

## HYDROLYSIS OF SUCCINYLCHOLINE SALTS

BY

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Glick (1941), whilst working with various esters of choline, demonstrated that the choline salt of succinic acid was hydrolysed by horse serum and by alkali. Bovet, Bovet-Nitti, Guarino, Longo, and Marotta (1949) and de Beer, Castillo, Phillips, Fanelli, Wnuck, and Norton (1951) showed that succinylcholine possessed powerful neuromuscular-blocking properties. The latter authors showed that eserine potentiated the paralysis of the cat gastrocnemius caused by succinylcholine. As compound 49-164, which does not inhibit cholinesterase, produced a similar potentiation they doubted whether cholinesterase was the enzyme responsible for the destruction of succinylcholine. This seemed worth re-investigation.

In the last two or three years many surgeons and anaesthetists have preferred succinylcholine to other neuromuscular-blocking compounds for many surgical procedures because of its very short duration of action and its freedom from undesirable side-effects. It is ideally suited for administration as an intravenous drip to give a rapidly controllable degree of muscle relaxation. It has also found favour for use with thiopentone in electroconvulsion therapy (Arnold, Böck-Greisau, and Ginzl, 1951; Richards and Youngman, 1952).

Mayrhofer (1952) has, however, stated that succinylcholine is stable in solution for several weeks outside the refrigerator and also when mixed with thiopentone. This was surprising, since succinylcholine is unstable in alkaline solution (Glick, 1941) and the pH of thiopentone solution is around 11.0. It thus seemed desirable to re-examine Mayrhofer's claims. The hydrolysis of succinylcholine at elevated temperatures, in alkaline solutions, and by pseudo-cholinesterase, has accordingly been studied. A quantitative comparison has also been made between succinylcholine and its first hydrolysis product, succinylmonocholine, which still preserves a degree of neuromuscular-paralysing activity.

### METHODS

*Toxicity.*—Groups of 5 or 10 albino mice of either sex were used, injections being made into the tail vein.

*Action on Nerve-Muscle Preparations.*—The isolated rat diaphragm-phrenic nerve preparation of Bülbring (1946) was used (a) as an *in vitro* test for concentration of succinylcholine, and (b) for comparison of the concentrations of succinylcholine and succinylmonocholine to give equal neuromuscular-paralysing action. The isolated organ was immersed in a Ringer-Locke solution in a 100 ml. bath, and square stimuli of 2 V. and of 0.23 msec. duration were applied at 5/min. to the nerve. Drugs were added from a tuberculin syringe and left in contact with the diaphragm for 3 min. This was followed by two washes with Ringer-Locke at 3 and 6 min. respectively, giving a total cycle of 10 min. In 3 min., 50% paralysis of the diaphragm twitch was produced by a concentration of about 15.0  $\mu\text{g./ml.}$  of succinylcholine.

Tests *in vivo* were performed on cats and rabbits of either sex. The cats were anaesthetized with pentobarbitone sodium (usually 40 mg./kg. i.p.), and the rabbits with urethane (amount required varied considerably). Respiration was recorded from the cannulated trachea by a tambour recorder. The blood-pressure tracing was obtained from the cannulated carotid artery and injections were made into the femoral vein (cat) or jugular vein (rabbit). The muscle—gastrocnemius in the cat, extensor digitorum longus in the rabbit—was stimulated via the sciatic nerve by 6 discharges/min. from a neon stimulator.

For the chick paralysis test of Buttle and Zaimis (1949) two- to three-week-old chicks of either sex were used, injections being made into the wing vein. The duration of paralysis was recorded.

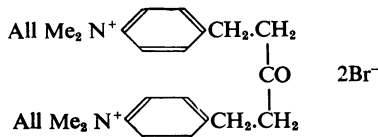
*Manometric Methods* (Warburg Respirometer).—Single side-arm flasks were used. The substrate was placed in the side arm and the enzyme preparation and Ringer-bicarbonate, to a total volume of 3.0 ml., in the base of the flask. Reactions were allowed to proceed in an atmosphere of 95%  $\text{N}_2$  : 5%  $\text{CO}_2$ . Corrections were made for extraneous temperature and pressure variations and for spontaneous hydrolysis of the substrate. The results were recorded as  $\mu\text{l. CO}_2$  evolved at 37° C. Ringer-bicarbonate was prepared by mixing

together, immediately before use, 50.0 ml. 0.9% NaCl, 15.0 ml. 1.26% NaHCO<sub>3</sub>, 1.0 ml. 1.2% KCl and 1.0 ml. 1.76% CaCl<sub>2</sub> (hydrated), and gassing for 10 min. with a 95% N<sub>2</sub>:5% CO<sub>2</sub> mixture.

**Cholinesterase Preparations.**—Human blood was withdrawn into a dry syringe and 1 part of a saturated solution of potassium oxalate was immediately added to 10 parts of whole blood. After gently mixing, this was centrifuged and the supernatant plasma drawn off. This provided the human plasma (pseudo) cholinesterase preparation which produced about 1,500  $\mu$ l./CO<sub>2</sub>/ml./hr. with benzoylcholine chloride (0.015M) as substrate. The packed red cells were washed twice with Ringer-bicarbonate, centrifuged, and the supernatant fluids discarded. The packed, washed red cells were then haemolysed by adding distilled water up to the original blood volume. This provided the human red blood-cell (true) cholinesterase preparation which produced about 2,000  $\mu$ l. CO<sub>2</sub>/ml./hr. with acetyl- $\beta$ -methylcholine chloride (0.03M) as substrate.

**Succinylcholine Salts.**—Succinylcholine dichloride was prepared by Burroughs Wellcome and Co. Succinylmonocholine monochloride was kindly prepared for me by Dr. Tudor Jones of the Wellcome Chemical Research Laboratories (see addendum).

**284C51.**—This compound (Copp, 1953) is 1: 5-bis(4-allyldimethylammoniumphenyl)-*n*-pentan-3-one dibromide,



It has been shown by Fulton and Mogeys (1954) to be a specific inhibitor of true cholinesterase with a *p*I 50 of 7.8 for true cholinesterase and a *p*I 50 of less than 3.0 for pseudo-cholinesterase.

## RESULTS

**Dose-Response Curve (Rat Diaphragm).**—The log dose-response curve for succinylcholine on the rat diaphragm was substantially linear over a wide range of responses. Fig. 1 gives 6 such curves chosen at random from over 50 examples. Three of these were multipoint log dose-response curves and three were pairs of curves taken from 2  $\times$  2 assays of "test" against "standard." The mean slope of these curves was 132 (i.e., 132% change in paralysis/tenfold dose increment). Calculation from this mean slope showed that a 50% fall in concentration of succinylcholine was accompanied by a 40% lowering of paralysis.

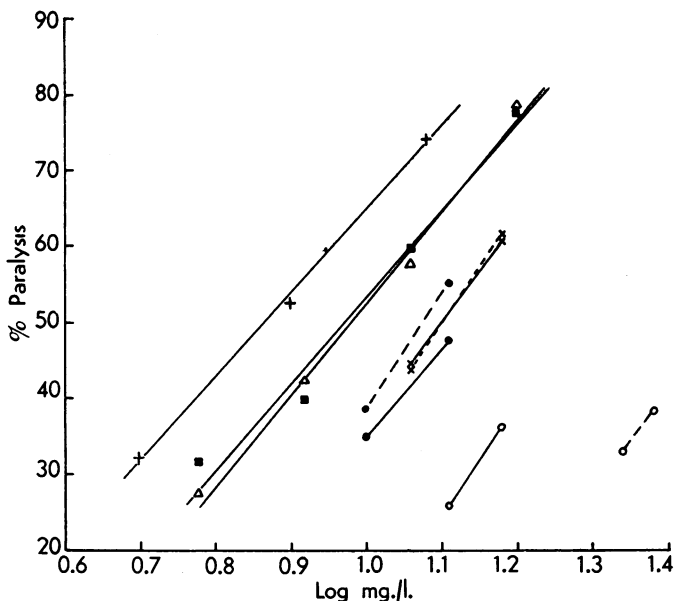


FIG. 1.—Dose-response curve for succinylcholine on isolated rat diaphragm. Ordinate: % paralysis of diaphragm in 3 min. Abscissa: log mg./l. succinylcholine. Three dose-response curves and three pairs of 2  $\times$  2 assays of "standard" against "test" are given. These were chosen at random from over 50 examples and have a mean slope of 132, i.e., 132% change in paralysis per tenfold dose increment.

**Effect of pH.**—Succinylcholine solutions were buffered at pH 5.0, 7.4, 8.2, 8.95, and 9.5 and maintained at 21° C. The residual succinylcholine activities of the various solutions were compared with that of the pH 7.4 solution on the isolated rat diaphragm-phrenic nerve preparation. The amount of control succinylcholine chosen was such as to give 70% paralysis in 3 min.

The difference between the % paralysis caused by the test and by the control solutions was plotted against the time in min. after solution of the succinylcholine in buffer (Fig. 2). Equivalent amounts of the buffers alone when added to the organ bath produced no change in the size of the muscle twitch.

These results showed no detectable hydrolysis of succinylcholine at pH 5.0, or at pH 7.4, but a slow hydrolysis at pH 8.2. In more alkaline solutions hydrolysis was more rapid, and at pH 9.5 there was a 50% loss of activity in just under 3 hr.

**Hydrolysis in Thiopentone Solution.**—Thiopentone alone on the isolated rat diaphragm caused a very marked increase in the size of the muscle twitch and in the sensitivity of the preparation to paralysis by succinylcholine. In view of this it was necessary to remove the thiopentone from a mixture of the two drugs before a satisfactory test of succinylcholine hydrolysis by thiopentone could be performed on the rat diaphragm.

## SUCCINYLDICHLORINE HYDROLYSIS

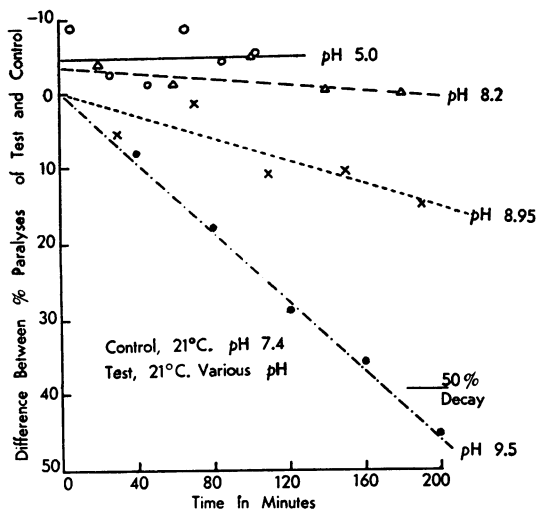


Fig. 2.—To show effect of buffered solutions at various pH's on destruction of succinylcholine *in vitro*. Rat diaphragm (isolated). Ordinate: difference between % paralysis caused by control and test samples of same initial concentrations. Abscissa: time in min. after preparation of solutions.

A solution in distilled water was prepared, each ml. containing 3.0 mg. succinylcholine and 10.0 mg. thiopentone. The pH of this mixture was 11.0 and it was maintained at room temperature (20° C.) until aliquots were neutralized to pH 7.0 with HCl. This resulted in precipitation of the thiopentone which was filtered off. (A control run in the absence of succinylcholine showed that sufficient thiopentone had been removed for such as remained to have no effect on the diaphragm.)

Half a ml. of filtrate was then added to the 100-ml. organ-bath and the paralysis occurring in 5 min. was noted. This concentration was found to give total paralysis of the diaphragm in 5 min. when neutralization was performed immediately after preparation of the solution. No paralysis was obtained when 90% of this amount of succinylcholine had been hydrolysed. The plot of % paralysis against log time of precipitation of the thiopentone after mixing of the two drugs, gave the straight line shown in Fig. 3. When a similar procedure was adopted using NaOH to pH 11.0 instead of thiopentone, total hydrolysis was obtained in under 10 sec. The significance of this will be discussed later.

**Effect of Temperature.**—In a series of tests similar to those for demonstrating the effect of pH, test and control solutions were buffered at pH 7.4. The control solution was maintained at 21° C. and the test solutions were incubated at temperatures of 40° C., 50° C., and 100° C. respectively.

Fig. 4 presents the mean results and demonstrates the instability of succinylcholine at tem-

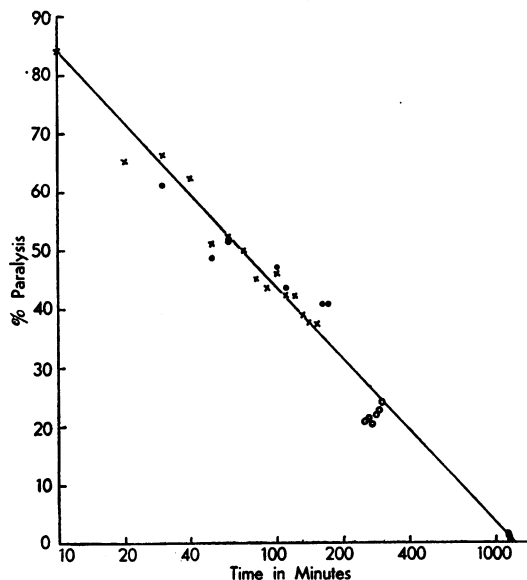


Fig. 3.—To show decay of succinylcholine in the presence of thiopentone (10.0 mg./ml.) pH 11.0. X, O, ●—three rat diaphragms. Ordinate: % paralysis. Abscissa: log time in min. between mixing of thiopentone and succinylcholine and precipitation of thiopentone by HCl to pH 7.0.

peratures higher than normal body temperature, 50% hydrolysis occurring in about 30 min. at 100° C.

**Effect of Cholinesterases.**—The action of cholinesterases on the paralyzing activity of succinylcholine was also studied. For the control, succinylcholine was buffered at pH 7.4 and maintained at 37° C. Test samples were made up in plasma, red blood cell haemolysate, whole blood, or in plasma to which eserine sulphate had been added to a final concentration of  $10^{-6}$ M and also incubated at 37° C.

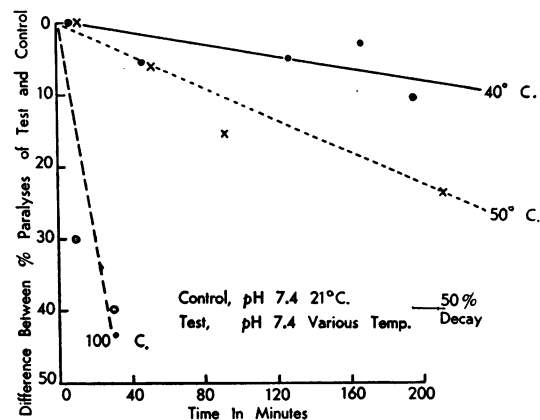


Fig. 4.—To show effect of temperature on solutions of succinylcholine buffered at pH 7.4. Rat diaphragm (isolated). Ordinate and abscissa as in Fig. 2.

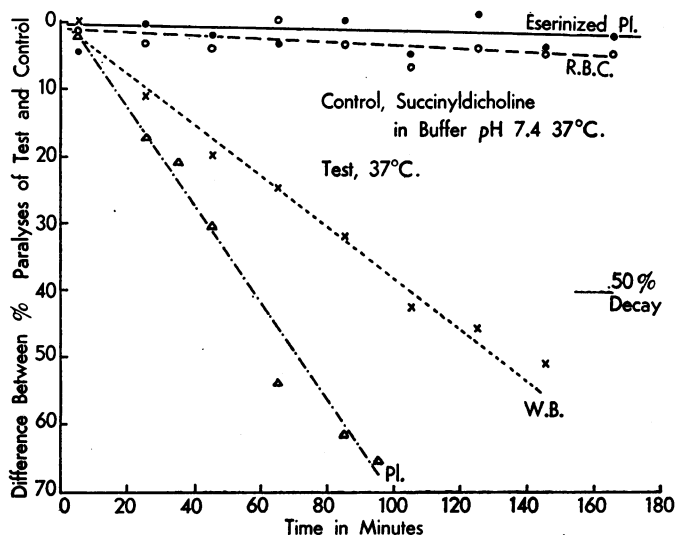
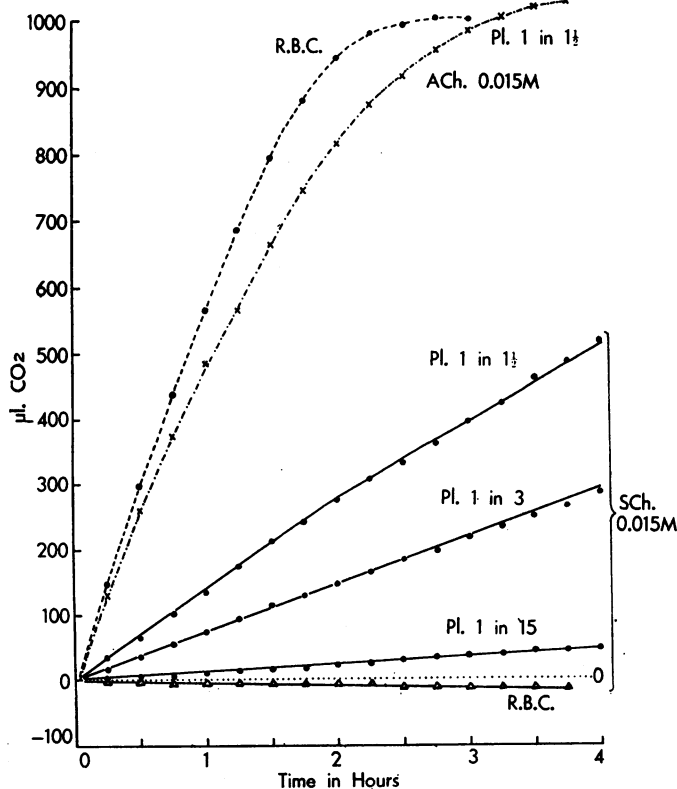


FIG. 5.—To show effect of cholinesterases on succinylcholine *in vitro*. Rat diaphragm (isolated). Ordinate and abscissa as in Fig. 2. Pl, human plasma (pseudo-cholinesterase). W.B., human whole blood (true and pseudo-cholinesterases). R.B.C., human red blood cell haemolysate (true cholinesterase). Eserinized Pl, human plasma pseudo-cholinesterase inhibited by eserine sulphate ( $10^{-4}M$ ).



Neither red blood cells nor eseriniz plasma destroyed succinylcholine (Fig. 5). Uninhibited plasma caused rapid hydrolysis of succinylcholine, whereas whole blood acted at a somewhat slower rate. The plasma volume in whole blood is 50–60% and, from Fig. 5, the rate of hydrolysis in whole blood was 60% of that in plasma. These results show that, *in vitro*, succinylcholine was hydrolysed by pseudo-cholinesterase but was unaffected by either true or inhibited pseudo-cholinesterase.

This was also demonstrated in the Warburg respirometer using three concentrations of human plasma, human red blood cell haemolysate, and eseriniz plasma. In Fig. 6 the hydrolysis curves for acetylcholine chloride have been included for comparison.

**Cat Gastrocnemius.**—Rapid destruction of succinylcholine *in vivo* was shown by the short duration of paralysis of the cat gastrocnemius muscle on intravenous injection of the drug. Further, identical injections repeated at 15-min. intervals caused the same degree (%) and duration of paralysis after the first few injections; hence there was no evidence of accumulation of succinylcholine in the animal.

In the experiment shown in Fig. 7, the cat was given a series of injections of succinylcholine (0.04 mg./kg.) until a constant response was obtained. At the arrow B, 0.2 mg./kg. 284C51 was given intravenously. This was sufficient to cause a considerable depression of true cholinesterase activity in the blood stream but to have no effect on the pseudo-cholinesterase. Following this by the standard injection of succinylcholine there was no change from the response obtained before 284C51. After 0.2 mg./kg.

FIG. 6.—To show hydrolysis of succinylcholine by cholinesterases *in vitro*. Warburg respirometer. Upper two curves show hydrolysis of acetylcholine (0.015M) by human plasma and red blood cells. Lower four curves show hydrolysis of succinylcholine (0.015M) by 3 concentrations of human plasma, and lack of hydrolysis by human red blood cells. Ordinate: corrected output of  $CO_2$  in  $\mu l$ . Abscissa: time in hr.

eserine sulphate, which gave 40% inhibition of the pseudo-cholinesterase, the paralysis caused by the standard dose of succinylcholine was increased and prolonged. Normal cat plasma had half the pseudo-cholinesterase activity of human plasma. Fig. 8 presents the results of a typical experiment, and shows how the maximum effect of the eserine in potentiating the succinylcholine was not achieved until about 1 hr. after the eserine was injected intravenously, despite the fact that this dose of eserine caused its maximum inhibition of the plasma pseudo-cholinesterase within 10 min. of the injection.

**Chick Paralysis.**—Intravenous injection of succinylcholine into the chick causes a spastic paralysis of the leg and neck muscles (Buttle and Zaimis, 1949). Using a dose of 0.03 mg./kg., a spastic paralysis lasting 3–4 min. was obtained. 0.5 mg./kg. 284C51 given half an hour before the succinylcholine produced no change in the duration of paralysis from that found in the controls. Similarly, eserine sulphate at 0.1 mg./kg. and 0.2 mg./kg. caused no significant prolongation of paralysis; but when 0.5 mg./kg. was given half an hour before the succinylcholine the duration

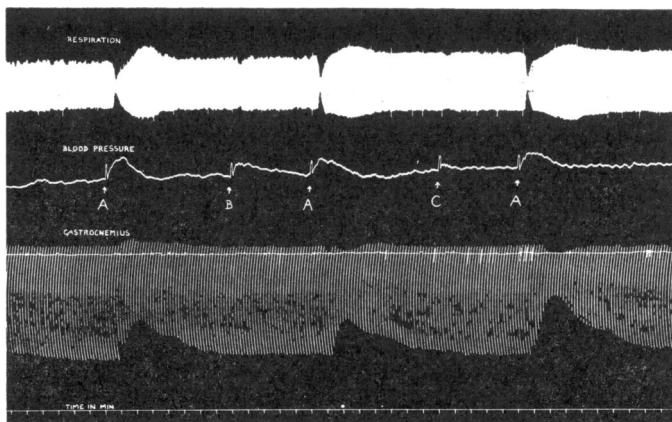


FIG. 7.—To show the potentiation of the succinylcholine paralysis of the cat gastrocnemius *in vivo* by eserine sulphate. Cat ♀, 5 kg. pentobarbitone sodium (40 mg./kg. i.p.). Gastrocnemius stimulated through sciatic nerve. At A, succinylcholine 0.04 mg./kg. i.v. At B, 284C51 0.2 mg./kg. i.v. At C, eserine sulphate 0.2 mg./kg. i.v. (see also Fig. 8).

of paralysis was extended to 7–8 min.—an increase of 80–100%. Normal chick plasma had 1/18 of the pseudo-cholinesterase activity of human plasma with benzoylcholine as substrate, and was also capable of hydrolysing succinylcholine at a slow rate, as is shown in Fig. 9.

**Recovery in Rabbits.**—Bourne, Collier, and Somers (1952) and Evans, Gray, Lehmann, and Silk (1952) have demonstrated a low pseudo-cholinesterase level in the plasma of patients who

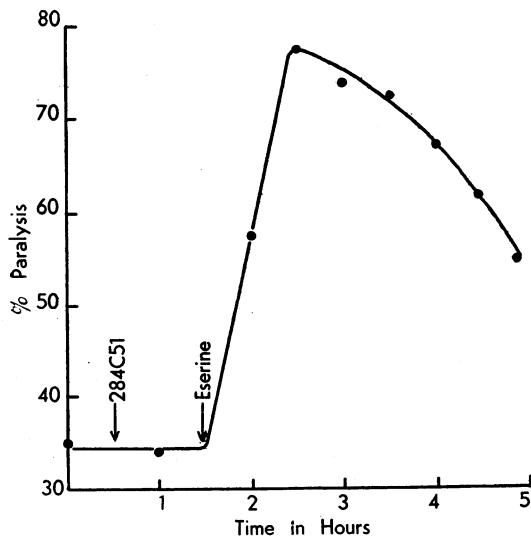


FIG. 8.—Graphical presentation of potentiation of succinylcholine paralysis of cat gastrocnemius by eserine. Ordinate: % paralysis of gastrocnemius muscle. Abscissa: time in hr. At first arrow: 284C51 0.2 mg./kg. i.v.; at second arrow: eserine sulphate 0.2 mg./kg. i.v. Each dot represents % paralysis of the muscle caused by 0.04 mg./kg. succinylcholine i.v.

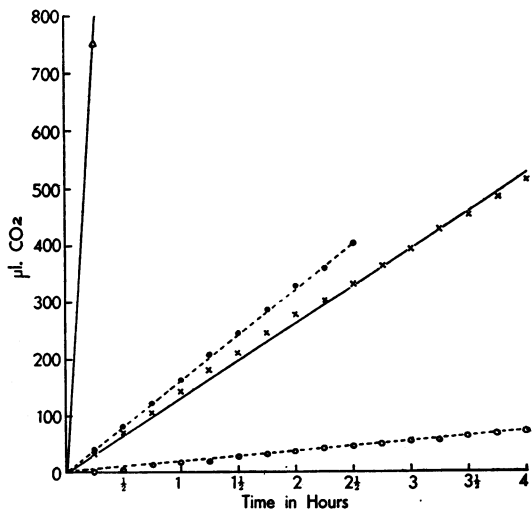


FIG. 9.—To show hydrolysis of succinylcholine by chicken plasma. Warburg respirometer. Ordinate:  $\mu\text{l. CO}_2$ . Abscissa: time in hr.  $\Delta$ — $\Delta$  Human plasma (1 in  $1\frac{1}{2}$ ) + benzoylcholine (0.015M). X—X Human plasma (1 in  $1\frac{1}{2}$ ) + succinylcholine (0.015M).  $\bullet$ — $\bullet$  Chicken plasma (1 in  $1\frac{1}{2}$ ) + benzoylcholine (0.015M).  $\circ$ — $\circ$  Chicken plasma (1 in  $1\frac{1}{2}$ ) + succinylcholine (0.015M).

had unusually prolonged recovery times from succinylcholine apnoea. In view of the findings of Koelle (1953), that rabbits fall into two distinct groups on the basis of their plasma pseudo-cholinesterase levels, rabbits were used to determine whether the low-activity group would take longer to recover from succinylcholine paralysis than the high-activity group. Some 20 rabbits from each group were used and the extensor digitorum longus muscle was stimulated via the sciatic nerve. It was not possible to demonstrate any relationship between plasma pseudo-cholinesterase level, sensitivity to succinylcholine, and time for recovery of the muscle from 66% paralysis to 33%. Enzyme estimations performed on this muscle failed to show any appreciable pseudo-cholinesterase activity.

**Succinylmonocholine Monochloride.**—This compound, which is the first hydrolysis product of

TABLE I  
COMPARISON OF ACTIVITIES OF SUCCINYLDICHOLINE  
AND SUCCINYLMONOCHOLINE

Test Animal	Criterion for Comparison	Dose of Drug		Ratio of Activity of Succinyl-dicholine to Succinyl-monocholine
		Succinyl-dicholine	Succinyl-monocholine	
Mouse	i.v. LD50 (mg./kg.)	0.455	4.66	10:1
Rat	50% paralysis diaphragm in 3 min. (mg./100 ml.)	0.9	10.0	11:1
Cat	50% paralysis of gastrocnemius muscle (mg./kg.)	0.025	1.0	40:1
Chick	3-4 mins. duration spastic paralysis (mg./kg.)	0.03	15.0	500:1

succinylcholine, had similar qualitative actions. Quantitatively, however, it differed, the ratio of activities varying according to the test animal (Table I).

In the Warburg respirometer, succinylmonocholine was hydrolysed by human plasma pseudo-cholinesterase (see Fig. 10). The ratio of the rate of hydrolysis of succinylcholine to that of succinylmonocholine was 1.4. No hydrolysis was obtained with human red blood cells or with eserinized plasma (eserine  $10^{-5}$  M).

## DISCUSSION

Using the isolated rat diaphragm, it has been shown that succinylcholine is unstable in alkaline solutions and at temperatures above about 40° C. Increasing alkalinity or increasing temperature led to a faster rate of destruction of the succinylcholine. In Figs. 2 and 4, summarizing these results, a straight line was obtained by plotting % paralysis of the rat diaphragm against incubation time. Fig. 1 showed that, on the rat diaphragm, % paralysis was directly proportional to log concentration, and hence the hydrolysis of succinylcholine both by alkali and by heat, was a first-order reaction. The same deduction also applied to the hydrolysis by pseudo-cholinesterase (Fig. 5). The reaction with thiopentone was different. Firstly, the rate of destruction of succinylcholine by thiopentone at pH 11.0 was very much less rapid than that obtained with NaOH at pH 11.0; secondly (Fig. 3), a straight line was only obtained when % paralysis was plotted against log time—thus this was a second-order

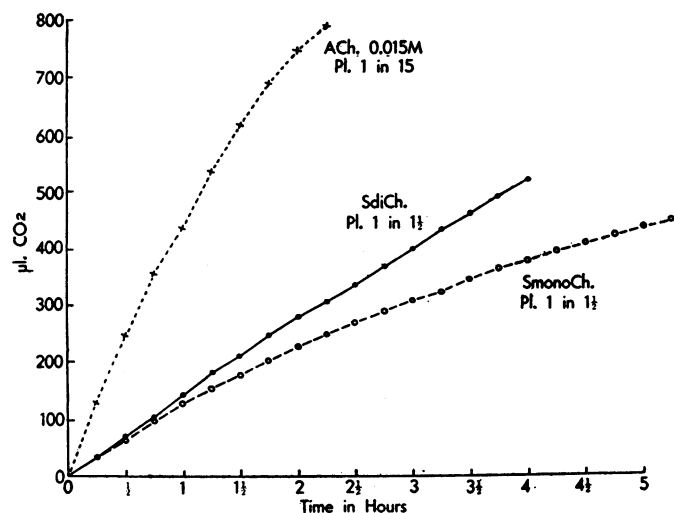


FIG. 10.—To show hydrolysis of succinylmonocholine by pseudo-cholinesterase *in vitro*. Warburg respirometer. Ordinate:  $\mu\text{l. CO}_2$ . Abscissa: time in hr. X---X Human plasma (1 in 15)+acetylcholine (0.015M). ●—● Human plasma (1 in 1½)+succinylcholine (0.015M). O---O Human plasma (1 in 1½)+succinylmonocholine (0.015M).

reaction. The result was suggestive of a reaction occurring between the succinylchlorine and the thiopentone as well as the straightforward alkaline hydrolysis. The rate of 50% loss of activity in the first 90 min. was sufficiently fast to make it inadvisable to mix thiopentone and succinylchlorine in the one syringe for intravenous injection.

The *in vitro* enzyme experiments agreed with those of previous authors (Glick, 1941; de Beer *et al.*, 1951; Whittaker, 1951; Evans *et al.*, 1952) that succinylchlorine salts were destroyed by pseudo-cholinesterase, but differed from those of Bovet-Nitti (1949) and Tamme (1953), who demonstrated hydrolysis by both cholinesterases. *In vivo* experiments in the cat and chick also showed that destruction of the true cholinesterase had no effect on the sensitivity to succinylchlorine, whereas inhibition of pseudo-cholinesterase considerably potentiated the paralysis caused by a standard injection of the drug. Further, in the cat, maximum inhibition of pseudo-cholinesterase in the plasma occurred in under 10 min. after intravenous injection of 0.2 mg./kg. eserine sulphate. However, maximum potentiation of succinylchlorine did not occur until 1 hr. after the injection of eserine (Fig. 8). It thus appeared that the pseudo-cholinesterase level in the plasma was not the only factor determining the duration of action of succinylchlorine *in vivo* (cf. de Beer *et al.*, 1951). Further support was provided by the rabbit experiments, where it was not possible to demonstrate any relationship between plasma pseudo-cholinesterase level, sensitivity to succinylchlorine and time for recovery of the extensor digitorum longus muscle from 66% paralysis to 33%. Enzyme estimations performed on this muscle showed only slight pseudo-cholinesterase activity. It is suggested that, although the plasma enzyme level plays an important part in determining the effect of an injection of succinylchlorine, other factors—for example, the pseudo-cholinesterase concentration in the muscle—may be equally important.

Succinylmonochlorine was similar in its actions to succinylchlorine but much less active (Table I). The ratio of 40:1 on the cat gastrocnemius was confirmed by Collier and Macauley (1953) on the cat tibialis. In the hydrolysis by human plasma pseudo-cholinesterase (Fig. 10) there was a slight discrepancy between these results and those reported by Lehmann and Silk (1953). Calculation from Lehmann and Silk's figures gave a ratio of the rate of hydrolysis of succinylchlorine to that of succinylmonochlorine, of 1.67 at 0.055M concentration of substrates, and of 3.8 at 0.0055M,

as compared with 1.4 at substrate concentrations of 0.015M in the experiments reported here. The concentration of enzyme used was, however, approximately three times greater in Lehmann and Silk's experiments and this probably accounts for the difference in the ratios.

#### SUMMARY

1. Succinylchlorine is unstable in alkaline solutions, the rate of destruction increasing with increasing alkalinity. The reaction is of the first order, and at pH 9.5 there is 50% loss of activity in just under 3 hr.

2. Mixed with thiopentone, succinylchlorine is rapidly hydrolysed, with 50% loss of activity in 90 min. There is apparently a reaction between the succinylchlorine and the thiopentone, since the decay curve shows it to be a second-order reaction.

3. Succinylchlorine is unstable in solution at temperatures above 40° C. At 100° C. there is 50% loss of activity in 30 min. The reaction is of the first order.

4. *In vitro*, succinylchlorine is hydrolysed by pseudo-cholinesterase but not by true cholinesterase or eserine-inhibited pseudo-cholinesterase.

5. *In vivo*, destruction of true cholinesterase by injection of 284C51 (a specific inhibitor of true cholinesterase) does not alter the response to succinylchlorine, whereas inhibition of the pseudo-cholinesterase by eserine considerably sensitizes the animal to succinylchlorine. Since maximum sensitization of a muscle is not achieved until 1 hr. after the eserine has been given intravenously, and since in rabbits there was no correlation between plasma pseudo-cholinesterase level and sensitivity of a muscle to succinylchlorine, it is suggested that factors other than plasma pseudo-cholinesterase concentration assist in determining the final effect of succinylchlorine on the muscle.

6. Succinylmonochlorine has been compared with succinylchlorine and found to be qualitatively similar in its actions but considerably less potent.

I wish to acknowledge the help and advice given by Dr. A. C. White, and the competent technical assistance of Miss L. S. Brooks and Messrs. F. Huggins and D. J. Tulett.

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## ADDENDUM

## PREPARATION OF SUCCINYLMONOCHOLINE ESTER SALTS

BY TUDOR S. G. JONES AND R. H. PAIN

The preparation of succinylmonocholine ester salts has been recently described by Phillips (1953), but before this a method was tried independently in order to provide material for the above and other work. Phillips found two methods to give satisfactory yields of salts of the ester: (1) esterification of 2-dimethylaminoethanol with acetic anhydride, followed by quaternation with methyl iodide, (2) direct esterification of choline iodide with acetic anhydride, in a melt at 140° C. A third attempt by partial hydrolysis of the di-ester iodide with alkali gave only some unchanged di-ester, succinic acid and sodium iodide. Our experiments, including examination of the products by paper chromatography, are in complete agreement with this last finding. The method of preparation adopted by us involved the discovery of a solvent—nitromethane—which dissolved both choline chloride and succinic anhydride.

*Preparation of Succinylmonocholine Ester Chloride.*—Choline chloride (7 g.) dried over KOH *in vacuo* and succinic anhydride (10 g., 2 equiv.) were dissolved in nitromethane (250 ml.) and refluxed for 3 hr. The solvent was removed by distillation. The residue was extracted under reflux successively with 350 ml. and 200 ml. of anhydrous ethyl ether and finally washed with dry ether. The product was dissolved in a minimum of hot ethanol and ether added to incipient crystallization. On cooling, a fine crystalline precipitate (10.7 g.) was obtained, filtered off and washed with dry ether. On recrystallization the yield was 9.9 g., m.p. 150–151°. It is readily recrystallized from ethanol. (Found: C, 45.1; H, 7.2; N, 5.9; Cl, 14.80.  $C_9H_{18}NO_4Cl$  requires C, 45.1; H, 7.5; N, 5.9; Cl, 14.80%.)

*Preparation of Succinylmonocholine Ester Perchlorate.*—The chloride (1.7 g.) was dissolved in water and an equivalent volume of silver perchlorate solution added. After boiling to coagulate the AgCl, which was removed by filtration, the solvent was evaporated under reduced pressure and the residual oil dried azeotropically with ethanol and benzene. Crystalline material was obtained from strong ethanol solution by seeding a strongly cooled solution, m.p. 116–117°. (Found: C, 35.6; H, 5.8; N, 4.8.  $C_9H_{18}NO_6Cl$  requires C, 35.6; H, 6.0; N, 4.6%.)

## REFERENCES

- Arnold, O. H., Böck-Greisau, W., and Ginzel, K. H. (1951). *Wien. med. Wschr.*, **101**, 492.  
 de Beer, A. J., Castillo, J. C., Phillips, A. P., Fanelli, R. V., Wnuck, A. L., and Norton, S. (1951). *Ann. N.Y. Acad. Sci.*, **54**, 362.  
 Bourne, J. G., Collier, H. O. J., and Somers, G. F. (1952). *Lancet*, **1**, 1225.  
 Bovet, D., Bovet-Nitti, F., Guarino, S., Longo, V. G., and Marotta, M. (1949). *R.C. Ist. sup. Sanità*, **12**, 111.  
 Bovet-Nitti, F. (1949). *Ibid.*, **12**, 357.  
 Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 48.  
 Buttle, G. A. H., and Zaimis, E. J. (1949). *J. Pharm. Pharmacol.*, **1**, 991.  
 Collier, H. O. J., and Macauley, B. M. (1953). *Brit. med. J.*, **1**, 1279.  
 Copp, F. C. (1953). *J. chem. Soc.*, 3116.  
 Evans, F. T., Gray, P. W. S., Lehmann, H., and Silk, E. (1952). *Lancet*, **1**, 1229.  
 Fulton, M., and Moge, G. A. (1954). *Brit. J. Pharmacol.*, **9**, 138.  
 Glick, D. (1941). *J. biol. Chem.*, **137**, 357.  
 Koelle, G. B. (1953). *Biochem. J.*, **53**, 217.  
 Lehmann, H., and Silk, E. (1953). *Brit. med. J.*, **1**, 767.  
 Mayrhofer, O. K. (1952). *Ibid.*, **1**, 1332.  
 Phillips, A. P. (1953). *J. Amer. chem. Soc.*, **75**, 4725.  
 Richards, H., and Youngman, H. R. (1952). *Brit. med. J.*, **1**, 1334.  
 Tammelin, L.-E. (1953). *Acta chem. scand.*, **7**, 185.  
 Whittaker, V. P. (1951). *Experientia*, **7**, 217.