STABILITY OF ATROPINE SOLUTIONS: BIOLOGICAL AND CHEMICAL ASSAYS

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Atropine solutions stored at room temperature for 2 to 6 years were assayed biologically using the anti-acetylcholine action as end point. The activity of the 6-year old solutions was about 25 per cent below the labelled strength. The loss of activity was proportional to the duration of storage. Colorimetric assays on some of these samples confirmed these results. The spasmolytic and lethal activities of two other samples of partially hydrolysed atropine solutions were also determined. The presence of the hydrolysis products did not alter the ratio of these two activities of atropine. But, the biological activities of the sample, in which the hydrolysis amounted to 44 per cent, were greater than those attributable to the unhydrolysed atropine.

There is a growing practice to store atropine solutions for use in accidental poisonings with certain pesticides. It was therefore desirable to ascertain the stability of these solutions. Using a chemical method, Kondritzer and Zvirblis[1] found that atropine solution was relatively stable in acidic media. They estimated that at a temperature of 30° and pH 4·5, the hydrolysis of atropine would amount only to 25 per cent in about 100 years. On the other hand, Huycke[2] noted that one-third of the pharmacological activity of the atropine solutions tested was lost after the solutions had been stored for 4 to 5 years.

Schriftman and Kondritzer have reported that tropine and tropic acid, the hydrolysis products, did not interfere with the chemical analysis of atropine[3]. The effects of these substances on the biological activities of atropine have not been ascertained.

Experimental

Material. Samples of five batches of atropine tartrate solution and two batches of atropine sulphate solution of commercial origin were used. The atropine tartrate samples contained 2 mg./ml. in terms of atropine sulphate and in addition 2·4 per cent (w/v) glycerol and 0·4 per cent (w/v) phenol. The atropine sulphate samples contained 0·3 mg./ml. and in addition, sodium acetate 2·177 mg./ml. and acetic acid 0·48 mg./ml. The pH of all samples was about 4·5.

Two large samples of partially hydrolysed atropine sulphate solution were prepared by adjusting the pH to 8·8 with NaOH and heating at 70°. Heating was discontinued when approximately the desired degree of hydrolysis, as shown by chemical analysis, was achieved. These samples were subsequently acidified with hydrochloric acid, and untreated atropine sulphate solution was then added to adjust to the final concentration and

degree of hydrolysis to the preselected values. The mixtures were assayed colorimetrically. The provisional standard used was Merck's atropine sulphate powder.

Biological assay. This was made on the isolated guinea pig ileum. The ileum was suspended in the tissue bath of an automatic biological assay apparatus (Casella Electronics). The tissue bath of about 6 ml. in capacity was filled with Tyrode's solution which was kept at 37° and aerated with a mixture of 95 per cent of oxygen and 5 per cent CO_2. The control unit of the apparatus was set so that the following events took place in each cycle: (1) Wash out and refill of the tissue bath with Tyrode's solution, (2) Rest I, 30 seconds, (3) Wash out and refill, (4) Rest II, 30 seconds, (5) Wash out and refill with Tyrode's solution containing acetylcholine (10^{-8}) and (6) Contact, 40 seconds. The duration of each cycle was about 2 minutes.

The intestinal contraction induced by acetylcholine during Event 5 and 6 of each cycle was recorded by means of an electrical stylus on a kymograph. The power supply to the stylus and the kymograph were automatically turned on before Rest II had turned off at the end of Contact. After the response of the intestine to acetylcholine had become stabilised, different concentrations ($2–5 \times 10^{-9}$) of atropine were added during Event 3. The depressant effect of atropine on the response to the subsequent dose of acetylcholine was used as the criterion for the assay. A dose of atropine was given only after every five acetylcholine doses to ensure nearly complete recovery of the intestine from the effect of atropine. The sample to be tested and the standard solution of atropine were given alternately and 1×2-dose design[4] was adopted in these assays. Each batch was assayed twice and the weighted mean potency and its confidence limits were estimated.

In addition to the spasmolytic activity, the lethal activity of the two large samples of partially hydrolysed atropine was compared to that of the standard. This was done on adult male rats of the Wistar strain. Their weight was between 150 and 200 g. The rats were randomly distributed in nine groups of 15 each. Three graded doses of each sample were given to these rats. The mortality rates were converted to probits. The LD50 and the relative potencies were computed by probit analysis[5,6].

Chemical assay. The chemical assay was made by the Vitali-Morin method[7], except that dimethyl formamide was used instead of acetone. The sample, neutralised if alkaline, was placed in a 20 ml. beaker and evaporated to dryness over a steam bath. After the addition of 0·3 ml. of fuming nitric acid (Merck reagent grade, sp.gr. 1·5, 90 per cent HNO_3) the solution was evaporated as before. After cooling the beaker, 5·00 ml. of dimethyl formamide (Anachemia) was added to dissolve the nitrated product and 3·00 ml. of the resulting solution was transferred to a 1 cm. cuvette. Colour was developed by the addition of 164·8 μl. of 3 per cent KOH dissolved in methanol. The solutions were mixed rapidly and the absorbance determined at 555 mμ exactly 60 seconds after mixing. Absorbance decreased approximately 2 per cent per minute. A blank was run using water instead of atropine solution. Beer's law was followed

up to 50 μg./3·16 μl. of final coloured solution. The blank was renewed when it began to show turbidity.

Results and Discussion

A section of the kymographic tracing of a typical biological assay is reproduced in Figure 1. The intestinal contractions were induced by acetylcholine. After the first dose of atropine, the standard preparation

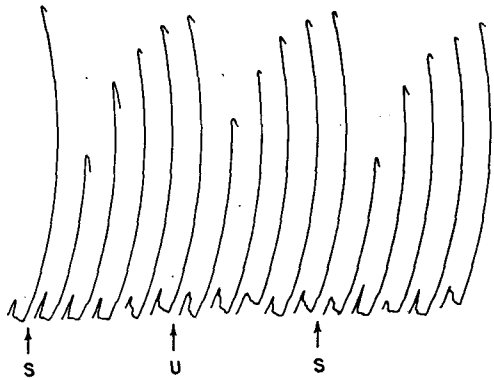

FIG. 1. A portion of the tracing of a typical biological assay. The intestinal contractions were induced by acetylcholine (10^{-8}). At the two S marks, Tyrode's solution containing the atropine standard (3×10^{-9}), instead of plain Tyrode's solution, was introduced into the tissue bath, before Rest II. This was washed out before adding the subsequent doses of acetylcholine. At U, Tyrode's solution containing a sample of aged atropine (4×10^{-9}) was added.

(S), the contraction was reduced by 47·6 per cent. The intestine gradually recovered after repeated doses of acetylcholine. The second dose of atropine was one of the aged samples (U) and it depressed the contraction by 34·2 per cent. Another dose of the standard (S) induced a 47·3 per cent depression of the intestinal contraction. The experiment was continued in this manner with a single concentration of the atropine standard, while two concentrations of the aged atropine sample were given alternately. The two concentrations of the aged sample were so selected that one would produce a greater and the other a smaller spasmolytic effect than the standard.

In Table I are listed the results obtained in the biological as well as the chemical assays. It may be noted that there was no significant difference between these two methods. These results also indicate that there was a definite inverse relation between the strength of the sample and its duration of storage. The regression equations calculated from the biological and chemical assays, according to the least square principle[8] are respectively:

$$E = 100{\cdot}8 - 0{\cdot}3376\ X \text{ and}$$
$$E = 92{\cdot}4 - 0{\cdot}2086\ X,$$

E being the estimated strength and X the duration of storage in months. From these equations, the spasmolytic activity of a 5-year old atropine

solution is estimated as 80·5 per cent of the original potency and the chemical strength 82·0 per cent. These two estimates are not significantly different.

Since the clinical dose of atropine may vary considerably, a 20 per cent decrease in potency was considered insignificant; it would only necessitate the administration of a slightly larger dose to obtain the same effect when

TABLE I

THE STRENGTHS OF AGED ATROPINE SOLUTIONS AS DETERMINED BY BIOLOGICAL AND CHEMICAL ASSAYS. THE VALUES (WEIGHTED MEAN AND CONFIDENCE LIMITS) ARE PERCENTAGES IN TERMS OF THE STANDARD

Sample*	Age**	Biological	Chemical
1	73	73·4 (68·4–78·7)	—
2	70	73·8 (66·8–81·5)	—
3	67	77·4 (72·9–82·2)	74·6 (69·7–79·9)
4	66	79·9 (73·4–86·9)	80·6 (74·9–86·8)
5	53	94·3 (85·8–103·6)	84·0 (77·9–90·6)
6	30	92·8 (85·2–101·2)	84·5 (81·8–87·2)
7	25	86·7 (81·6–92·2)	87·5 (85·0–90·0)

* Samples 1 and 2 are atropine sulphate solutions, and the others are atropine tartrate solutions.
** Age in months from date of packaging to date of assay.

5-year old atropine solution is used. However, old atropine solutions would be more undesirable if the hydrolysis products would augment the toxic but not the therapeutic action of atropine. According to the results summarised in Table II, these two biological activities of atropine were increased slightly and almost equally by the hydrolysis products when they were present in higher proportions.

The LD50 of the atropine sulphate standard in these rats was 255 mg./kg. with confidence limits (P = 0·05) at 232–281 mg./kg. This is similar to the LD50 of 280 (225–350) mg./kg. reported by Cahen and Tvede[9].

TABLE II

THE EFFECT OF THE HYDROLYSIS PRODUCTS ON THE SPASMOLYTIC (ISOLATED GUINEA PIG ILEUM) AND LETHAL (LD50 IN RATS) ACTIVITIES OF ATROPINE. THE VALUES (WEIGHTED MEAN AND CONFIDENCE LIMITS) ARE PERCENTAGES IN TERMS OF THE STANDARD (SAMPLE 1)

Sample	Unhydrolysed atropine per cent	Spasmolytic activity	Lethal activity
1	100	100	100
2	70·8	74·4 (68·4–80·8)	71·2 (62·8–80·6)
3	56·2	63·8 (58·4–69·8)	69·4 (64·8–74·4)

Five of the seven samples assayed on the ileum (Samples 3–7, Table I) had been assayed by Huycke[2] on the mouse pupil (Sample 4–8 in his paper). It is evident from these results that there was a smaller loss in the spasmolytic activity than the mydriatic. The difference is especially notable since the spasmolytic tests were carried out about 15 months after mydriatic tests. The reason for this discrepancy is unknown. However, it is possible that the hydrolysis products may affect these two biological actions of atropine in different ways.

The rate of deterioration of atropine as indicated by the biological and chemical assays, although slower than that reported by Huycke, is faster than that estimated by Kondritzer and Zvirblis[1]. Whether the excipients in the commercial atropine solutions have any effect on the rate of hydrolysis is unknown.

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