SCIENCE PAPERS AND DISCUSSIONS

THE STABILITY OF INJECTION OF SUCCINYLCHOLINE CHLORIDE

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From the Wellcome Chemical Works, Dartford

Received July 2, 1954

THE use of *d*-tubocurarine chloride for skeletal muscular relaxation during surgical operations has led, during the past 5 years, to much work by organic chemists in search of synthetic muscle relaxants. A notable contribution in this field was made by Ing and Barlow[1] who, working on the assumption that the two cationic groups in the *d*-tubocurarine molecule are separated by an optimal distance, prepared a series of compounds in which two quaternary ammonium groups were separated by polymethylene chains—$(CH_2)_n$. Maximum activity was produced when "n" was 10 and as a result salts of decamethonium (I) were introduced into medicine.

$$X - (CH_3)_3N - (CH_2)_{10} - N(CH_3)_3 - X$$

(I)

In extension of this work a number of workers[2,3,4,5] almost simultaneously studied salts of dicholine succinate (II) which bears an obvious structural resemblance to decamethonium.

$$\begin{array}{l} CH_2{\cdot}CO{\cdot}O{\cdot}(CH_2)_2N(CH_3)_3 - X \\ | \\ CH_2{\cdot}CO{\cdot}O{\cdot}(CH_2)_2N(CH_3)_3 - X \end{array}$$

(II)

Succinylcholine chloride (II; X = Cl) is a powerful muscle relaxant, but owing to its rapid destruction by enzymes of the body its action is of short duration. It is to the latter property that succinylcholine chloride owes its clinical use. The drug has been given the approved name suxamethonium chloride and is likely to be included in the Addendum to the B.P. 1953.

Injection of succinylcholine chloride, containing 50 mg./ml. is available commercially and is usually supplied in 2 ml. ampoules or 10 ml. rubber capped vials. The drug is a choline ester which will be hydrolysed by the pseudo-cholinesterase of the serum with which it will come in contact when injected into the body and it may also be hydrolysed, although more slowly, when aqueous solutions such as the injection are stored. A study of the hydrolysis of succinylcholine is therefore of great clinical and pharmaceutical interest.

Whittaker[6,7] has studied the hydrolysis of succinylcholine in the presence of cholinesterase, while Evans *et al.*[8] have correlated the sensitivity of patients to succinylcholine chloride and the enzyme activity of their blood. Tammelin[9] has reported an extensive investigation on the hydrolysis of succinylcholine iodide (Celocurin) in which the functions of

concentration, *p*H and temperature on the rate of hydrolysis were observed. At *p*H 7·3 and at temperatures between 25° C., and 100° C. the rate of hydrolysis increased with rise of temperature, while at 75° C. solutions were most stable at *p*H 4 to 5. In Tammelin's experiments the concentration of the drug had little effect on the rate of hydrolysis.

As far as we are aware no publication on the stability of the injection, under normal storage conditions, has appeared. In order to supply such information we have during the past 2 years carried out stability tests and our results are described in the present communication.

Injection of Succinylcholine Chloride

The injection used in our investigations was prepared from succinylcholine chloride, $C_{14}H_{30}O_4N_2Cl_2,2H_2O$, according to the following formula.

Succinylcholine chloride (Anhydrous salt)	50 g.
Water for injection to produce	1000 ml.

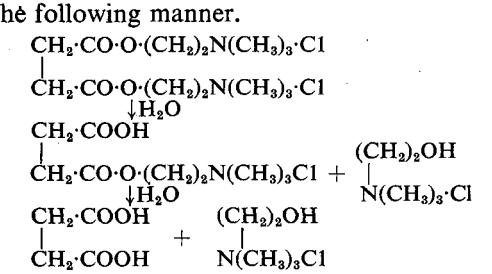
When required for 10 ml.-multidose containers, 0·1 per cent. of chlorocresol was added. After filling the injection was sterilised by heating in an autoclave at 10-lb. pressure of steam for 30 minutes. The product had *p*H 3·4.

Storage Conditions

The major part of our work was carried out with samples of a production batch of 10-ml. vials of injection of succinylcholine chloride. Equal numbers of vials were stored in the laboratory at room temperature and in a cupboard maintained at 37° C. Samples were examined at intervals and tested (1) chemically for hydrolysis, (2) chromatographically for the presence of hydrolytic products and (3) biologically for potency.

Estimation of Extent of Hydrolysis

When succinylcholine chloride is in aqueous solution it may undergo hydrolysis in the following manner.



Providing that no buffering agent is present the extent of hydrolysis may be assessed by determination of the free acid liberated. Such conditions occur in the injection, employed in our experiments, and the presence of 0·1 per cent. of chlorocresol did not interfere with the estimations which were based upon the following reaction.

$$3\,(CH_2{\cdot}COOH)_2 + KIO_3 + 5KI = 3\,(CH_2{\cdot}COOK)_2 + 3I_2 + 3H_2O$$

KIO_3/KI Reagent

10 g. of potassium iodide and 0·36 g. of potassium iodate dissolved in water and the volume adjusted to 100 ml. Mix well.

Estimation

Transfer 2 ml. of injection, accurately measured, to a 150-ml. flask, add 10 ml. of 0·005N sodium thiosulphate solution and 2 ml. of the reagent. Allow to stand for 10 minutes, add 1 ml. of mucilage of starch and titrate the excess of 0·005N sodium thiosulphate with 0·005N iodine. Carry out a blank determination on the reagents.

Each ml. of 0·005N iodine is equivalent to 0·000295 g. of $(CH_2.COOH)_2$. Record the extent of hydrolysis in terms of total acidity, calculated as succinic acid.

The above method was used when hydrolysis amounted to no more than 10 per cent. of the succinylcholine present. As hydrolysis increased the assay was suitably amended; 0·025N sodium thiosulphate and 5 to 15 ml. of KIO_3/KI reagent being employed.

TABLE I

PERCENTAGE HYDROLYSIS OF INJECTION OF SUCCINYLCHOLINE CHLORIDE (50 mg./ml.)

Period of storage	Stored at room temperature	Stored at 37° C.
Freshly prepared	3·13 per cent.	3·13 per cent.
7 weeks	4·65 „ „	13·4 „ „
12 „	5·85 „ „	24·5 „ „
16 „	6·0 „ „	31·7 „ „
25 „	8·1 „ „	50·0 „ „
36 „	11·4 „ „	83·0 „ „
52 „	22·0 „ „	100·0 „ „

Table I summarises the results obtained when samples were examined by this iodimetric process. The percentage hydrolysis of the succinylcholine has been calculated on the assumption that the acidity of the solution is due entirely to the presence of free succinic acid.

Chromatographic Examination

Separation and identification of the hydrolytic products present in injection of succinylcholine chloride by paper partition chromatography afforded a useful confirmation of our iodimetric results. Our early experiments were carried out using the methods of Whittaker[6,7]. 0·01 ml. of injection was placed on a Whatman No. 1 paper and the chromatogram developed by irrigation with a solvent consisting of *n*-propanol (5 vols), benzyl alcohol (2 vols) and water (2 vols). Both ascending and descending techniques were employed for development, which was allowed to proceed for about 18 hours. After air drying the chromatograms at room temperature they were immersed in 0·05 iodine solution and the spots thereby located. The following approximate R_F values were observed, using the ascending technique:—choline, 0·25; succinylcholine, 0·105.

No difficulty was experienced in following the hydrolysis occurring in the injection. When hydrolysis was less than 10 per cent., as assessed iodimetrically, little choline was observed on the chromatograms, but at

20 per cent. hydrolysis choline was easily detectable. At approximately 25 per cent. hydrolysis a third spot, which was not definitely identified but which might be due to the mono-choline ester of succinic acid, appeared. As hydrolysis proceeded the choline spot increased in intensity while that due to succinylcholine correspondingly diminished. When the iodimetric method indicated complete hydrolysis no trace of succinylcholine could be detected chromatographically.

In later experiments we found that Partridge's[10] solvent prepared by mixing *n*-butanol (4 vols.), acetic acid (1 vol.) and water (5 vols.), gave rather better results when used for development of the chromatograms on which the spots may also be located by use of Dragendorff's reagent. Choline and succinylcholine give violet and orange spots respectively with the latter.

Biological Assay

Although it might be expected that biological activity would decrease as succinylcholine underwent hydrolysis, it was obviously desirable that the results of our chemical experiments should be correlated with a series of biological assays. For this purpose assays were carried out on samples of the injection using the phrenic nerve-diaphragm method, described in the B.P. 1953 for the biological assay of *d*-tubocurarine chloride, and employing a sample of pure succinylcholine chloride as the standard preparation. The results of these experiments are included in Table II in which the loss in potency of each sample is recorded as a percentage of its stated potency, based upon the amount of drug used in the preparation of the injection.

TABLE II

PERCENTAGE LOSS IN POTENCY OF INJECTION OF SUCCINYLCHOLINE CHLORIDE (50 mg./ml.)

Storage	Conditions of storage	Loss in potency per cent.	Limits of error (P=0·95) percent.
Freshly prepared	—	4·7	1·8 – 7·5
12 weeks	37° C.	28·9	25·9 – 31·8
25 ,,	,,	58·5	56·4 – 60·4
36 ,,	,,	88·8	88·0 – 89·6
52 ,,	,,	97·1	96·9 – 97·3
12 ,,	Room temperature	4·9	0·9 – 8·8
52 ,,	,, ,,	19·5	16·7 – 23·1

Discussion

The present investigation has established that injection of succinylcholine chloride undergoes deterioration on storage, a conclusion fully supported by our chemical, chromatographic and biological experiments. These results have been confirmed when samples of the injection, purchased on the open market, and stocks returned by customers have been examined. Deterioration of the injection occurred equally in sealed ampoules and in rubber capped vials and contact with rubber did not increase the rate of hydrolysis of the succinylcholine, when the vials were stored in an inverted position.

The rate of hydrolysis at room temperature must be disturbing to the

pharmaceutical profession and we have considered what steps could be taken to reduce the loss in potency of the injection. Rise of temperature increases substantially the hydrolysis and it was therefore of interest to obtain information concerning the reaction rate at temperatures below that of the room. In this connection Dr. P. J. Fraser, of The Wellcome Research Laboratories, Beckenham, has kindly provided particulars of an experiment in which some unsterilised injection solution was kept for 46 weeks in a refrigerator at 0° C. At the end of this period no loss of potency was detected when the solution was tested biologically using the rat diaphragm-phrenic nerve preparation.

Many pharmacies will be unable to provide storage conditions at 0° C. and injection of succinylcholine chloride will usually be kept at room temperature. Injection of *d*-tubocurarine chloride, which loses little potency after storage at room temperature for a number of years, must bear an expiry date on the container as it is controlled by regulations made under the Therapeutic Substances Act. The need for an expiry date for injection of succinylcholine chloride, which does not come under the T.S.A. regulations, is far greater and we suggest that it should be labelled:— "This preparation when stored in a cool place below 10° C. may be expected to retain satisfactory potency for 12 months after its date of manufacture."

Summary

1. An iodimetric method for estimating the extent of hydrolysis of succinylcholine occurring in injection of succinylcholine chloride has been developed.
2. The method has been employed to study the stability of injection of succinylcholine chloride when stored at room temperature and at 37° C.
3. After a year's storage almost complete hydrolysis occurred at 37° C. and about 20 per cent. hydrolysis at room temperature.
4. The iodimetric results have been confirmed by chromatographic and biological tests.
5. It is suggested that each batch of injection of succinylcholine chloride should be labelled with a date up to which it might be expected to retain satisfactory potency. A suitable statement is recommended for purposes of labelling.

We wish to thank the Directors of The Wellcome Foundation for permission to publish this paper.

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DISCUSSION

The paper was presented by MR. G. A. STEWART.

DR. K. BULLOCK (Manchester) asked why the authors had chosen 10° C. as the maximum temperature for storage. Table II showed a 20 per cent. decomposition at room temperature, and it might be argued that the chemical reaction would be halved by a fall of 10° C. so that there would not be more than 10 per cent. decomposition at 10° C. A single experiment showed that the biological test was not sufficiently sensitive to detect any decomposition which had occurred at 0° C.—not 10° C. Had any experiments been carried out on material stored at 10° C. to show the percentage decomposition?

MR. E. H. REID (Dagenham) said that his results, mainly on the bromide, agreed with those of the authors. His method differed since the free succinic acid was extracted with amyl alcohol and estimated by titration with standard alkali. He had estimated both succinic acid and monocholine ester and found in every case that, until a high degree of decomposition—55 to 60 per cent.—was reached, the content of monocholine ester was greater than that of the succinic acid. After 21 months there was approximately 17 per cent. of monocholine ester and 15 per cent. of succinic acid. A sample of monocholine ester had been shown biologically to be comparatively inactive. Had the authors done any chromatographic experiments which indicated the presence of monocholine ester?

DR. F. HARTLEY (London) pointed out that the instability of quaternary nitrogen compounds in aqueous solution had long been well known. As Tammelin had shown, the rate of hydrolysis followed the expected behaviour of unimolecular reactions, being governed by the speed of the slowest stage in the hydrolysis, which was normally hydrolysis of the monocholine ester. The rate of hydrolysis was independent of the concentration of the drug, and one would therefore expect the hydrolysis to be catalysed by hydrogen ions. Why had the authors used, as injection of succinylcholine chloride, a simple aqueous solution of the substance? No data had been given about the batch of the material used. What was the *p*H of the freshly made solution? Why did the authors decide to sterilise the solution by heating in an autoclave and what happened to the *p*H during the process? He considered more work must be done before any recommendation could be made about the formula, and the conditions of storage, of an injection of succinylcholine chloride.

MR. W. SMITH (Ware) said that in his experience, when freshly prepared, a 5 per cent. solution of succinylcholine chloride had a *p*H between 4 and 4·5. Table I showed the percentage hydrolysis of freshly prepared material as being 3·13. Did "freshly prepared" refer to the solution before, or after, autoclaving?

Dr. G. E. FOSTER, in reply, said that work had been done with material stored at room temperature and at 37° C. except for the one sample tested biologically after storage for 46 weeks at 0° C. It was obvious that

there was a very steep temperature coefficient to this hydrolytic reaction, and thus, had there been time, it would have been desirable to complete the work by carrying out further experiments below room temperatures. He agreed there was no direct evidence to support the statement that it should be stored at temperatures below 10° C., but there was evidence that the preparation would be more stable under the conditions recommended than if stored at room temperature. The authors had also made experiments with the bromide and the acid radical did not greatly affect the rate of hydrolysis. There was some indication from chromatographic experiments that monocholine ester was present, and there was evidence that it had some activity. Replying to Dr. Hartley, he said that a simple aqueous injection of succinylcholine chloride had been suggested for inclusion in the B.P. Addendum. The *p*H of the solution when freshly made was about 4; after autoclaving it dropped to 3·4. The phrase "freshly prepared" in the table meant after autoclaving. He had figures for the analysis of the material used. Experiments had been made in an effort to achieve stability, but these had been unsuccessful. The work done was part of a wider investigation into the stability of choline esters. What was required was something which was hydrolysed by pseudocholinesterase in the serum but which gave a stable product in solution.