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PHARMACOLOGICAL STUDY OF INJECTED CHOLINESTERASE

BY

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The aim of producing muscular relaxation during abdominal operations has been achieved with curare and other neuromuscular blocking agents, but unfortunately the use of such drugs is not always without risk, and the reversal of their action by neostigmine cannot be relied upon.

The possibility was considered that cholinesterase in excess might inhibit the transmission of nervous impulses to the muscle. Its use would have the advantage that its paralytic action could be immediately nullified by the administration of anticholinesterase drugs. To the best of the author's knowledge, no experiments have been carried out to explore this possibility.

If it could be proved that injected cholinesterase blocks neuromuscular transmission, another proof might also be established in favour of the theory of chemical transmission between nerve and muscle, although failure to produce such evidence would not invalidate this theory.

Most of the work published on cholinesterase concerns the inhibition of its action by different drugs, and very few studies deal with the augmentation of the acetylcholine-hydrolysing capacity of the organism, which should follow the injection of cholinesterase. Mendel and Hawkins (1943) reported on the effects of injection into rats of horse serum pseudocholinesterase, dog pancreas pseudocholinesterase, and true cholinesterase prepared from the electric organ of the torpedo. They observed that the chromodacryorrhoea produced by subcutaneous injections of acetylcholine could be prevented if horse serum or dog pancreas pseudocholinesterase was injected intravenously before the administration of acetylcholine. Furthermore, they were able to show that these enzymes were not only capable of annulling the effects of injected acetylcholine but also that liberated at nerve synapses. They reached this conclusion on the basis that these enzymes interrupted the pupillary light reflexes. They obtained similar results in a few experiments with the true cholinesterase prepared from the electric organ of the torpedo.

Recently Lesuk (1949) prepared a very potent true cholinesterase from bovine red blood cells. No pharmacological work on its action is known to the author apart from two recent short notes by Koppanyi and Karczmar (1948, 1949); their results will be referred to later. In this study Lesuk's preparation will be referred to as the

red cell enzyme.* Cholinesterase, if meant generally, is referred to as such throughout this work.

METHODS

Preparation of solutions of red cell enzyme.—The dried material was dissolved in sterile 2 per cent (w/v) gelatin in distilled water to make a solution of 1,000 units per ml. Withdrawals were carried out under sterile conditions. The container was kept in a refrigerator. No decrease in activity was observed during storage over six weeks. The activity of the preparation was in reasonable agreement with that stated by the manufacturer.

Determination of cholinesterase.—The acetylcholine employed in all experiments was the chloride obtained from Hoffman-LaRoche. An amount of 0.5 ml. of the enzyme, or the enzyme-containing blood or tissue extract, was put into the side arm of a Warburg vessel and the substrate (acetylcholine, final concentration 0.01 M) into the main compartment. The Ringer solution in which the acetylcholine was dissolved had the same composition as that described by Birkhaüser (1940). The total volume of fluid in the Warburg vessel was 2 ml. The quantity of red cell enzyme or cholinesterase which liberated 1 μl. of CO_2 (equivalent to 8 μg. acetylcholine base) in one minute was considered as one unit. Readings were made every 5 minutes for 30 minutes when the cholinesterase activity of blood, and every 10 minutes for 1 hour when that of muscle extracts, were determined. Each result given is the mean of two parallel determinations.

Choice of animals.—In most experiments male and female rats weighing about 300 g. were used. Rats were chosen for the experiments because of their relatively small size and the resultant economy of enzyme. Care was taken in each series of experiments that about half the animals were males and half females in view of the report by Sawyer and Everett (1947) of a sex difference of serum cholinesterase levels. In some experiments cats were used. The animals were anaesthetized with 70 mg. chloralose per kg. intravenously after an ether induction. As an anticoagulant 1 mg. sodium heparin per kg. was used.

Electrical stimulation.—In all the experiments where nerves were stimulated, square wave stimuli of 3 milliseconds' duration were applied, though frequency duration and amplitude varied in different kinds of experiments.

RESULTS

Blood cholinesterase activity after intravenous injection of 1,000 *units of red cell enzyme*

It is known that injected horse serum pseudocholinesterase remains for several days, and dog pancreas pseudocholinesterase only for about one hour, in the blood of rats (Mendel and Hawkins, 1943).

About 1 ml. of blood was withdrawn by intracardiac puncture from rats weighing about 300 g. Of this blood 0.5 ml. was added to 1.5 ml. distilled water containing 66 μg. heparin per ml. to prevent coagulation. One thousand units of red cell enzyme were then injected into a cannula tied in the jugular vein and washed in with 0.5 ml. saline solution; 2, 5, or 10 minutes after the injection 1 ml. of blood was withdrawn by cardiac puncture and diluted in the same way as the first sample. Not more than two blood samples (before and after injection of red cell enzyme) were removed from the same animal in order to avoid excessive disturbance of the blood volume. Determinations of cholinesterase content were carried out by the manometric method described above, 0.5 ml. of the diluted blood being used; as

* The author is indebted to Dr. M. L. Tainter, director of Winthrop-Stearns, Inc., for a supply of Lesuk's preparation.

0.125 ml. of blood was in the vessel, the results were multiplied by 8 in order to obtain cholinesterase values for 1 ml. of blood.

After the injection of 1,000 units of red cell enzyme, the cholinesterase activity of whole blood is increased for the first two minutes but rapidly falls off and may even be decreased ten minutes after injection (Table I). The average increase in the total

TABLE I

No.	Weight and sex of rat	Cholinesterase activity before injection of 1,000 units red cell enzyme. Units/ml. blood	Time between injection and withdrawal of second blood sample	Cholinesterase activity after injection of 1,000 units red cell enzyme. Units/ml. blood	Difference in units/ml. blood before and after injection of enzyme
1	300 g. ♂	16.4		77.9	+ 61.5
2	330 g. ♂	16.8	2	33.1	+ 16.3
3	270 g. ♀	25.4	minutes	65.0	+ 39.6
4	240 g. ♀	15.8		55.7	+ 39.4
Mean		18.6		57.9	+ 39.2 ± 9.2*
5	260 g. ♀	21.0		17.6	− 3.4
6	270 g. ♀	19.2	5	23.1	+ 3.9
7	295 g. ♂	25.5	minutes	25.4	− 0.1
Mean		21.9		22.0	+ 0.1 ± 2.1*
8	270 g. ♂	16.1		15.5	− 0.6
9	290 g. ♀	25.9	10	24.4	− 1.5
10	320 g. ♂	20.5	minutes	17.5	− 3.0
Mean		20.8		19.1	− 1.7 ± 0.7*

* Standard error.

blood cholinesterase activity of 39.2 units/ml. indicates a total increase of 800 units if it is assumed that a 300-g. rat has a blood volume of about 20 ml. This means that two minutes after the injection practically all the injected red cell enzyme was still in the circulating blood. On the other hand, five minutes after the injection, nearly all the injected enzyme had disappeared from the blood.

The rather big error in the results at five minutes may be explained by the fact that five minutes is the approximate limit of the increased cholinesterase activity of the blood. In some animals there is still a slight increase; in others the activity has returned to normal or even a negative value may be found. It may seem paradoxical that after injection of red cell enzyme the total cholinesterase activity should decrease (rats Nos. 5, 8, 9, and 10 in Table I), but this is probably due to dilution of the blood. This dilution is due (1) to the loss of the 1 ml. blood sample drawn for the first analysis, which represents approximately 5 per cent of the total blood volume and is presumably replaced by extracellular fluids poorer in cholinesterase than blood; (2) to the 1.5 to 2 ml. fluid injected with the red cell enzyme (1 ml. containing 1,000 units and 0.5 to 1 ml. saline to wash it in through the cannula). These two factors together account for 12 to 15 per cent dilution, which covers very well the " loss " of cholinesterase, once the originally injected enzyme has disappeared.

Muscle cholinesterase activity after intravenous injection of 1,000 *units of red cell enzyme*

It was necessary to know whether injected enzyme reaches the muscle if an attempt to evaluate its action on neuromuscular conduction was to be made.

The skin over both gastrocnemius muscles of male and female anaesthetized rats was incised, and after an interval of ten minutes the right gastrocnemius was cut out with as little trauma as possible. Then 1,000 units of red cell enzyme were injected intravenously, and after 2, 5, or 10 minutes the left muscle was removed. Extracts from these were prepared as follows: about 1 to 2 g. muscle was washed for 30 seconds with Ringer solution (Birkhaüser, 1940), dried with filter paper, and dissected free of connective tissue. After being weighed it was cut into small pieces, placed on a wire gauze, and again washed with Ringer solution; 6 ml. of Ringer solution were now added for each gramme of tissue, and the muscle was ground with glass powder in a mortar (a 1-g. muscle piece was ground for 6 min. and 2 g. for 12 min.). The ground material was filtered through two layers of muslin. For each determination 0.5 ml. (= 83.3 mg. muscle) of the filtrate was used.

Because of the limited quantity of red cell enzyme available the author was not able to carry out many experiments, but the results in Table II probably indicate the trend of events, at any rate. It will be seen that the cholinesterase activity of muscle is increased at least during the first ten minutes. This is not due to operative shock, because if no enzyme is injected the second muscle tends to have a lower activity than the first muscle (animals Nos. 7, 8, and 9). The relatively high value two minutes after the injection is probably due to the high enzyme content of the blood at this time (Table II).

TABLE II

No.	Weight and sex of rat	Cholinesterase activity before injection of 1,000 units red cell enzyme. Units/g. muscle	Time between injection and taking the second muscle sample for test	Cholinesterase activity after injection of 1,000 units red cell enzyme. Units/g. muscle	Difference in units/g. muscle before and after injection of enzyme
1	275 g. ♂	2.51	2 minutes	4.33	+1.82
2	330 g. ♂	2.67	5 minutes	3.24	+ 0.57
3	310 g. ♂	2.06		2.54	+ 0.48
4	220 g. ♀	5.81		7.05	+ 1.24
Mean		3.51		4.27	+ 0.76 ± 0.24*
5	330 g. ♂	3.12	10 minutes	3.57	+ 0.45
6	300 g. ♀	4.81		5.71	+ 0.90
Mean		3.96		4.64	+ 0.67 ± 0.22*
7	310 g. ♂	5.24	No enzyme injected; 2nd muscle excised 7.5 min. after the 1st	4.60	− 0.64
8	230 g. ♀	4.40		4.35	− 0.05
9	250 g. ♂	3.96		3.50	− 0.46
Mean		4.53		4.15	− 0.38 ± 0.17*

* Standard error.

Though the total cholinesterase activity in the muscle is increased after injection of red cell enzyme the results do not discriminate between an increase taking place in the muscle fibres themselves and that in the extracellular fluid.

Acetylcholine hydrolysis in vivo *by red cell enzyme as measured by the effect of injected acetylcholine and of vagus stimulation on the blood pressure of rats*

The carotid artery of anaesthetized rats was cannulated and the blood pressure measured by a mercury manometer with the help of the amplifier described by Bayo, Wilbrandt, and Lauener (1948) for amplifying intestinal pressure changes. With this method only relative changes of blood pressure are determined. In some experiments the apparatus was calibrated in terms of absolute pressures. Injections, followed by saline, were made into the jugular vein. In other experiments the vagus was prepared and stimulated at 60 per sec. for 10 sec. with a strength sufficient to produce a marked fall in blood pressure.

After establishing the hypotensive reaction by different doses of intravenously injected acetylcholine, 1,000 to 2,000 units of red cell enzyme were administered intravenously, and the acetylcholine test was repeated several times.

It was found, as was to be expected from the blood cholinesterase activity values, that after the injection of red cell enzyme (Table I) the action of injected acetylcholine was reduced only during the first few minutes after the injection of the enzyme, but not after five minutes (Fig. 1). In none of the experiments was the acetylcholine action completely abolished.

The hypertension after acetylcholine injection shown in Fig. 1, which precedes the typical depressor effect, is due to the amount of injected liquid. The acetylcholine

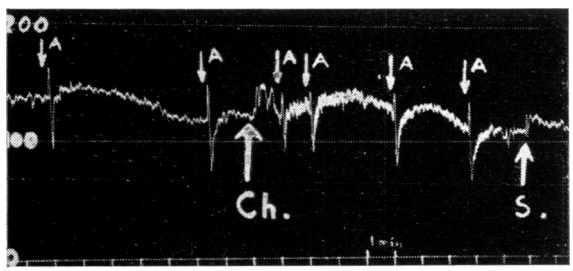

FIG. 1.—(Exp. 28.) Rat. ♂ 290 g. Chloralose. B.P. record. Time in minutes. A = 0.01 μg. acetylcholine i.v. Ch = 1,000 units red cell enzyme followed by 1 ml. saline. S = 1 ml. saline.

was washed into the jugular vein through the cannula with 1 ml. saline, which is about 5 per cent of the blood volume. The hypertension after injection of enzyme (Ch) is probably due to the same cause. It may be seen that saline alone can have the same effect (S). In animals which are less sensitive to an increase of blood volume this hypertension does not take place (Fig. 2*a*), so that the conclusion seems justified that red cell enzyme does not affect the blood pressure. This is in agreement with Sarkar, Maitra, and Ghosh (1942), who showed that even large amounts of cobra-venom cholinesterase do not affect the blood pressure and respiration of the rabbit. They observed an increase of beat amplitude on the toad's heart. Schachter (1945), on the other hand, found that cholinesterase raised the blood pressure of shocked dogs.

The increase of oscillations shown in Fig. 1, two minutes after injection of enzyme, may be due to the increased blood volume or superimposed respiration, but not to the red cell enzyme itself. This phenomenon was not found in other experiments (see Figs. 2*a* and 2*b*).

In Figs. 2*a* and 2*b* it may be seen that red cell enzyme is totally ineffective in decreasing the effects of vagus stimulation on the heart, whether the nerve be stimulated immediately (2*b*) or 5 to 45 min. after injection (2*a*). (These results

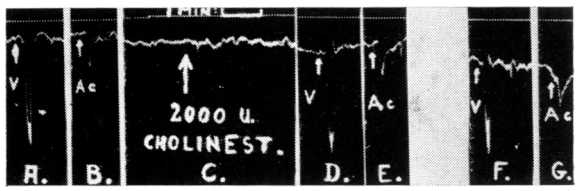

FIG. 2*a*.—(Exp. 6.) Rat. ♀ 330 g. Chloralose. Blood pressure. Drum rate in section C. *V* = Vagus stimulation: 60 per sec., for 10 sec., strength 1 volt. *Ac* = Acetylcholine chloride: 0.1 μg. intravenously. (A) and (B) before injection of cholinesterase. (C) 2,000 units red cell enzyme intravenously. (D) 5 min., (E) 8 min., (F) 45 min., and (G) 48 min., after the injection in (C).

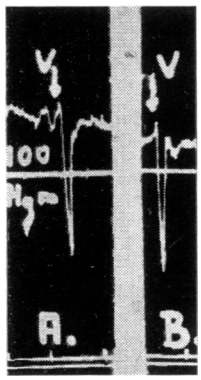

FIG. 2*b*.—(Exp. 30.) Rat. ♂ 290 g. Chloralose. Blood pressure. Time in minutes. *V* = Vagus stimulation: 60 per sec. for 10 sec., strength 7 volts. (A) Before injection of cholinesterase. (B) 2 min. after the injection of 1,000 units red cell enzyme.

contradict those of Koppanyi and Karczmar (1948), but are in accordance with later work of the same authors (1949).) The explanation of this will be discussed in connexion with neuromuscular transmission in striated muscle.

The action of red cell enzyme on neuromuscular transmission in vivo

Two different methods have been employed:

(*a*) The sciatic nerve of anaesthetized rats was prepared and the Achilles tendon freed and tied to one arm of an isometric torsion-spring myograph. The length of this arm was 1.5 cm. The other arm, 22.5 cm. long, formed the writing lever. Calibration was done with weights. The tibia was held in a clamp in order to immobilize the hind limb, with the nerve resting on silver wire electrodes; submaximal or supramaximal stimuli were applied at a rate of 2 per min. Injections of 1,000 to 2,000 units of red cell enzyme and of neostigmine sulphate were made through a cannula tied into the external jugular vein.

The twitch tension of the gastrocnemius did not change after 1,000 or 2,000 units of intravenously injected red cell enzyme. In order to be sure that the preparation had not deteriorated, and that other substances were still able to produce their effect, 50 μg. neostigmine sulphate per kg. was injected intravenously at the end of every experiment: it produced a prompt increase in twitch tension (Fig. 3).

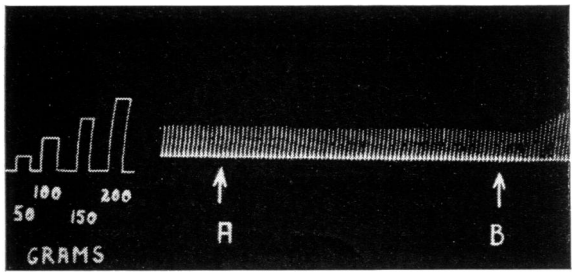

FIG. 3.—(Exp. 16.) Rat. ♀ 290 g. Chloralose. Isometric recording of the gastrocnemius. Submaximal stimulation of the sciatic nerve. One stimulus every 30 sec. Strength 7 volts. At A, 2,000 units red cell enzyme. At B, 33 min. later 50 μg. neostigmine sulphate intravenously.

(*b*) In order to increase the possibility of the enzyme reaching the endplates it was injected directly into the artery supplying the cat's tibialis anterior muscle and the isometric contractions of the muscle were recorded. In three experiments Brown's method (1938) was used and in one the enzyme was injected into the femoral artery, all the branches of the latter except that going to the tibialis anterior muscle having been tied. Stimulation at the rate of 6 per min. was applied in all four experiments to the peripheral end of the tibialis nerve; in two of supramaximal and in two of submaximal strength. With this technique a relatively high concentration of enzyme reached the muscle in a short time, and, if the enzyme were able to reach the endplate, a greater quantity should have done so than in the previous experiments. One to two thousand units of enzyme were injected in one and a half seconds. In none of the four experiments did any change in the twitch tension occur after the injection of the enzyme. One of the records is reproduced in Fig. 4.

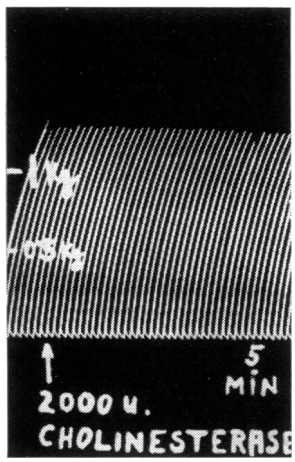

FIG. 4.—(Exp. 25.) Cat. ♂ 3.35 kg. Chloralose. Isometric recording of the tibialis anterior muscle. Supramaximal stimulation of the tibialis nerve, once every 10 sec. Strength 2 volts. At arrow, 2,000 units red cell enzyme. Close arterial injection; duration of injection 1.5 sec.

Action of red cell enzyme on neuromuscular transmission in vitro

In order to increase further the possibility of the enzyme reaching the muscular endplate two different isolated preparations were studied.

(*a*) *Isolated sciatic gastrocnemius preparation of the frog.*—The sciatic nerve and the gastrocnemius were prepared by the usual technique. The external fascia of the gastrocnemius was clipped open to allow easier penetration of the enzyme to the neuromuscular junction. The gastrocnemius was fixed at the knee in a 50-ml. bath containing Ringer solution, the composition of which was as follows: NaCl = 0.65 g.; KCl = 0.02 g.; $CaCl_2$ (anhydr.)

= 0.02 g.; 98 ml. distilled water; 2 ml. of phosphate solution (2.86 g. Na_2HPO_4 in 80.6 ml. 0.1 N-HCl and 19.4 ml. distilled water, *p*H = 7.1).

The contractions were recorded isometrically. The sciatic nerve was stimulated with single shocks twice per minute at supra- or sub-maximal strength. After stabilization of contraction had occurred, different quantities of red cell enzyme were added to the bath and their effects on the contractions observed.

(*b*) *Isolated phrenic nerve-diaphragm preparation of rat.*—For this experiment a preparation described by Bülbring (1946) was used. The stimulation of the phrenic nerve was 1 per sec. or 1 per 10 sec., supra- or sub-maximal, with the electrodes described by Bülbring. The bath contained 50 ml. of modified Tyrode solution aerated with 95% O_2 + 5% CO_2 (Taugner and Fleckenstein, 1950). Contractions are more stable and steadier in this fluid than in normal Tyrode. The isotonic contractions were recorded with a well-balanced light frog heart lever. The contractions before and after addition of red cell enzyme to the bath were observed.

Though the concentration of red cell enzyme in the experiments of both series was very high (20 to 40 units/ml.), no changes in contractions occurred after its addition to the bath (Fig. 5).

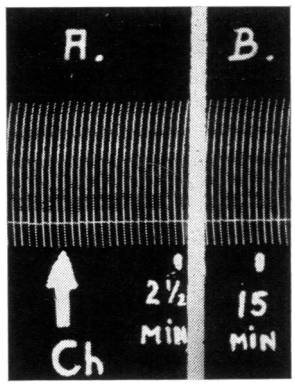

FIG. 5.—(Exp. 32.) Isotonic recording of the rat phrenic nerve-diaphragm preparation *in vitro*. Muscle bath containing 50 ml. modified Tyrode solution at 37° C. Supramaximal stimulation of the phrenic nerve once every 10 sec. Strength 5 volts. (A) *Ch* = 1,000 units red cell enzyme added to the bath. (B) 15 min. after addition of enzyme to the bath.

The action of red cell enzyme on the muscular contractions caused by close arterial injections of acetylcholine

Two experiments were performed with the method described by Brown (1938). In order to avoid the muscarinic effects of acetylcholine the cats were injected with 0.5 mg. atropine per kg.

In one experiment 10 μg. and in the other 20 μg. acetylcholine in 0.2 ml. saline were injected into the prepared tibialis artery of the cat. Intravenous injections of red cell enzyme did not affect the tension developed significantly (Fig. 6) despite the large amount of enzyme injected (9,000 units in one experiment and 10,000 in the other).

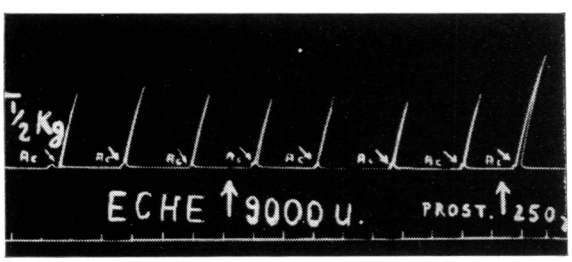

FIG. 6.—(Exp. 36.) Cat. ♀ 2.05 kg. Chloralose. Isometric recording of the tibialis anterior muscle. Atropine sulphate 0.5 mg./kg. i.v. Time in minutes. Ac = close arterial injections of 20 μg. acetylcholine chloride. ECHE = intravenous injection of 9,000 units red cell enzyme. Prost = intravenous injection of 250 μg. neostigmine sulphate.

In order to check the responsiveness of the muscle 250 μg. neostigmine sulphate were injected intravenously at the end of the experiments and the expected increase in tension of the contraction occurred.

The influence of red cell enzyme on chromodacryorrhoea induced by acetylcholine injections

It was of interest to know whether the experiments of Mendel and Hawkins (1943) could be repeated with this preparation of cholinesterase. The short duration of increased esterase activity in blood obtained after the injection of the red cell enzyme decreased this possibility since, according to the authors cited, cholinesterases which remain for a longer time in the blood counteract for a longer period the " bloody tears " effect of acetylcholine than those which remain for only a short time. For this reason the red cell enzyme could not be expected to cause more than a retardation of the appearance or a diminution of the abundance of the chromodacryorrhoea.

The experiments were carried out with the technique of Mendel and Hawkins; 1 mg. acetylcholine chloride was injected subcutaneously into rats weighing about 50 g. and the chromodacryorrhoea was observed by touching the eye with the edge of a filter paper. The coloration of the filter paper and the size of the drop after contact with the eye served as a measure of the acetylcholine action. This measurement was repeated at different intervals one to twenty minutes after the injection of acetylcholine, depending on the amount of chromodacryorrhoea. Five animals were tested without previous injection of enzyme; five others two minutes after injection of the enzyme. After one week a cross-over test was carried out. The red cell enzyme (37.5 to 1,000 units) was injected intravenously or intracardially.

It was observed that the chromodacryorrhoea produced by the subcutaneous injection of 1 mg. acetylcholine generally appeared in the first minute and reached its maximum about five minutes after the injection. Its usual duration was fifteen minutes, though occasionally it lasted longer.

In three animals out of ten the chromodacryorrhoea appeared to be slighter if the acetylcholine was injected after the red cell enzyme, but the response appeared within the normal time limit. In two animals it appeared to be greater when acetylcholine was injected after the enzyme, and in five other animals the injection of enzyme did not affect the course of chromodacryorrhoea produced by acetylcholine. Red cell enzyme seems, therefore, to have no effect upon this phenomenon.

No change in pupil diameter was caused by the red cell enzyme nor was the response to light modified.

Discussion

The rapid disappearance of the injected red cell enzyme from the blood stream is puzzling. Is it destroyed or inactivated, does it simply diffuse into the tissue spaces, does some special organ store it or does rapid excretion cause this quick disappearance? If it is destroyed the destruction does not occur in the blood; this is shown by the fact that, although all determinations of blood cholinesterase were done several hours after the blood sample had been taken, yet, as may be seen in Table I, when the blood was withdrawn two minutes after the injection, about 80 per cent of the total amount of injected enzyme was recovered; if the blood had continued to circulate, the total amount would have disappeared within five minutes. Diffusion cannot account for a

complete disappearance of the enzyme from the blood. The relatively small increase in muscle cholinesterase at a time when no increased activity in the blood remains removes the possibility of equal distribution in the body by diffusion. Had the injected cholinesterase been distributed equally throughout the extracellular fluid, or throughout the whole of the body water, the increase in muscle cholinesterase should have been of the order of about 3 units per g. muscle; instead, the mean increase observed was only 0.76 units per g. muscle. An appreciable excretion of red cell enzyme in the urine or faeces in so short a time is improbable. The only remaining possibility is inactivation of the enzyme or its accumulation by a special tissue. Koppanyi and Karczmar (1948) state that the liver is probably involved in the removal of the enzyme from the blood, but the experiments they mention indicate only that the disappearance is due chiefly to some process which occurs below the diaphragm.

During the short period in which the blood cholinesterase is increased the injection of acetylcholine produced a subnormal effect on the blood pressure. Evidently a portion is hydrolysed by the circulating enzyme.

The period in which the enzyme is effective against acetylcholine injections was much shorter in the present experiments (maximum 5 min.) than in those recently reported by Koppanyi and Karczmar (1948) (" less than 30 minutes ").

The negative nature of the results of the experiments in which the vagus, the sciatic of rats, and the tibialis anterior of the cat were stimulated, and of the experiments with the isolated gastrocnemius and diaphragm preparations, requires consideration. The most obvious explanation in all these experiments is the possible failure of the enzyme, injected into the general circulation, into the artery supplying the tibialis muscle, or applied directly to muscle and nerve, to reach the point essential for the elicitation of an effect. The conditions for penetration seem especially favourable in the diaphragm preparation; the muscle is very thin and the concentration of enzyme in the bath does not diminish as in the experiments in which the enzyme was injected intravascularly. It may be that some penetration did occur but that the excess of enzyme at the vital points is normally so high that the increases in concentration which may have occurred were too small to be detectable. It is known from the experiments of Eccles, Katz, and Kuffler (1941), Eccles and Kuffler (1941), and Kuffler (1942) that in skeletal muscle the endplate potential must be reduced to one-third of its original value before neuromuscular conduction fails appreciably. A study of the endplate potential changes after injection of the enzyme might throw some light upon the extent to which penetration actually occurs. The fact that even " close " injections of acetylcholine (into the tibialis artery) produced the same twitch of the tibialis muscle regardless of whether enzyme was injected or not indicates that the injection did not raise the enzyme concentration appreciably at the point essential for its action. A calculation based upon the extent to which the enzyme concentration of muscle is increased by the injection of 1,000 units of red cell enzyme in 300-g. rats shows that the increase in the entire tibialis anterior muscle of the cat is sufficient to hydrolyse only 0.3 μg. acetylcholine per quarter second. Since an injection of 10 to 20 μg. acetylcholine was made in a fraction of a second there could not have been enough destruction to affect its action.

These negative results are reminiscent of the persistent lack of response that Felix (1938) and Best and McHenry (1940) found on injection of their highly active histaminase preparation. Only Karady and Brown (1939) have been able to find that

injected histaminase counteracted histamine and anaphylactic shock, but Knoll (1940) and Youngner, Freedmann, and Nungster (1941) were unable to confirm their results.

SUMMARY

1. The effects of intravenously injected bovine erythrocyte true cholinesterase (red cell enzyme) were studied.

2. The cholinesterase level in blood of 300 g. male and female rats is increased in the first two minutes and returns to normal five minutes after the intravenous injection of 1,000 units of red cell enzyme.

3. The muscle cholinesterase activity is increased for two to ten minutes or longer.

4. The hypotensive action of intravenously injected acetylcholine on the rat's blood pressure is partly reduced, but not abolished, immediately after the intravenous injection of 1,000 units of red cell enzyme. No reduction in the hypotensive effect of acetylcholine occurs five minutes after injection of the enzyme.

5. The blood pressure level of rats is not affected by the intravenous injection of 1,000 units of red cell enzyme.

6. The effect of vagus stimulation on the blood pressure of rats is not affected by the intravenous injection of 1,000 units of red cell enzyme.

7. Red cell enzyme injections do not influence neuromuscular transmission *in vivo* in the sciatic gastrocnemius preparation of the rat or in the tibialis anterior of the cat.

8. Red cell enzyme does not influence neuromuscular transmission in the isolated sciatic-gastrocnemius preparation of the frog or in the isolated phrenic-diaphragm preparation of the rat.

9. The twitch produced by close intra-arterial injection of acetylcholine is not affected by the administration of red cell enzyme.

10. The chromodacryorrhoea in rats produced by subcutaneous injection of acetylcholine is not affected by this preparation of cholinesterase, nor does it dilate the pupils of rats.

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