A COMPARISON OF THE ACTIVITY OF SOME DRUG PRODUCTS INJECTED IN HYDROLYSED CALCIUM GELATINATE AND IN DISTILLED WATER

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Received June 6, 1950

During the past few years a number of pharmaceutical companies have incorporated into some of their formulations substances which are known to prolong the action of the active ingredient following parenteral injection. This type of formulation has resulted in some cases in a reduction in the amount of active ingredient necessary to attain a desired blood level for adequate medication. Where the active ingredients are rapidly eliminated or detoxified in the body these substances have been most useful in maintaining adequate blood levels. Special diluents have also been advocated for the same purpose.

The purpose of this paper is to report on an investigation on Hydrolysed Calcium Gelatinate, a diluent which has been reported to prolong the action of a number of drugs, such as morphine, penicillin, certain vitamins and sex hormones[1]. The drugs which were chosen for this study were morphine sulphate, *d*-tubocurarine chloride and œstrone.

A comparison of the analgesic activity of morphine sulphate in distilled water and hydrolysed calcium gelatinate in the rat, and also determinations of the free morphine content of rabbit blood at various intervals after intramuscular injection were made. The activity of the *d*-tubocurarine chloride in distilled water and hydrolysed calcium gelatinate was compared and a similar comparison on œstrone was also made.

Methods

The analgesic tests in rats were conducted according to the method described by D'Amour and Smith[2] and further modified by Miller[3]. The apparatus used for this study was one designed and made to the specification of Wolff, Hardy and Goodell[4]. The free morphine determinations in rabbit blood were done by the silicomolybdic acid method as proposed by Shideman and Kelley[5]. The activity of the *d*-tubocurarine chloride solutions was determined by a sloping screen method which has been used in this laboratory for testing the strength of *d*-tubocurarine solutions[6]. The details of the method for the assay of œstrone has been previously described[7].

For all these assays, with the exception of the free morphine determinations in blood, the object of the experiments was to determine the activity of the product in both diluents and to ascertain if possible if the diluent delayed the onset and prolonged the effect.

For the analgesic tests the observations were made at various intervals after the injections were made in order to find out the approximate time of the onset and the duration of analgesia.

The rats used in the *d*-tubocurarine chloride assays were observed continuously for several hours. It has been found that rats injected

with *d*-tubocurarine chloride in distilled water respond before 20 minutes has elapsed or do not respond at all[6]. In these tests the period of observation was extended to several hours in order to find out if the onset of effect might be delayed. The computations for all the assays reported in Tables I, III and IV were done by the methods of Bliss[8,9].

TABLE I

COMPARISON OF THE ANALGESIC EFFECT OF MORPHINE SULPHATE INJECTED INTRAMUSCULARLY IN DISTILLED WATER AND HYDROLYSED CALCIUM GELATINATE
RADIANT HEAT-TAIL METHOD

Morphine sulphate in hydrolysed calcium gelatinate diluent in terms of morphine sulphate in distilled water Minutes after Injection							
30		60		90		120	
[a]Per cent and Confidence Limits, P= ·05							
133	112–159	107	94–122	90	75–107	68	67–110

NOTE.—Instrument setting, 350–400 millicalories. Cut off time, 3·0–5·4 secs. [a]Weighted mean of 2 or more assays.

RESULTS AND DISCUSSION

In Tables I, III and IV comparative results are shown of the analgesic

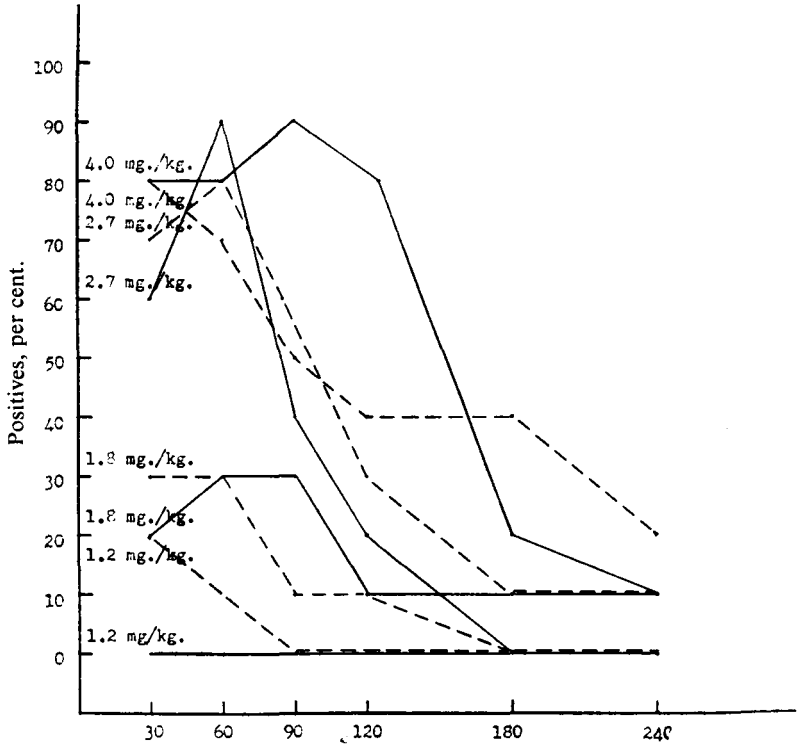

FIG. 1. Minutes after injection—tail method.
——— Morphine sulphate in aqueous solution
– – – Morphine sulphate in hydrolysed calcium gelatinate diluent

potency of morphine in distilled water and hydrolysed calcium gelatinate and the activity of *d*-tubocurarine and œstrone in both diluents.

For the analgesic tests there was no evidence that those rats injected with hydrolysed calcium gelatinate showed greater analgesic effect at any time during the observation period, which in some cases lasted for several hours. The number of rats responding positively at the end of the observation period were about the same for groups which received the same dose of the drug. This may be seen from an examination of the results of a typical experiment (Fig. 1). If the drug had been slowly absorbed one would have expected that a greater proportion of the rats injected with morphine in distilled water would have shown an analgesic effect on the first readings, and on the later readings a greater proportion of the rats injected with morphine in hydrolysed calcium gelatinate would have shown an analgesic effect. The results do not show this trend as may be seen from an examination of the comparative estimates of potency shown in Table I.

As may be seen in Table II the determinations of free morphine in rabbit blood at various intervals after injection shows a somewhat

TABLE II

COMPARISON OF THE FREE MORPHINE CONTENT OF RABBIT BLOOD FOLLOWING INTRAMUSCULAR INJECTION OF MORPHINE SULPHATE IN DISTILLED WATER AND HYDROLYSED CALCIUM GELATINATE

[a] Free morphine concentration in blood, (mg. per cent., ±S.E.) after intramuscular injection of 50 mg./kg. of morphine sulphate in hydrolysed calcium gelatinate diluent and distilled water

Minutes after Injection					
5		15		45	
2·62±0·33[b]	4·23±0·53[c]	3·15±0·47[b]	3·47±0·38[c]	2·04±0·22[b]	1·50±0·22[c]

NOTE :—*a* 14 rabbits were used on each diluent. The results at each time interval are the means of 14 determinations.

b refers to determination of morphine sulphate in distilled water.

c refers to determination of morphine sulphate in hydrolysed calcium gelatinate.

similar agreement in results. At 5 minutes after the injections were made the free morphine recovered from blood was significantly greater in those rabbits injected with morphine in distilled water, but at 15 and 45 minutes after injection there was no significant difference in the amount of free morphine recovered in the respective groups. If the morphine had been released slowly a greater amount of free morphine should have been recovered at the 45-minute interval after injection. Determinations were made at later times but there was not sufficient present in the blood to be detectable.

In Tables II and IV are shown the results of comparative assays on *d*-tubocurarine chloride and œstrone in distilled water and hydrolysed calcium gelatinate diluent. As was mentioned previously the rats on the *d*-tubocurarine chloride test were observed continuously for several hours from the time the injections were made, but in no case was any effect observed after an elapsed time of 20 minutes. The rats that

responded did so within 20 minutes and the effect did not last longer on the rats injected with *d*-tubocurarine chloride in hydrolysed calcium gelatinate diluent. As may be seen from Table III there was no difference in the activity of *d*-tubocurarine chloride in the two diluents. If the action had been delayed and prolonged in those rats injected with *d*-tubocurarine chloride in hydrolysed calcium gelatinate diluent the relative activity for *d*-tubocurarine chloride in the two diluents would have been different.

TABLE III

COMPARISON OF THE ACTIVITY OF *d*-TUBOCURARINE CHLORIDE FOLLOWING INTRAMUSCULAR INJECTION IN DISTILLED WATER AND HYDROLYSED CALCIUM GELATINATE

[a]Potency of *d*-tubocurarine chloride in hydrolysed calcium gelatinate diluent in terms of *d*-tubocurarine chloride in distilled water, per cent.	Confidence Limits P = ·05
102·5	96·7–108·8

NOTE :—[a]Weighted mean of 2 assays.

A similar result was obtained on œstrone. The proportion of rats showing œstrus after injection of œstrone in both diluents was about the same for those groups receiving the same doses and this was evident from the computed activity of œstrone in distilled water in terms of œstrone in hydrolysed calcium gelatinate diluent, as may be seen from the results in Table IV. There was no evidence of a prolonged action

TABLE IV

COMPARISON OF THE ACTIVITY OF ŒSTRONE INJECTED SUBCUTANEOUSLY IN DISTILLED WATER AND HYDROLYSED CALCIUM GELATINATE

[a]Potency of œstrone in hydrolysed calcium gelatinate diluent in terms of œstrone in distilled water, per cent.	Confidence Limits P = ·05
110·4	87·8–137·5

NOTE :—[a]Weighted mean of 2 assays.

as the rats injected with œstrone in both diluents returned to their preinjection state at the same time.

SUMMARY

1. A comparison of the analgesic effect of morphine sulphate and the activity of *d*-tubocurarine chloride and œstrone in distilled water and hydrolysed calcium gelatinate diluent is presented.

2. No appreciable prolongation of analgesic effect of morphine nor activity of *d*-tubocurarine chloride and œstrone in hydrolysed calcium gelatinate was found.

3. Free morphine blood levels except at 5 minutes after injection were about the same following intramuscular injection of morphine in distilled water and hydrolysed calcium gelatinate diluent.

The assistance of Miss Elizabeth Carmichael, Mrs. Sybil Jaffray, Miss

Beverley Garland, and Messrs. Alphonse Lavallee and Edward Parliament is gratefully acknowledged.

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