ON THE EFFECT OF
LONG-ACTING CORTICOTROPHIN PREPARATIONS

By
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ABSTRACT

The effect of addition of carboxymethyl cellulose, gelatine, polyphloretin phosphate and zinc hydroxide to corticotrophin was studied by means of fluorimetric plasma corticosteroid determinations in man and rats. The plasma corticosteroid level was stabilized at a low level by the administration of dexamethasone. Two days after a 3 day period of dexamethasone, 2 mg × 4 orally, the volunteers had a normal diurnal rhythm of plasma corticosteroid level indicating that short term dexamethasone treatment may be given without any serious risks. For each type of experiment, the same corticotrophin was employed to avoid dose differences. Each subject was injected with each preparation at weekly intervals. Significant differences between individuals were observed. The effect of polyphloretin phosphate exceeded that of gelatine, both substances significantly exceeding that of carboxymethyl cellulose and zinc hydroxide 8 hours after the injection and subsequently. Similar results were obtained in animal experiments.

The instability of injected corticotrophin is a seriously limiting factor in the clinical use of this hormone. Several substances have therefore been added to corticotrophin in order to enhance or prolong its action, principally by an effect at the site of the injection. The effect of these prolonging substances has been studied by many methods, both in man and in laboratory animals.

Evaluations based on the eosinophil cell count may be considered less accurate, due to the considerable spontaneous variation (Foss-Abrahamsen 1958) and low specificity. More accurate seems to be the determination of urinary steroid excretion which has been used e. g. by DiRaimondo & Forsham (1958). This method, however, gives only scanty information on the question whether an added repository agent increases the intensity as well as the duration of the effect of corticotrophin. The steroid content of one diurnal
volume of urine may vary spontaneously from day to day (Hamburger 1954). These facts seem to limit the value of urinary steroid determinations as a parameter for the effect of corticotrophin. The effect of prolonged corticotrophin preparations on the adrenal ascorbic acid response has been investigated by Hamburger (1952) who found an enhanced effect of corticotrophin on addition of polyphloretin phosphate. The same parameter was also used by Rerup (1958) who found an unchanged potency of a corticotrophin preparation irrespective of whether it was dissolved in saline, gelatine or carboxymethyl cellulose. This parameter may, however, be regarded as being less suitable for a time-response curve as outlined below.

The determination of the effect of corticotrophin by the measurement of the level of plasma corticosteroids offers certain advantages in the evaluation of long-acting corticotrophin preparations both in animals and in man. The effect of a single injection of corticotrophin may be determined more accurately by this parameter both with regard to intensity and duration because of its more rapid return to control values following stimulation of adrenocortical activity as compared with the adrenal ascorbic acid response (Hedner & Rerup 1962 b). Moreover, for experiments in man the plasma corticosteroid response is the only method available. A source of error in the evaluation of corticotrophin preparations may be sought in the differences in potency between preparations assayed by different manufacturers and by different methods as pointed out by Hangård et al. (1960) who followed the effect of long-acting commercial preparations of corticotrophin on the plasma corticosteroid level in man. Other difficulties in an investigation of this type may be the spontaneous variation of the plasma corticosteroid level found e.g. in the diurnal rhythm, and in the different sensitivity of individuals to corticotrophin (Bayliss & Steinbeck 1954).

Experiments with depot corticotrophin preparations in man were designed to avoid these disturbing factors as far as possible. Similar experiments were performed in rats to investigate the value of animal experiments in the study of these preparations. Carboxymethyl cellulose, gelatine, polyphloretin phosphate and zinc hydroxide were employed, representing different principles for long-acting agents.

MATERIAL AND METHODS

Experiments in man

The material consisted of healthy male volunteers aged 20–32 years. There were no restrictions of diet or activity. Corticotrophin preparations were made originating from the same batch assaying 94.7 IU per mg* by the subcutaneous ascorbic acid

* Kindly supplied by Ferring A.B., Malmö.
depletion method against the U. S. P. Corticotropin Reference Standard. The same
prolonging agents in the same concentrations as mentioned below were used in the
preparation of solutions containing 30 IU of corticotrophin per ml. Phenol in a con-
centration of 0.5 per cent was also added. In addition, a zinc hydroxide preparation
was made by adding corticotrophin in a 0.5 per cent phenol solution to zinc chloride
in a solution of 0.5 per cent phenol and glycerine. 0.1 N NaOH was then added to
obtain a pH of 6.8. The final solutions contained 30 IU of corticotrophin and 3.5 mg
zinc per ml. A dose of 1 IU per kg body weight was injected subcutaneously in the
upper arm at 8 a.m. after a blood sample had been taken by venepuncture into centri-
fuge tubes prepared with heparin. New blood samples were taken 4, 8, 12 and 16 hours
after the injection. Dexamethasone* in a dose of 2 mg orally was given at the time
of injection and also 8 hours before and 6 and 12 hours after the injection, the total
dose during each experiment being 8 mg. Each subject was injected with each cor-
ticotrophin preparation at intervals of one week. The blood samples were centrifuged
within 20 minutes and the plasma frozen. The plasma corticosteroid determination
was performed according to the fluorimetric method of Silber et al. (1958) slightly
modified by Hedner (1961). The results are expressed as µg cortisol per 100 ml plasma.
The fluorescence was read after 5 minutes development (De Moor et al. 1960).

Experiments in animals

Male albino rats weighing 150–200 g from the same breeding colony were used
throughout. Hypophysectomy was replaced by dexamethasone blockade, 1 mg per rat
being given subcutaneously as a 2 mg per ml suspension in saline 3 hours before the
experiment (Hedner & Rerup 1962a). Subcutaneous assays were performed using
corticotrophin in doses of 20 and 80 mU per 100 g rat, blood samples being taken
1 hour after the injection under ether or pentobarbital anaesthesia. The plasma cor-
ticosteroid parameter was used throughout, and when more than one blood sample
was taken from the same animal, tail blood was used as described by Rerup & Hedner
(1961). The blood samples were centrifuged within 20 minutes and the plasma analysed
for corticosterone according to the fluorimetric method mentioned above. The results
are expressed as µg of corticosterone per 100 ml plasma. The same corticotrophin
preparation was used in each type of experiment. The dry substance was dissolved
in a small volume of saline adjusted to pH 5 by addition of 0.1 N acetic acid. Dilution
to final concentrations was then performed by solutions of
1. 0.9 per cent NaCl.
2. 1 per cent carboxymethyl cellulose.
3. 16 per cent gelatine.
4. 1 per cent polyphloretin phosphate.

In time response experiments, the effect of these, subcutaneously injected, long-acting
preparations, in doses of 50 mU per 100 g rat, was checked hourly for 5–6 hours, only
one blood sample being taken from each animal in each group.

RESULTS

Experiments in man

For a more accurate evaluation of the effect of injected corticotrophin a
stable base of the plasma corticosteroid concentration was obtained by oral

* Kindly supplied by Pharmacia A. B., Uppsala.
Mean plasma corticosteroid level in 5 healthy subjects after oral treatment with dexamethasone 2 mg × 4 for 3 days. The bars indicate the standard deviation of the single observation.

dexamethasone treatment. The effect of 2 mg dexamethasone × 4 is shown in Fig. 1. The plasma corticosteroid level was already depressed after 8 hours and kept at a stable level, suitable for the study of exogenously administered corticotrophin. In this experiment the dexamethasone administration was continued for 3 days. After the next 2 days without medication, the subjects had a diurnal variation in their plasma corticosteroid concentration as found normally (Fig. 1) with the fluorimetric method used here. No subjective symptoms were registered.

For time response experiments, 9 male volunteers were injected. The dexamethasone administration was started 8 hours prior to the subcutaneous injection of corticotrophin, which was given at 8 a.m. Blood samples for plasma corticosteroid determination were drawn every 4th hour after the injection. Every subject was injected with each preparation at intervals of one week in the order given in Table 1. Dose differences between preparations were avoided by using the same corticotrophin for all preparations. The results of the plasma corticosteroid determinations appear in Table 1. A survey is given in Table 2 which gives the mean values reported in Table 1.

It can be seen from the table that the plasma corticosteroid levels at 0 hours were uniformly about the same as those shown in Fig. 1 during dexamethasone treatment, indicating an efficient blockade present at each injection. A statistical analysis of the figures in Table 1 revealed:

at 4 hours: A consistent increase of the plasma corticosteroids occurred after
Table 1.

Plasma corticosteroid values expressed as µg cortisol per 100 ml in 9 male volunteers under dexamethasone treatment after the subcutaneous injection of 1 IU of corticotrophin in different media per kg body weight at 0 hours. The interval between the injections was one week.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Hours after inj.</th>
<th>Subject</th>
<th>Mean</th>
<th>s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.4 3.1 2.8 2.2 8.0 3.1 6.4 7.4</td>
<td>4.4</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>28.0 52.5 27.4 46.0 32.2 37.8 29.7 37.5 43.4</td>
<td>37.2</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>8 5.0 24.2 22.4 28.0 11.4 28.6 26.4 21.2 17.5</td>
<td>20.5</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3.4 13.2 14.5 11.2 6.2 24.2 9.9 8.5 4.4</td>
<td>10.6</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>2.2 6.0 5.6 6.1 6.2 7.7 6.0 4.4 4.4</td>
<td>5.4</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.8 5.8 5.8 6.9 4.7 5.4 2.3 5.9 2.9</td>
<td>5.1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Carboxymethyl</td>
<td>4 29.9 54.1 30.9 54.1 29.7 38.9 51.8</td>
<td>40.3</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>cellulose</td>
<td>8 11.5 27.6 19.4 26.3 29.2 30.3 28.0 31.6</td>
<td>34.0</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5.8 8.1 16.1 8.6 11.7 15.1 16.0 10.5 19.9</td>
<td>12.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>2.9 5.8 9.2 9.2 1.1 6.5 3.7 3.4 0.6</td>
<td>4.7</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.4 2.2 4.6 2.7 6.0 5.4 6.5 7.3 4.6</td>
<td>4.6</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Polyphloretin</td>
<td>4 40.9 49.7 39.2 38.5 30.1 52.0 34.6</td>
<td>37.1</td>
<td>42.2</td>
<td></td>
</tr>
<tr>
<td>phosphate</td>
<td>8 30.1 43.2 39.2 34.9 26.2 49.7 34.6</td>
<td>42.9</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>18.0 42.1 33.4 36.1 13.2 46.0 40.0</td>
<td>34.8</td>
<td>42.2</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>9.6 27.0 18.6 26.5 15.6 45.5</td>
<td>40.0</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td>Gelatine</td>
<td>8 32.9 49.6 39.2 44.0 36.2 41.7 44.5 34.8 21.3</td>
<td>38.2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>17.8 53.0 34.5 35.2 16.5 45.2 40.0 14.9 10.1</td>
<td>29.7</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>8.8 19.2 24.2 27.1 11.0 33.0 18.3 3.4 6.7</td>
<td>16.9</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Zinc hydroxide</td>
<td>0 2.2 3.4 3.4 2.2 5.5 2.3 5.6 5.6</td>
<td>3.6</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>34.7 38.4 23.0 44.0 41.7 37.1 10.7 44.8 50.3</td>
<td>36.1</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16.7 33.0 18.4 48.3 39.5 25.1 5.3 19.2 34.6</td>
<td>26.7</td>
<td>4.3</td>
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<td>12</td>
<td>10.1 16.0 11.5 47.2 14.3 19.4 4.3 13.9 22.4</td>
<td>17.7</td>
<td>4.1</td>
<td></td>
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<tr>
<td>16</td>
<td>3.4 6.7 6.9 34.0 8.0 10.3 3.4 15.6 11.2</td>
<td>11.1</td>
<td>3.1</td>
<td></td>
</tr>
</tbody>
</table>

All preparations. No significant differences between preparations were found as judged from the over all analysis of variance. However, individual analyses of variance as compared with saline showed a hardly significantly higher value for gelatine but not for the other preparations \((0.025 < P < 0.05)\). If the common variance of the total material was used, no significant difference between preparations was found. Differences between individuals were significant at \(P < 0.01\), each individual showing similar plasma corticosteroid levels from preparation to preparation.
Table 2.
Mean plasma corticosteroid values (µg per 100 ml), taken from Table 1, in male volunteers under dexamethasone treatment after the subcutaneous injection of 1 IU corticotrophin in different media per kg body weight at 0 hours. The interval between the injections was one week.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Hours after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>4.4</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>5.1</td>
</tr>
<tr>
<td>Polyphloretin phosphate</td>
<td>4.6</td>
</tr>
<tr>
<td>Gelatine</td>
<td>4.4</td>
</tr>
<tr>
<td>Zinc hydroxide</td>
<td>3.6</td>
</tr>
</tbody>
</table>

at 8 hours: The mean plasma corticosteroid values were still significantly above the control values for all preparations but significantly lower than the values obtained at 4 hours with the exception of gelatine and polyphloretin phosphate, which retained the full corticotrophin effect. In comparison with saline, carboxymethyl cellulose, gelatine and polyphloretin phosphate preparations exerted a significantly greater action. The effect of gelatine and polyphloretin phosphate significantly exceeded that of carboxymethyl cellulose. No significant differences between individuals were found at this time.

at 12 hours: The mean plasma corticosteroid levels were still significantly higher than the control levels for all preparations. Significantly lower values were recorded compared with the level at 8 hours with the exception of polyphloretin phosphate, the effect of which was not even significantly different from the effect at 4 hours. There was no significant difference between the effects of gelatine and polyphloretin phosphate preparations, but these were significantly higher than those of the saline, carboxymethyl cellulose and zinc preparations. A significant difference between individuals at the level of $P < 0.05$ was found.

at 16 hours: The saline and carboxymethyl cellulose preparations did not differ significantly from the control values while there was still an effect with the other preparations. A significant decrease compared with the 12 hour levels was noted for all preparations. The effect of polyphloretin phosphate was significantly greater than that of gelatine and zinc, between which there was no significant difference. No significant difference between individuals was found.

No samples were drawn 20 hours after the injection for practical reasons. Blood samples 24 hours after the injection were not considered necessary as no preparation revealed any effect at this time as judged from a preliminary
experiment. Pain at the site of the injection, rapidly disappeared after polyphloretin phosphate and was more persistent after zinc hydroxide. No other side reactions were registered.

Experiments in animals

In order to investigate the possibility that the activity of corticotrophin is increased by the addition of prolonging agents, assays were performed on dexamethasone treated rats. The different agents were assayed against a saline preparation containing the same amount of corticotrophin. Both parallel line and twin cross over assays were used. The time between the subcutaneous injection and the blood sampling was 1 hour (cf. Fig. 2). The mean index of precision ($\lambda$) was 0.15. The results of the individual assays are shown in Table 3. It can be seen from the table that none of the agents listed exerted any significant effect on the potency of the subcutaneously injected corticotrophin.

The duration of the effect of subcutaneously injected corticotrophin was found to depend on the dose, which is shown in Fig. 2. For time response experiments, a dose of 50 mU per 100 g body weight was chosen. In these

![Graph](https://via.placeholder.com/150)

**Fig. 2.**

Mean plasma corticosteroid levels in dexamethasone blocked rats after subcutaneous injection of corticotrophin dissolved in saline in doses of 25 mU (x-x) and 50 mU (o-o) per 100 g body weight at 0 hours. The bars indicate the standard deviation of the single observation.

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Table 3.

Results of 4 point \((2 + 2)\) parallel line assays and twin cross over assays of prolonged against saline preparations containing the same amount of corticotrophin. 1 hour plasma corticosteroid response to subcutaneous injections into dexamethasone blocked rats.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Assay design</th>
<th>Estimated potency (expected: 100 per cent)</th>
<th>Range ((P = 0.05))</th>
<th>Index of precision(^1)</th>
<th>Weight factor(^2) ((W_1))</th>
<th>(R_{EV}) (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxymethyl</td>
<td>Twin cross over</td>
<td>114.0</td>
<td>97.6 – 133.4</td>
<td>0.09</td>
<td>1229</td>
<td>1.92</td>
</tr>
<tr>
<td>cellulose</td>
<td>4 point ((2 + 2))</td>
<td>90.8</td>
<td>49.6 – 162.5</td>
<td>0.22</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Gelatine</td>
<td>Twin cross over</td>
<td>92.8</td>
<td>57.3 – 147.0</td>
<td>0.24</td>
<td>119</td>
<td>0.96</td>
</tr>
<tr>
<td>Polyphloretin</td>
<td>4 point ((2 + 2))</td>
<td>105.0</td>
<td>86.0 – 128.5</td>
<td>0.09</td>
<td>590</td>
<td></td>
</tr>
<tr>
<td>phosphate</td>
<td>4 point ((2 + 2))</td>
<td>96.0</td>
<td>36.6 – 242.1</td>
<td>0.35</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

1) For 4 point parallel line assay \(\bar{x} = \frac{s}{b}\). For twin cross over assay
\[
\bar{x} = \frac{s}{b} (Rerup & Hedner 1961).
\]

2) \(W_1 = \left(\frac{2t}{L}\right)^2\) where \(t\) is »Student's« \(t\) at \(P = 0.05\) and the appropriate degrees of freedom and \(L\) the confidence interval at \(P = 0.05\) in log units (Rerup 1959).

3) Ratio of efficiency based on variance comparison (Rerup 1960).

Experiments too, only one corticotrophin preparation was used throughout to avoid dose differences due to standardisation errors. The plasma corticosteroid level was controlled hourly for 5–6 hours after the injection. Maximal corticosteroid increase was always obtained 1 hour after the injection irrespective of the preparation. In these experiments, carboxymethyl cellulose did not differ significantly from saline. The gelatine and polyphloretin phosphate preparations maintained high plasma corticosteroid levels for 5 to more than 6 hours as compared with 3 hours for the saline preparation. No prolonged
effect was obtained with a zinc preparation where a concentrated solution containing 30 IU of corticotrophin and 3.5 mg zinc per ml was diluted 300 times. This initial solution was effective in man. On the other hand, if the final corticotrophin solution to be injected contained 3.5 mg zinc per ml no corticotrophin effect was found at all.

**DISCUSSION**

A difficulty in the evaluation of the duration of action of corticotrophin on the plasma corticosteroid level is the spontaneous variation seen e. g. in the diurnal rhythm and probably due to a variable release of endogenous corticotrophin. To stabilize this the subjects were treated with dexamethasone. The danger of corticosteroid administration, especially for the feed back mechanism, has been revised to some extent and it is commonly agreed at present that short term steroid treatment is without any serious risks (Paris 1961). This is supported by our findings that 2 days after a 3 day period with dexamethasone (2 mg × 4) the subjects had a normal diurnal rhythm as measured by the fluorimetric method for plasma corticosteroid determination used here (Fig. 1). It is apparent, that dexamethasone is a powerful depressant of the pituitary-adrenocortical system which produces full depression of the plasma corticosteroid level within 8 hours of the first oral dose (2 mg). Thereafter, on continued dexamethasone administration, the plasma corticosteroid level was kept stable and low, giving a good basis for the study of exogenously administered corticotrophin.

From the data obtained in this study it may be concluded that polyphloretin phosphate is the most effective prolonging agent, followed by gelatine, zinc hydroxide and carboxymethyl cellulose. These results differ from those of Geller et al. (1957) and Siegel et al. (1958) who found zinc hydroxide preparations more effective than gelatine preparations. A possible explanation may be different methods for the preparation of the zinc hydroxide. In general, differences between investigations on long-acting corticotrophin preparations may be due to:

1. Individual variations in the response to corticotrophin (Bayliss & Steinbeck 1954). This was also seen in this investigation as shown by subjects 1 and 2 in Table 1. This fact may play a role in a small material where the subjects were not injected with all preparations.

2. Different batches of corticotrophin may be labeled with different degrees of accuracy due to variable standardization errors. With different origins of preparations the results may be biassed both with regard to the extent of the plasma corticosteroid response (Hangård et al. 1960) and to its duration as DiRaimondo & Forsham (1958) found for a gelatine preparation that each
doubling of the corticotrophin dose prolonged the increase in steroidogenesis by approximately 1 hour.

3. The type of corticotrophin used. Purified corticotrophin has been reported to be more resistant to enzyme degradation than «crude corticotrophin» (Pincus et al. 1952; Raben et al. 1952). This may to some extent influence the action of added repository agents.

4. Results obtained with subcutaneous and intramuscular injections cannot be easily compared. Differences due to the route of injection have been reported by Hangård et al. (1960) who found that the subcutaneous injection was more effective for carboxymethyl cellulose and hyaluronic acid preparations.

In this investigation, the gelatine preparation produced a higher effect of doubtful significance compared with the saline preparation 4 hours after the injection. The two preparations may, however, induce maximal responses at different times after the injection and this was not recorded here as sufficient blood samples could not be obtained for practical reasons. No significant difference in potency was found by the assay experiment on rats.

Regarding the design of the experiment shown in Table 1 the choice fell on a parallel design, i.e. each preparation was tested simultaneously in all subjects, to facilitate the practical procedure. A possible bias might be introduced by a varying susceptibility to corticotrophin within individuals from time to time, chiefly as a carry over effect of a preparation on the effect of the next one. This was considered to play a minor role with respect to the relatively mild and short lasting adrenocortical stimulation, with plasma corticosteroid values which only moderately exceeded normal levels. Moreover, the time elapsing between each injection was one week, allowing of a period of several days without any effect from corticotrophin or dexamethasone.

Differences between preparations in this study may chiefly be ascribed to the added prolonging agents only, and their duration of action may be directly compared. However, not only are the absolute duration times to be considered but also the patterns of the corticosteroid concentration curves elicited by the preparations. The polyphloretin phosphate and gelatine preparations kept the level high for at least 16 hours, the difference compared with saline, carboxymethyl cellulose and zinc hydroxide being significant at 8 hours and thereafter. The value of the protective effect of polyphloretin phosphate and gelatine is thus not only a prolongation of the duration time but also an ability to maintain the plasma corticosteroids at a high level for a considerable part of the period of action. This is of interest from a therapeutic point of view and both factors ought to be considered in evaluating long-acting corticotrophin preparations.

The experiments in dexamethasone blocked rats revealed that the plasma corticosteroid responses were much shorter than in man. In spite of this, the
general shape of the plasma corticosteroid curves was similar for each type of preparation irrespective of species. This points to the possibility of using rats in preliminary studies of prolonging agents of the type used in our animal experiments. However, a definitive evaluation should be based on experiments in man.

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REFERENCES


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