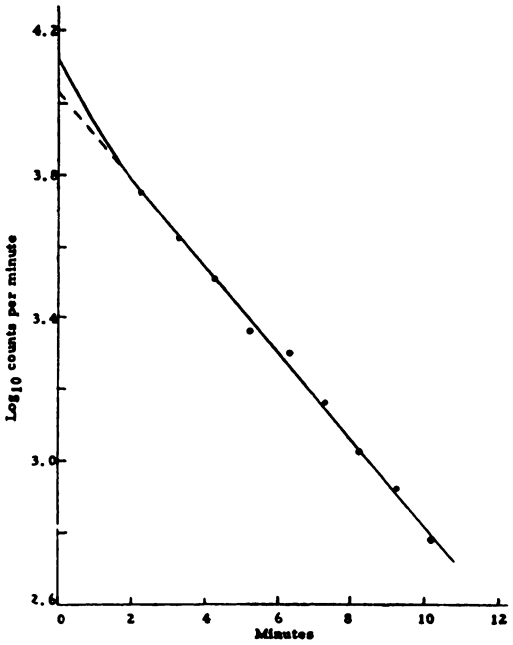


FIG. 7. Rapid phase of outward movement of iodocholinium.

The points are the difference between the curve and extrapolated line in figure 4, and represent the loss of drug from the extracellular spaces. The deviation from linearity which occurs during the first 2 minutes is obtained as described in the text.

the drug has left the interspaces (Creese, 1954). Therefore, the data can be treated as the sum of 2 exponentials after the first 2 minutes, by which time over 40% of the drug in the extracellular space has been washed out. This provides a basis for retropolation to the origin in figure 4.

For the points after 12 minutes (fig. 4) a straight line was fitted and extrapolated to zero time. The height of this line, at each time, was then subtracted from the corresponding points during the first 12 minutes and plotted (fig. 7). This curve then represents the washout of extracellular drug. These data can be used to estimate the apparent diffusion coefficient of iodocholinium through the intercellular spaces.

If f is the fraction of extracellular drug that remains after time (t), then for a flat muscle assuming diffusion kinetics,

$$f = \frac{8}{\pi^2}\left(e^{-kt} + \frac{1}{9}e^{-9kt} + \frac{1}{25}e^{-25kt} + \cdots\right) \quad (1),$$

where k is $\pi^2 D'/4d^2$. The apparent diffusion coefficient of the labeled drug in the interspaces is D', and d is half the muscle thickness.

After about 40% of the intercellular drug has been lost the curve becomes exponential and now

$$\log_{10} f = -0.091 - \frac{k}{2.3}t \quad (2).$$

If the regression line of figure 7 is given by

$$y = a + bx \quad (3),$$

then the slope b is $-k/2.3$ and therefore k is $-2.3b$. From figure 7 the value of b is 0.121 min^{-1}; and this gives the value of k as -0.00464 sec^{-1}. Also, the half-time for the exponential component of the rapid outflow is about 2.5 minutes.

The thickness of the muscle can be obtained from its area, weight, and specific gravity. Since D' is 4 kd^2/π^2 the value of D' comes out as 2.27×10^{-6} cm^2 sec^{-1} for the experiment in figure 7. In a series of six muscles the values for the apparent diffusion coefficient of labeled drug in the interspaces varied between 1.46×10^{-6} and 4.21×10^{-6}, the mean value being 2.5×10^{-6} cm^2 sec^{-1} $\pm$ 0.4×10^{-6} (S.E.). In this analysis the muscles are considered to be a wide slab and diffusion through the ends and cut sides is ignored.

The volume of the space in the muscle containing the rapidly moving drug can also be estimated. By extrapolation of figures 4 and 7, the initial radioactivity of the rapid phase can be estimated and from the activity of the saline its volume can be determined. This can then be expressed as a fraction of the muscle volume. The mean value for this space for six muscles was 0.30 $\pm$ 0.03 (S.E. of 6).

DISCUSSION. *Uptake experiments.* Chemical analysis indicates that about 10% of the chlorocholinium is converted to iodocholinium during the exchange process. The pharmacology of the mixture therefore is chiefly that of chlorocholinium, whereas the uptake measurements and outflow are exclusively of the iodocholinium present in the mixture.

Although it is possible to set up a block with C-10 which resembles that produced by curare in that it is antagonized by neostigmine, potassium ions and a fall in temperature of about 10°C, (Jenden *et al.*, 1951, 1954; Zaimis, 1953), the action of C-10 under these conditions occurs in two phases. The first (phase I) consists of a neuromuscular block of rapid onset and short duration, associated with a diminished response to direct

stimulation and followed by spontaneous partial recovery; the second (phase II) is a block which slowly progresses to a steady state and is not associated with an alteration in response to direct stimulation. It is phase II which resembles curare.

Moreover, Jenden *et al.* (1954) presented evidence that the sites of action of curare and C-10 in phase II are not the same. A concentration of curare sufficient to produce a steady state of partial block in an isolated slip of rat diaphragm *in vitro* approaches very close to this steady state in about half an hour whereas C-10, although it is a smaller molecule and diffuses faster, takes five to ten times as long to produce a similar steady state. From the standpoint of diffusion the behavior of C-10, the lepto curares (Bovet *et al.*, 1951) and, we might add, iodocholinium, is quite different from curare. Acetylcholine produces a similar dual effect on a muscle which has been treated with neostigmine (Jenden, 1955). Phase I block corresponds to the depolarization block described by Burns and Paton (1951); and, although the phase II block again resembles the action of curare, it differs in that its rate of onset is, like that of C-10, much slower.

To explain the tachyphylaxis to repeated doses of C-10, Burns and Paton (1951) suggested that the drug might be entering the muscle fiber and that entry of depolarizer might be an essential part of the depolarizing process. Some evidence for the entry of C^{14} labeled decamethonium into the muscle fibers in the region of the end-plate has been presented by Waser (1960), and the results of the present work are not inconsistent with this.

It is likely from the work of Castillo and Katz (1955) that there are surface receptors for acetylcholine at the end-plate. Acetylcholine depolarizes if applied externally, and the same quantities produce no action if injected at the end-plate region beneath the cell membrane. In our experiments the uptake of labeled drug after 12 hours is such that 1 g of muscle contains the labeled drug from 5 ml solution. The experiments of Castillo and Katz are not evidence that iodocholinium does not contribute to phase II block because its concentration inside the muscle in our experiments is much higher.

An alternative model has been proposed by Castillo and Katz (1957) in which competitive interaction between drug, receptor and esterase is used to interpret the results obtained by ionophoretic application at the end-plate. In this model the depolarizing substance does not penetrate the membrane. The possibility that tachyphylaxis, depolarizer-induced end-plate resistance, and secondary block are different aspects of the same basic phenomena has been discussed (Taylor, 1959).

Outflow experiments. In isolated mammalian preparations of voluntary muscle, there is sometimes an initial short-lived increase in contraction height probably due to reversal of oxygen lack sustained by the muscle during dissection. This is followed by a small, fairly rapid decrease in height, after which many hours of useful, reliable performance can be obtained. During the period of soaking, however, there is usually some loss of potassium and a gain in sodium and water (*e.g.*, Creese, 1954). This process would be hastened by damage to muscle fibers caused by cutting, and for this reason the lumbrical muscle was used as well as diaphragm.

In some preparations that had to be set up for long periods, a sharp increase in rate of loss of drug was noted and in general rate of exit was less from muscles which had been soaked for shorter time intervals. Nevertheless, the half-times taken as a whole were very variable and it is possible that these rates may be sensitive indicators of muscle membrane integrity.

The overall model of drug washout, commencing with the rapid exit from the interfiber spaces governed by simple diffusion, followed by a slower leak from inside the fibers gives an estimate of the total interfiber spaces and of the apparent diffusion constant of iodocholinium.

The inulin space for rat diaphragm is 0.28, expressed as the fraction by volume into which inulin will penetrate (Creese, 1954). The similarity of this figure with that obtained above (0.30) gives some confidence that the rapid portion of the exit curve represents drug in the interspaces, and suggests that very little iodocholinium is loosely adsorbed to the outside of the muscle fibers or endplates.

The apparent diffusion coefficients for intercellular diffusion through rat diaphragm at 38°C expressed as $cm^2\ sec^{-1} \times 10^{-6}$ are 5.2 for potassium and 0.56 for inulin (Creese, 1954). The present value of 2.5 for labeled iodocholinium (molecular weight 617, calculated as the dichloride salt) is of the approximate order of mag-

nitude to be expected from these findings. A correction which takes account of the loss of drug from the cut sides as well as from the surfaces of the muscle would tend to reduce the above estimate of the apparent diffusion coefficient. These results also indicate that the radioactive iodine is leaving the interspaces as part of the iodocholinium ion and not as iodide ions freed by decomposition of the parent molecule.

One of the characteristics of phase II block produced by depolarizers (Jenden *et al.*, 1951, 1954) is that, although recovery from paralysis upon changing the bath fluid is rapid, the recovery of original response to depolarizer requires much more prolonged washing suggesting the presence of drug at deeper levels. In these studies, the slow clearance of drug from inside the muscle may be a different manifestation of the same phenomenon. If this is true, phase II block requires some superficial occupation of receptors by depolarizer; but since it takes so long to develop, it also requires the presence of depolarizer inside the fiber. It seems possible that, if enough drug got in, the block might persist after superficial receptor clearance; but this has not been demonstrated yet. If depolarizer entry is a necessary part of depolarization, as suggested by Burns and Paton (1951), then subsequent exit will be necessary for the maintenance of normal neuromuscular transmission, and anything which impeded exit might contribute to incipient paralysis.

The experiments described in this paper show that curare has no detectable influence on the release of iodocholinium from muscles. Attempts to detect such effects by comparing paired muscles, one of which was curarized, were discontinued because of the variability in outflow rates from muscle to muscle. In those experiments where the alternating technique was used, an initial period of drug exit of sufficient duration to define its rate of loss was recorded. This was followed by a period of exposure to curare more than adequate to reach equilibrium with curare. Since the action of curare requires 30 to 45 minutes to produce a steady state of paralysis (Holmes *et al.*, 1951), the muscles therefore were fully curarized for over an hour before the curare-free wash solution was restored. The curare did not appear to displace any detectable amount of iodocholinium from receptors nor did it produce any slowing of the outward movement of the drug. If curare had had an effect comparable to that seen in the uptake studies, a clear change in slope would have resulted.

It appears that the entrance and exit of iodocholinium are by different mechanisms.

SUMMARY

Uptake of a neuromuscular blocking agent labeled with I^{131} has been recorded in various muscles and in tendon. The compound used, iodocholinium, was an iodo-ethyl derivative of decamethonium (C-10).

In skeletal muscle, the uptake was comparatively high in the 12-hour period during which block was increasing, while in smooth muscle and tendon, uptakes were much lower.

Inorganic iodine as NaI^{131} was concentrated by skeletal muscle to a very low extent compared with that of the organically-bound I^{131} in all other tissues studied.

d-Tubocurarine (DTC) markedly inhibited the uptake of iodocholinium in skeletal muscle, although there was no detectable effect on smooth muscle and tendon.

Outward movement of labeled iodocholinium has also been studied in isolated guinea-pig diaphragm and rabbit lumbrical muscle and has been shown to occur in two phases. There is an initial, rapid outward movement, virtually complete in 20 minutes, of an amount of drug which corresponds to that contained in the interfiber spaces (0.30 by volume of the muscle). The half time for this process is about 2½ minutes. The apparent diffusion coefficient of labeled iodocholinium through the interspaces has been estimated to be 2.5×10^{-6} cm^2 sec^{-1}. The remainder of the drug is lost at a much slower rate and appears to be coming from inside the muscle fibers, and though variable has a half-time of about 4 to 6 hours.

Curare added to the preparation during release of iodocholinium has no detectable effect, in contrast to its effects on iodocholinium uptake.

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