EXPERIMENTAL STUDIES ON THE FATE OF DECAMETHONIUM*

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THE DIFFERENCES between the mode of action of lepto- and pachy-curares are as yet poorly understood. Among the various obscure points still awaiting a convincing explanation, a most important one is, in our opinion, that related to the manner in which a lepto-curare, after its pharmacological action, is released from the myoneural junction. It is well known that, while the more complex substances such as pachy-curares usually induce long-lasting paralysis, the lepto-curares, which have a simpler structure, exert a shorter muscle relaxant effect. No conclusive explanation for this has been given, at least regarding the methonium salts.

Now, if we admit that the active group in the case of both lepto- and pachy-curares is the quaternary ammonium, as generally admitted, it seems reasonable to assume that the shorter duration of muscle paralysis may be due to the fact that simpler substances are chemically degraded and more promptly inactivated than more complex compounds.

This hypothesis has been experimentally confirmed by several authors[1, 2, 3, 4, 5] in the case of succinylcholine (succinyldicholine). This drug does in fact exert a short duration of action (not more than a few minutes), which is strictly dependent upon the high rate of hydrolysis by cholinesterase. The prevailing view with respect to methonium salts, on the other hand, is that they are released unchanged from the endplate, and even excreted unchanged[6, 7] as happens for the pachy-curares. This hypothesis is based chiefly on the experimental evidence given by Zaimis,[8] who by means of the ammonium reineckate precipitation method has shown that pentamethonium is excreted unchanged into the urine after its intravenous administration. Pentamethonium, however, exerts low curarizing activity, whereas its higher homologue, decamethonium, which exerts marked muscle relaxant properties, cannot be adequately estimated by the use of such method. The data obtained by Zaimis with pentamethonium cannot therefore be directly applied to its higher homologues, except on a purely tentative basis. To acquire further information in this field we undertook a series of investigations with the aim of finding more sensitive chemical or physical methods for the detection of methonium salts. Thus we have been able to develop a specific turbidimetric procedure[9] and we have shown that with this method it is possible to estimate amounts of methonium salts of the order of 5 μg., while with the previous method, as the reineckate reaction, it was not possible to detect less than 10 mg. On the other hand, by means of a radioisotope technique using C^{14}

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labelled C_{10}, we have quantitatively determined the distribution of decamethonium in the animal body.[10] In the present paper, we discuss the results of further observations in which the curve of decamethonium blood concentration levels has been investigated under different experimental conditions.

Material and Methods

The decamethonium (C_{10}) used in our experiments was decamethonium bromide with methyl groups uniformly labelled with C^{14}. The hot synthesis of the compound was performed at the Radiochemical Centre, Amersham, England. The sample employed was 99 per cent pure, and had a 5 μC/mg. activity.

Wistar rats, 200–300 gm. in body weight, received the required amount of decamethonium by intravenous injection. At suitable time intervals, 0.1 ml. blood samples were withdrawn from the femoral vein, and desiccated on aluminum discs. Radioactivity measurements were carried out using a thin window (1.5 mg./cm.^{2}) Geiger counter with lead shield. An E.K.C.O. Scaler Mod. 530A was used for counts over 1000 sec. In some of the experiments the contractions of the faradically stimulated masseter muscle were graphically recorded. At higher doses of decamethonium, when apnoea occurred, tracheotomy was done. Into the trachea of the animal was inserted a plastic tube connected with an automatic respirator supplying a flow of pure oxygen at a pressure of 2 cm. of water and at the rate of 20–30 respirations per min.

Results

The pattern of blood radioactivity following intravenous injection of different amounts of C^{14} labelled decamethonium is shown in Figure 1. Two peaks are present; the first one occurring immediately after the injection and the second one after 20–25 minutes. The second peak corresponds to the beginning of the decurarization phase (shown by the first arrow, while the second arrow shows the end of decurarization). The pattern is almost identical in all of the four curves, the first peak appearing to be proportional to injected amounts only in the three lower curves. The lower curve was obtained following administration of half of the minimal paralyzing dose to a rat; the upper curve was obtained following injection of an amount greater than the paralyzing dose. It is interesting to note that blood concentration levels of intravenously injected d-tubocurarine decrease continuously from the maximum level attained shortly after administration, whereas the decamethonium blood concentration curve shows a second peak occurring at the very beginning of the decurarization phase. This second peak would indeed remain inexplicable, should we accept the classical view that C_{10}, like d-tubocurarine, is released as such from the endplate. If such an assumption is correct, increased blood levels of the active compound should result in enhanced muscle relaxant effect instead of its end. Nor, on the other hand, can it be argued that "desensitization" of the endplate might have taken place, since if more decamethonium is injected at the time of decurarization, a new "paralysis" immediately occurs. The second peak must therefore be caused by a substance containing the carbon atoms of the original methyl groups of C_{10}, which are the carriers of radioactivity, but not by the whole decamethonium molecule. In

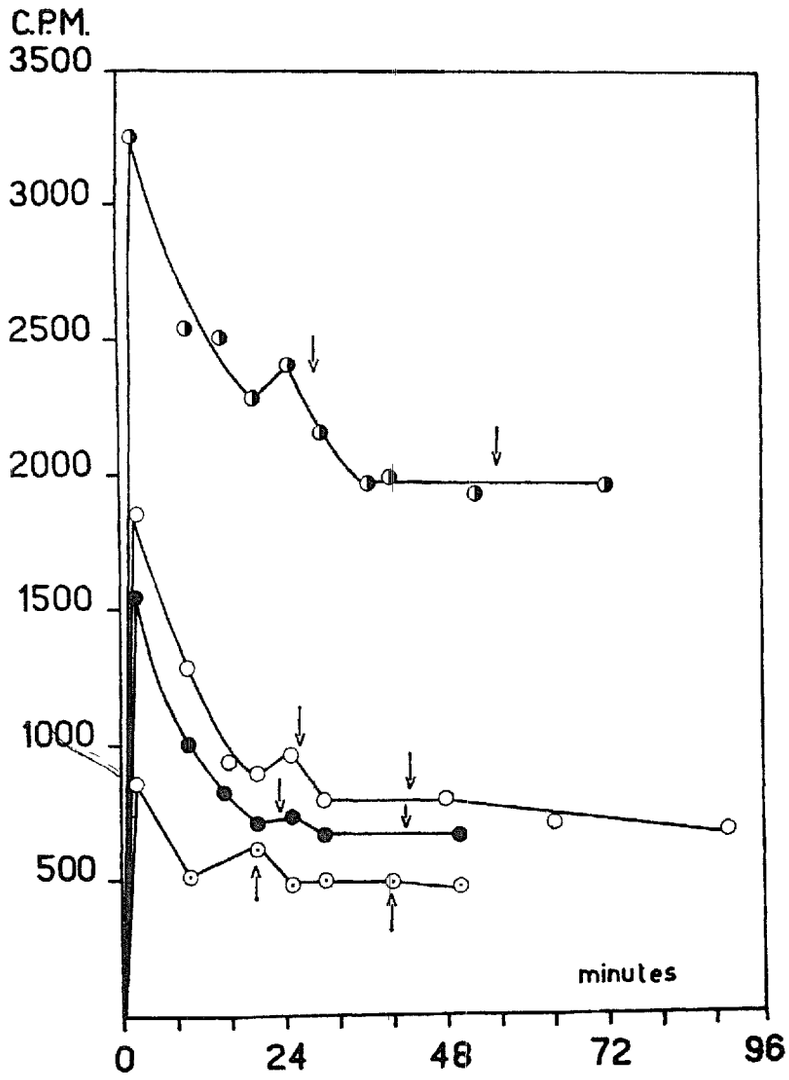

FIGURE 1. ⊙–⊙ 1,2 mg./kg.; ◐–● 1,8 mg./kg.; ○–○ 2,0 mg./kg.; ◑–◑ 2,8 mg./kg. intravenously injected decamethonium labelled with C^{14}.

addition, the drop in d-tubocurarine blood content occurs much earlier than that observed with decamethonium; thus, only 15 per cent of the injected amount of d-tubocurarine is still present in blood 40 minutes after injection, whereas more than 33 per cent of the original dose of decamethonium was found to be present after an equal period of time. This finding suggests that d-tubocurarine is eliminated through the kidney and/or the liver much more rapidly than decamethonium, although the latter's action is of much shorter duration, thus providing further evidence in support, even indirectly, of our view.

It seems reasonable to assume that decamethonium, which is slowly eliminated, should maintain muscle paralysis for a longer time than d-tubocurarine, if both drugs, in their active form, are released from the endplate into the bloodstream. On the other hand, if C_{10} is inactivated by the endplate, the presence of an inactive derivate in the blood would not affect the course of "paralysis."

Another feature of some interest lies in the fact that, while the first peak varies depending on the injected dose, the second peak is always nearly the same, irrespective of the dosage employed. In this connection, it should be pointed out that the amount of drug we used in all of the experiments ranged from one-half to a whole paralysing dose. If the degree of paralysis is regarded as the expression

of the number of specific effectors blocked at the myoneural junction, as many as 50 to 100 per cent of them may be considered to have been blocked in the course of our experiments. Accordingly, it is reasonable to assume that the larger the number of effectors blocked, the greater should be the amount of inactivated C_{10} being re-introduced into the bloodstream; but, also, that the higher the initial decamethonium blood levels, the greater the number of effectors thus blocked. Now, the second peak is in absolute value highest when the initial injected dose is elevated, whereas its relative value (i.e., the amount of decamethonium re-introduced into bloodstream minus the decamethonium amount already present in the blood) is practically constant. The relationship between the second decrease in decamethonium blood levels and decurarization may be better understood if we observe Figure 2. In this figure two different graphs are reported: one

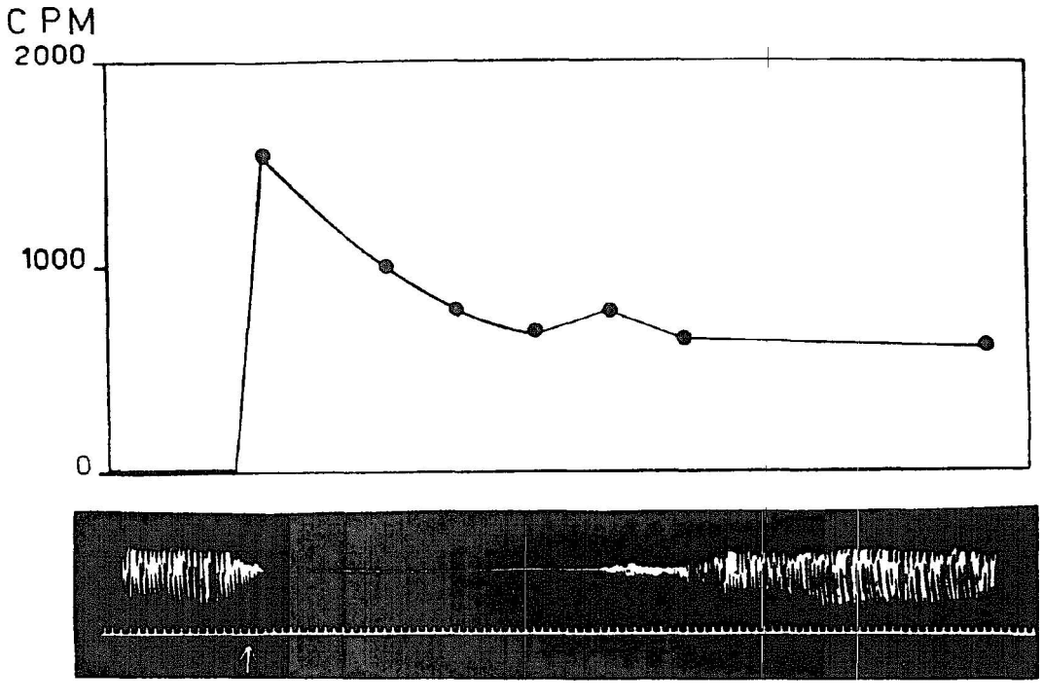

FIGURE 2. ●–● 1,8 mg./kg. intravenously injected decamethonium labelled with C^{14}.

has been obtained by recording the contractions of the faradically stimulated masseter muscle of a rat treated with C_{10}; the other has been obtained by plotting the radioactivity as a function of the time. Since the time units are the same in both measurements, it is clear that the first rise in level of radioactivity corresponds to the onset of paralysis, whereas the second one begins shortly before recovery of muscle excitability.

An additional experiment was carried out to further clarify our hypothesis. One-half the paralysing dose of non-labelled decamethonium was injected first, and an equal dose of radioactive decamethonium was again injected after three minutes. The data obtained are reported in Figure 3, where it may be seen that in the animal pre-treated with non-labelled C_{10} the initial peak is much higher than in the control animal. This is most likely to be due to the fact that non-labelled decamethonium has been fixed by the endplate, thus preventing the fixation of the radioactive compound. Afterwards a decrease of the radioactivity

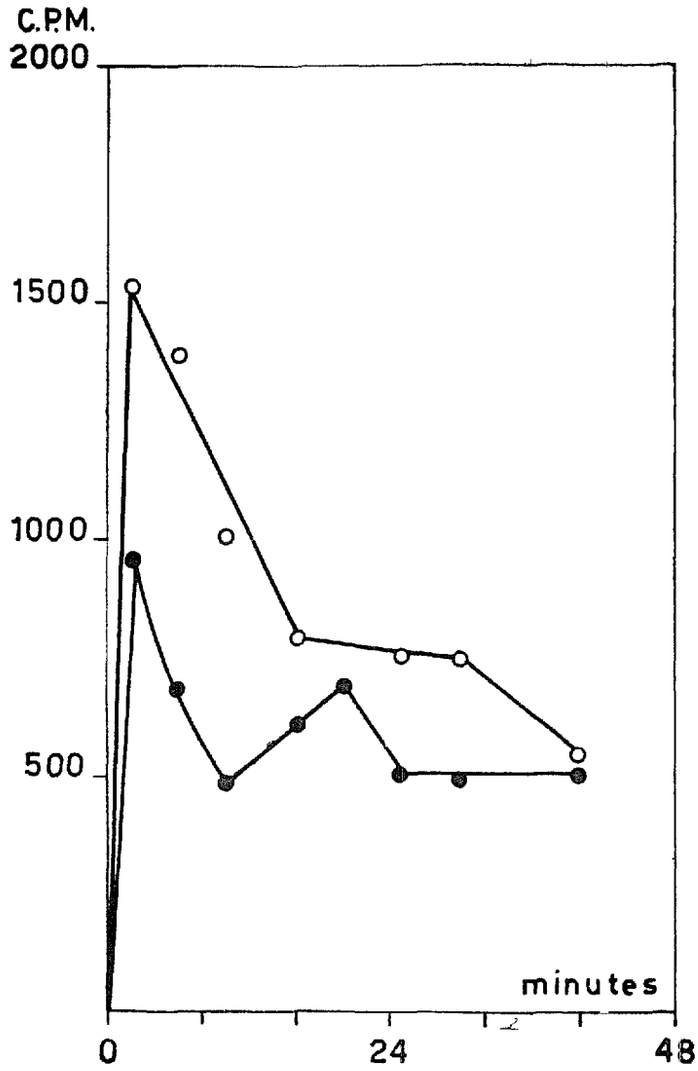

FIGURE 3. ●–● 1,2 mg./kg. intravenously injected decamethonium labelled with C^{14}, ○–○ 1,2 mg./kg. intravenously injected decamethonium labelled with C^{14} 3′ after the injection of an equal amount of non-labelled C_{10}.

appears, and it is followed by a plateau corresponding to the second peak in the control animal. The presence of this plateau is probably the result of superimposition of two curves displaced in three minutes; that is to say, the lapse of time between the first and the second decamethonium injection.

If d-tubocurarine is first administered, and followed after seven minutes by injection of labelled decamethonium, the pattern of blood radioactivity is the same as that obtained from the control animal treated only with an equal amount of C^{14}–labelled C_{10} (Fig. 4). The curve, however, is markedly displaced upwards, with respect to the control, as an effect of injection of a higher dose. It seems, therefore, that both decamethonium and d-tubocurarine act on the same structures, or at least that both compounds are taken up by the same receptors, as shown by the fact that d-tubocurarine injection is followed by a decreased decamethonium uptake. Moreover, it is clear that the mode of elimination of d-tubocurarine differs from that of C_{10}, inasmuch as pre-administration of d-tubocurarine does not modify the decamethonium elimination pattern.

In order to get a more complete picture, it was thought of interest to investigate the pattern of blood radioactivity, following intramuscular injection of C^{14}

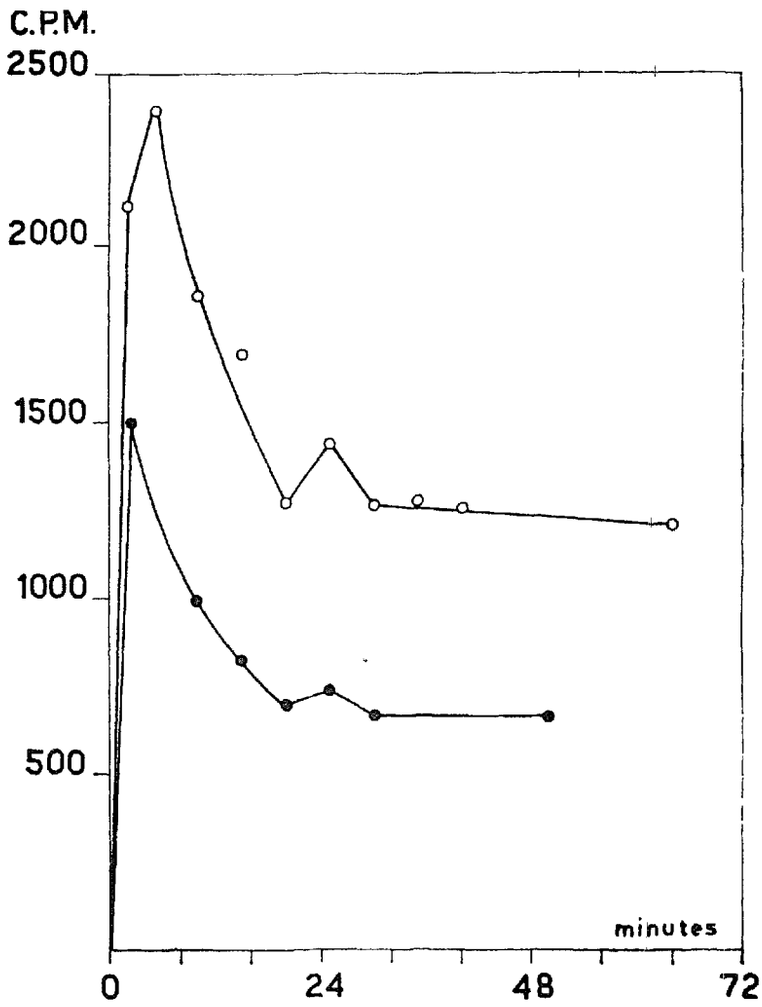

FIGURE 4. O–O 1,8 mg./kg. intravenously injected decamethonium labelled with C^{14}, 7′ after the injection of 0,1 mg./kg. of d-tubocurarine; ●–● 1,8 mg./kg. intravenously injected decamethonium labelled with C^{14}.

labelled C_{10}. The results thus obtained are shown in Figure 5. The typical upper curve was obtained after intravenous injection, the lower curve after an intramuscular injection. As shown by Paton and Zaimis[7] the intramuscular injection is two to three times less effective than the intravenous one and causes a much slower induction of paralysis. In the curve obtained after the intramuscular injection, on the other hand, there is a progressive rise of radioactivity up to the sixtieth minute, followed by a slow decrease. After intramuscular injection in rats there occurred a slight curarization between the thirtieth and sixtieth minute.

To explain the difference in decamethonium blood levels after intravenous or intramuscular administration, it should be recalled that, while in the first instance the whole of the drug is immediately introduced into the bloodstream, with the latter the drug goes into the blood gradually. We believe that, of the two peaks occurring in the case of intravenous injection at the beginning and at the end of curarization, respectively, the first might be tentatively explained as the effect of introduction of C_{10} into the bloodstream, and the second to the release of an inactive degradation product of the drug from muscles, where it is mainly stored. Further support to such hypothesis is given by the data obtained after the intramuscular injection. In this case, the first peak (corresponding to the introduction

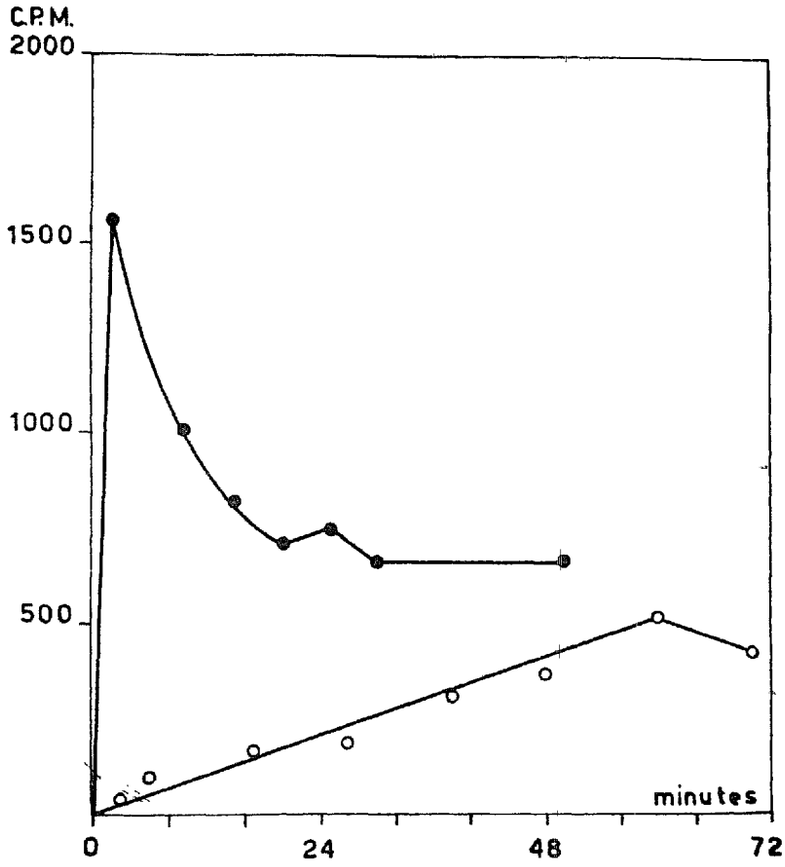

FIGURE 5. O–O 4,1 mg./kg. intramuscularly injected decamethonium labelled with C^{14}; ●–● 1,8 mg./kg. intravenously injected decamethonium labelled with C^{14}.

of radioactive material into the bloodstream) is not present and is replaced by a slow increase in blood concentration levels, as decamethonium is being gradually released into the bloodstream, whereas a peak occurs corresponding to the beginning of the decurarization phase. This phase actually begins at a level of blood redioactivity much higher than that coinciding with the beginning of curarization. This level will be reached much later after the animal has been completely decurarized for several minutes. The only possible explanation for this finding appears to be that only part of the radioactivity is due to the whole decamethonium molecule, the rest of it being caused by an inactive fragment of the same molecule.

CONCLUSIONS

The results of the present study thus provide fairly comprehensive evidence of the fact that decamethonium is inactivated by the endplate, while it seems no more admissible that this drug behaves in a manner similar to d-tubocurarine.

Our findings support the view that decamethonium, following introduction into the bloodstream, becomes fixed by the endplates, where it undergoes degradation which leads to the loss of its pharmacological properties. In this connection, it should be emphasized that the second rise in blood radioactivity following intravenous injection of labelled decamethonium, which corresponds with the beginning of the decurarization phase, cannot be regarded as being due to an active curarizing substance. Also, the subsequent pattern of the curve, if compared to

that previously obtained by other workers with d-tubocurarine, cannot possibly agree with the view that decamethonium is released from the endplate unchanged and thereafter pharmacologically active. It has, in fact, been observed that, although the length of action of this drug is approximately half of that of d-tubocurarine, decamethonium blood levels are maintained for a much longer period of time. An additional point which strongly supports our view is the prompt decurarization of animals treated with C_{10}, in contrast to the slow and gradual recovery of muscle activity noted in those treated with d-tubocurarine. The behaviour of this latter drug is clearly that of a substance which is not fixed in any part of the body and whose effects are merely dependent on the rate of elimination through the kidney or liver. On the other hand, indirect evidence suggests that elimination of decamethonium is likely similar to that of succinylcholine, which is degraded both in blood and muscles by cholinesterase, thus accounting for its extremely short duration of action.

In the case of decamethonium, we have a type of pharmacological action which is intermediate between that of d-tubocurarine and that of succinylcholine. In view of its pharmacological behaviour, and on the basis of our findings, it seems most likely that inactivation of decamethonium occurs at the myoneural junction. In a previous paper,[10] we have shown that when decamethonium blood levels decrease, the drug is accumulated in muscles. When it is re-introduced into the bloodstream, decurarization begins: this fact obviously proves that the drug is released from muscle as an inactivated derivative. The second rise in blood radioactivity corresponds to the release of C_{10} from muscles. The evidence discussed herein indirectly supports the view we have offered. Direct confirmation will be provided only by identification of the compound which is responsible for the second rise in blood radioactivity, or at least by demonstration that it is not identical with C_{10}. Research is now in progress with the aim of developing a new chromatographic procedure for isolation and detection of methonium salts with different length of molecule, and we hope this may be of help in explaining a part of this fascinating problem.

Summary

The decamethonium blood concentration pattern following intravenous injection of C^{14} labelled C_{10} is characterized by two well-distinguished peaks, the first occurring at the time of injection, and the second after variable periods of time, depending on the dosage employed. Thus, the second peak was found to shift from 20–30 min. after injection when the administered dose was increased from 1.8 to 3.0 mg./kg., its time of onset fairly closely coinciding with the initial stage of decurarization, as shown by parallel experiments using graphic recording of faradically stimulated masseter contractions. Based on these findings, and from previous studies by the same workers, an attempt was made to elucidate the causes for the unusual behaviour of C_{10} as compared to d-tubocurarine—the latter, as is well known, exhibiting a simple elimination curve with but one initial peak followed by gradual decrease of blood concentration levels.

Pre-treatment with a large dose of non-labelled decamethonium (5 mg./kg.) results in disappearance of the second peak, which is replaced by an extended

"plateau," whereas pre-treatment with 100 mg./kg. d-tubocurarine causes displacement of both peaks towards the right. The evidence thus substantiates the authors' previously suggested opinion that the initial drop of decamethonium blood levels is associated with its fixation by muscle receptors, and that the second peak, corresponding to the decurarization stage, is due to re-introduction of either inactivated decamethonium or its radioactive degradation products from the muscles into the bloodstream.

Finally, the identity of receptors for decamethonium and d-tubocurarine is discussed.

Résumé

La courbe de la concentration sanguine au décaméthonium à la suite de l'injection intraveineuse de C^{14} marqué C_{10} est caractérisée par deux pics bien distincts le premier survenant immédiatement après l'injection, et le deuxième après des délais variables selon la dose employée. Ainsi, nous avons réalisé que le deuxième pic se déplace de 20 à 30 minutes après l'injection, lorsque nous augmentons la dose de 1.8 à 3.0 mg./kg.; le moment de son début coincide à très peu près avec la phase initiale de décurarisation, comme le prouvent des expériences parallèles où l'on a fait un graphique des contractions des masseters stimulés par un courant faradique. Forts de ces données et à la suite d'autres études par les auteurs, nous avons étudié les causes des effets différents du C_{10} et de la d-tubocurarine, cette dernière, on le sait, donne une courbe d'élimination ordinaire ne montrant qu'un pic suivi d'une diminution graduelle des taux de concentration dans le sang.

Un traitement préalable avec une grosse dose de décaméthonium non marqué (5 mg./kg.) a entraîné la disparition du deuxieme pic a été remplacé par un plateau prolongé, alors qu'un traitement préalable avec 100 mg./kg. de d-tubocurarine a entraîné le déplacement des deux pics vers la droite.

Ces faits confirment l'opinion émise au préalable par les auteurs que la première diminution des taux décaméthonium dans le sang est liée à sa fixation par les récepteurs musculaires, et, que le second pic, survenant à la phase de décurarisation, est dû à l'apparition dans le courant sanguin soit du décaméthonium inactivé, soit de ses produits radioactifs dégradés par les muscles.

Finalement, nous discutons de l'identité de récepteurs pour le décaméthonium et la d-tubocurarine.

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