THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE ON DERMAL SPREAD

BY

HANS CARSTENSEN and HAKAN LINDERHOLM

Several hormones are known to influence the spread of dyes in dermal connective tissue. Anterior pituitary extract decreased the spread of Indian ink when injected together with testicular extract (Weinstein, 1940). An inhibition of the spread of the dye was also found after treatment with posterior pituitary hormone (Favilli, 1939). In these cases it is impossible to exclude interference of hormones from the other pituitary lobe owing to the difficulty of isolating anterior and posterior pituitary hormones from each other.

Other hormones reported to influence the spread might have acted indirectly upon the pituitary. Thus, adrenaline (Favilli, 1939, Homburger, 1944) and oestrogens (Sprunt, McDearman & Raper, 1938, Sprunt & McDearman, 1939, 1940) inhibited the spread and also stimulated the output of adrenocorticotropic hormone (Vogt, 1944, Christensen, 1944, Long & Fry, 1945, Selye, 1946, Lurie, 1950).

In some recent papers (Opsahl, 1949 a, b, c, Opsahl, White & Duran-Reynals, 1950) adrenocortical extract and some synthetic glucocorticoids (Compounds A and E of Kendall) were reported to inhibit the spread while desoxycorticosterone was ineffective. The results seemed to be more unequivocal with hyaluronidase than without. It is thus possible that the pituitary hormones might have influenced the dermal spread via the adrenal glands.
An inhibition of the dermal spread after alarming stimuli (Linderholm, 1951), which produce a hyperactivity in the pituitary-adrenocortical system and the general adaptation syndrome (for ref. see Selye, 1950), is in agreement with the above mentioned hormonal effects.

The purpose of the present investigation was to study the effect of the adrenocorticotrophic hormone (ACTH) on dermal spread and the dose and time relationships of this effect.\(^1\) Some of the investigations on dermal spread in which the influence of the pituitary-adrenocortical system may be of importance were made with, and some others without, hyaluronidase (cf. Linderholm, 1951). It was therefore reasonable to start examining the effect of ACTH on dermal spread without using the enzyme.

It is assumed that the »early« or »initial spread« in living animals, i.e. the spread during the first few minutes, and the spread in dead animals depends essentially on the state of the ground substance of the connective tissue, while the »late spread«, i.e. the increase of the spreading area from 10–20 minutes after the injection of the wheal and thereafter, is markedly influenced by i.a. vascular factors (McMaster & Parsons, 1938, Parsons & McMaster, 1938, Linderholm, 1951). The influence of the last-mentioned factors can be excluded if recently killed animals are used. These principles will be applied here in an attempt to define the nature of the effect of ACTH on the spread.

METHODS

Animals.

Rabbits. Apparently healthy male albino rabbits, weighing 1.9–2.1 kg., were used. They were caged for one week after their arrival from the different breeders and given hay, oats and water ad lib. and

\(^1\) Growth hormone (Li et al., 1944), in some respects being antagonistic to ACTH (Baker et al., 1948), was also tested for its effect on dermal spread in a few experiments. Preliminary results indicated that it did not reduce the spread. Further investigations will be carried out in this laboratory on the subject.
kept at a temperature of 15—20° C. No animal was used more than once.

Rats. Male albino rats of the Wistar strain were obtained from one breeder. Most of the experiments were made in young adult rats weighing 80—120 gm. Before use they were kept in an airconditioned room at 25° ± 0.5 C. and received a special bread (Gard, 1944) and water ad lib. During the investigation the ascorbic acid content of the adrenals was frequently tested in some animals according to the method of Bessey (1938) in order to ensure that they were not exposed to any alarming stimuli, especially infections. In healthy rats the ascorbic acid content was between 450—550 mg. per 100 gm. fresh adrenal tissue.

Adrenalectomy in rats. Adrenalectomy was carried out by the abdominal route in order to leave the skin of the back and flanks intact for the spreading test. Ether was used as an anesthetic. After the operation the rats received water, containing 5 per cent glucose and 1 per cent sodium chloride, to drink. All rats were carefully inspected for adrenal residues at autopsy. As a control for ectopic adrenal tissue the survival time was registered in 15 adrenalectomized rats which received only tap water to drink from the fourth day after the operation. The standard bread ration was given. On this diet 14 out of 15 rats died 5—9 days after the withdrawal of the sodium chloride. The remaining rat was killed 26 days after the operation. No macroscopically visible residues of the adrenals were found. As the body weight had increased from 92 to 145 gm. the presence of an ectopic adrenal in this animal is very likely.

Hormone preparations.

ACTH. A highly purified ACTH protein from sheep hypophyses (Li et al., 1943) was used in one experiment. This preparation, here called ACTH:1, is claimed to be free from other pituitary hormones. The blood pressure response in rabbits showed that 1 mg. ACTH:1 contained less than about 0.007 I. U. of vasopressin. A more thorough test was impossible owing to the limited supply of the preparation.

In other experiments a less purified ACTH from hog hypophyses (Cortrophin, Organon) was used. This preparation (ACTH:2) was assayed for adrenocorticotrophic activity according to the adrenal ascorbic acid depletion method (Sayers et al., 1948) using ACTH:1 as a reference standard. The assays were based on 21 and 50 hypophysectomized rats, respectively. One µg. of the ACTH:1 preparation was estimated to have the same potency as 7.9 µg. of the ACTH:2 powder, with 5 per cent fiducial limits at 6.1 µg. and 10.4 µg. The vasopressor activity was assayed with the above-mentioned method.
and was found to be about 0.02 (0.01—0.04) I. U. of vasopressin for an amount of ACTH:2 which corresponded in activity to 1 mg. of ACTH:1. According to the manufacturer, some prolactin should be present but no thyrotrophic or gonadotrophic hormones.

An ACTH peptide mixture (ACTH:3), the preparation I. 2026 MS (Li et al., 1950, 1951), was used in one experiment.

Vasopressin. A commercial preparation, Hypadrin (Astra), was used in order to test the effect of vasopressin on the spread. Its vasopressor activity was compared with that of Pitressin (Parke, Davis) using the blood pressure response in rabbits. Later on, the same preparation of Hypadrin was assayed for adrenocorticotrophic activity. This was found to be considerable, corresponding to 45 µg. ACTH:1 per I. U. of vasopressin. Owing to the small number of animals used in the assay this figure is rather uncertain. The upper 5 per cent fiducial limit is about 3 times and the lower limit about \( \frac{1}{2} \) the mean.

Dosage. The ACTH was diluted with 0.9 per cent sodium chloride solution immediately before use. Intramuscular injections were given in the thighs of the rats in volumes of 0.20—0.25 ml. per injection. The rabbits received the ACTH as intravenous or intramuscular injections of about 1 ml. in volume. When no other special procedure is mentioned, the same volume of saline was injected in the control animals. In no case were any injections given later than 2 hours before the spreading test.

Dermal spreading test.

The method is essentially the same as described by Linderholm (1951).

Indicator solution. An isotonic solution of 10 gm. per cent of bovine hemoglobin, 155 mN with respect to sodium chloride and containing 15 mg. per cent of calcium, was sterilized by filtering through a Seitz filter and was found to keep well for some months at \(-15^\circ\). It was prepared as described by Edlund & Linderholm (1949).

Rabbit experiments. The animals were depilated on their backs and flanks with a paste containing barium sulphide. About 2 hours later 0.3 ml. of the hemoglobin solution was injected, on one side of a line drawn along the spine, at from four to six (usually five) points (control side). The areas of the hemoglobin wheals were measured 5, 10, 20, 60 and 120 minutes after the injection. After 16—20 hours hemoglobin solution was similarly injected in symmetrical sites on the other side (test side) and the areas of the wheals were measured. The rabbits treated with ACTH were injected at various times before the test side measurements (vide infra). Controls were
injected with 1 per cent egg albumin solution or saline intravenously 16—20 hours before the test side measurements.

*Rat experiments.* The rats were depilated as described in the case of the rabbits, but under light ether anesthesia in animals which were to be kept alive during the spreading test. When the test was performed in dead animals, they were killed with ether. Immediately after the depilation a 0.05 ml. hemoglobin solution was injected in 4—6 symmetrical wheals on the back.

*Measurement of the spread and statistical calculations.* The longest and shortest diameter of each hemoglobin wheal was measured with calipers. The area was calculated on the assumption that the wheals formed regular ellipses. In the rabbit experiments the mean wheal area of the control side, Ac, was compared with that of the test side, At, and the differences in per cent of the control side, D = 100 · (At—Ac)/Ac, were the variates used. The mean difference, D, of each group given ACTH was compared with the D of the corresponding control group. The method of *Fisher* (1944) for the treatment of small samples was applied to evaluate the significance of the difference between the groups. The P values were obtained from *Fisher & Yates* (1943).

In the rat experiments the mean wheal area of each rat was used as a variate and the hormone treated animals were compared with the corresponding control groups. The statistical evaluation of the results was performed as described for the rabbits.

**RESULTS**

*Preliminary experiments in the study of the time course of the effect of ACTH on the dermal spread in living animals.*

Four groups of rabbits with 3—7 animals in each group were given 6.8 μg. ACTH: 2 per 100 gm. body weight divided in 5 equal intramuscular doses. The spreading test was started 3, 6, 12 or 19 hours after the first injection of ACTH. An inhibition of the spread was found 1 and 2 hours after the injection of the wheal in the groups tested 12 and 19 hours after the first injection of ACTH (Fig. 1). No inhibition of the spread was found during the first 20 minutes after the injection of the wheal in any group, nor was there any significant inhibition of the spread in the groups tested 3 and 6 hours after the first injection of ACTH. When all the 11 ani-
Inhibition of the 2 hours' spread in rabbits at different times after the beginning of the treatment with ACTH:2 (6.8 µg. per 100 gm. body weight) given as the percentage difference between the test and the control side.

11 other rabbits, 7 of which received 1 ml. of a 1 per cent egg albumin solution intravenously, served as controls. The mean

\[ D = -12.4 \pm 2.85 \text{ per cent.} \]

1) Arithmetic mean ± standard error of the mean.
percentage difference between the test and control sides of the spread after 2 hours, D, was $+1.0 \pm 2.67^1$ per cent. The probability, P, that random factors caused the difference between the two groups is less than 0.01.

Two rabbits received 27 μg. ACTH: 2 per 100 gm. body weight intravenously divided in two equal doses 6 and 4 hours before the spreading test. They showed an inhibition of the spread after 2 hours of 17 and 27 per cent respectively. Though the results are not conclusive they seem to indicate that the effect of ACTH on dermal spread is more rapid when a larger dose is used. This is supported by an experiment on 4 female rats (175—200 gm. of body weight) which received 27 μg. ACTH: 2 per 100 gm. body weight divided in two equal doses, and given about 4 and 2 hours before the spread was tested in the living animals. Four female rats of the same weight served as controls. The spreading area after 1 hour in the ACTH-treated animals was 109 (107—111) mm² and in the controls 122 (121—125) mm².

These results seem to indicate that the inhibition of the spread found in living animals may occur quite rapidly, only a few hours after a large dose of ACTH, but that the effect requires a longer time to become fully developed.

The effect of ACTH on the spread in intact, living and dead, animals.

Groups of intact rats were injected with ACTH: 1 or ACTH: 2 and compared with controls of corresponding body weight which received saline injections. The dosage and the results are seen in Table 1, A.

The ACTH injections began 24 hours before the spreading test. It is evident that an inhibition of the spread is obtained in living animals. In dead animals, killed immediately before the injection of the wheal, no inhibition was found using a moderate dose of ACTH.

In order to see if a continued administration of ACTH would cause an inhibition even in dead animals, some rats were given
<table>
<thead>
<tr>
<th>Dose per 100 gm. body weight.</th>
<th>n</th>
<th>Body weight (ranging m.)</th>
<th>Spread M</th>
<th>Spread s²</th>
<th>Difference from controls</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH doses refer to ACTH:1 as standard. (Intramuscular injections)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A. Intact rats.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Living.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:1, 660 μg. divided in 6 doses with 4 hour intervals.</td>
<td>14</td>
<td>70-80</td>
<td>89</td>
<td>31.4</td>
<td>40</td>
<td>108.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2. ACTH:2, 13 μg. divided in 6 doses with 4 hour intervals.</td>
<td>16</td>
<td>170-175</td>
<td>106</td>
<td>121.2</td>
<td>23</td>
<td>38.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>3. ACTH:2, 6.5 μg. divided in 3 doses with 4 hour intervals.</td>
<td>6</td>
<td>170-180</td>
<td>104</td>
<td>105.4</td>
<td>25</td>
<td>17.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>4. Controls (Mean of a, b and c).</td>
<td>58</td>
<td>70-280</td>
<td>129</td>
<td>195.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>a. Untreated.</td>
<td>16</td>
<td>70-90</td>
<td>125</td>
<td>310.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>b. Untreated.</td>
<td>31</td>
<td>155-180</td>
<td>132</td>
<td>155.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c. Saline, 0.25 ml. × 6 with 4 hour intervals.</td>
<td>11</td>
<td>170-180</td>
<td>126</td>
<td>163.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>II. Dead.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:2, 13 μg. divided in 6 doses with 4 hour intervals.</td>
<td>12</td>
<td>160-180</td>
<td>90</td>
<td>30.3</td>
<td>1</td>
<td>1.4</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>2. Controls, untreated.</td>
<td>14</td>
<td>155-180</td>
<td>90</td>
<td>31.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>B. Adrenalectomized rats.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:2, 40.5 μg. divided in 3 doses with 4 hour intervals.</td>
<td>8</td>
<td>128-190</td>
<td>86</td>
<td>6.0</td>
<td>17</td>
<td>192.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2. ACTH:2, 6.5 μg. divided in 3 doses with 4 hour intervals.</td>
<td>5</td>
<td>122-186</td>
<td>89</td>
<td>9.7</td>
<td>14</td>
<td>74.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>3. ACTH:3, 800 μg. divided in 4 doses with 2, 3 and 4 hour intervals between the doses.</td>
<td>5</td>
<td>167-202</td>
<td>94</td>
<td>11.7</td>
<td>9</td>
<td>31.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>4. Controls, 0.2 ml. × 3 injections with 4 hour intervals.</td>
<td>26</td>
<td>115-190</td>
<td>103</td>
<td>10.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>The controls divided in different weight groups: a</td>
<td>8</td>
<td>115-140</td>
<td>102</td>
<td>11.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>b</td>
<td>9</td>
<td>140-160</td>
<td>103</td>
<td>8.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c</td>
<td>9</td>
<td>160-190</td>
<td>103</td>
<td>13.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

For explanation of the symbols see Table 2.
Table 2.
The spread of 0.05 ml. hemoglobin solution 15 min. after the intradermal injection. The rats were injected with 40.5 μg. ACTH:2 per 100 gm. body weight per day divided in three doses for 6—7 days and killed immediately before the injection of the hemoglobin solution.

<table>
<thead>
<tr>
<th>Dose per 100 gm. body weight. ACTH doses refer to ACTH:1 as standard. (Intramuscular injections)</th>
<th>n</th>
<th>Body weight (range in gm.)</th>
<th>Spread</th>
<th>Difference from controls</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>s²</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>93—155</td>
<td>59</td>
<td>1.6</td>
<td>8</td>
<td>147.4</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>96—175</td>
<td>67</td>
<td>2.5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>A. Intact rats.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:2, a total dose of 280 μg. during 7 days.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Controls with saline.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B. Adrenalectomized rats.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:2, a total dose of 240 μg. during 6 days.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Controls with saline.</td>
<td>10</td>
<td>105—150</td>
<td>60</td>
<td>6.2</td>
<td>-</td>
<td>54.9</td>
</tr>
</tbody>
</table>

*Explanation of the symbols used in the table:*

- n = number of rats.
- $\chi^2$ = variance ratio.
- M = mean spreading area in mm².
- $s^2$ = variance.
- P = the probability that the differences are caused by random factors.
Table 3.

The dermal spread in rats treated with vasopressin (Hypadrin).

*Group A:* Adrenalectomized rats treated with Hypadrin during 24 hours before the test. The wheal area was measured after 60 min. spreading in living animals.

*Group B:* Intact and adrenalectomized rats treated during 6 days with 3 daily injections of Hypadrin. The wheal area was measured after 15 min. spreading in dead animals.

<table>
<thead>
<tr>
<th>Dose of vasopressin in I. U. per 100 gm body weight. (Intramuscular injections)</th>
<th>n</th>
<th>Body weight (rangeingm.)</th>
<th>Spread</th>
<th>Difference from controls</th>
<th>v²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>s²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 0.024 I. U. divided in 3 doses with 4 hour intervals (containing a total amount of 1 µg. ACTH).</td>
<td>10</td>
<td>103–185</td>
<td>97</td>
<td>17.4</td>
<td>6</td>
<td>18.0 &lt; 0.001</td>
</tr>
<tr>
<td>2. 0.240 I. U. divided in 3 doses with 4 hour intervals (containing a total amount of 10 µg. ACTH).</td>
<td>10</td>
<td>95–185</td>
<td>90</td>
<td>9.3</td>
<td>13</td>
<td>111.3 &lt; 0.001</td>
</tr>
<tr>
<td>3. Controls with saline (= Tab. I, B 4).</td>
<td>26</td>
<td>115–190</td>
<td>103</td>
<td>10.2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Group B.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. 1. Intact rats. 0.24 I. U. (containing 11 µg. ACTH) per 24 hours divided in 3 doses with 4 hour intervals during 6 days.</td>
<td>4</td>
<td>115–140</td>
<td>57</td>
<td>11.0</td>
<td>10</td>
<td>58.3 &lt; 0.001</td>
</tr>
<tr>
<td>2. Controls with saline.</td>
<td>9</td>
<td>96–175</td>
<td>67</td>
<td>2.5</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>II. 1. Adrenalectomized rats. 0.24 I. U. (containing 11 µg. ACTH) per 24 hours divided in 3 doses with 4 hour intervals during 6 days.</td>
<td>6</td>
<td>120–135</td>
<td>49</td>
<td>2.2</td>
<td>11</td>
<td>88.4 &lt; 0.001</td>
</tr>
<tr>
<td>2. Controls with saline.</td>
<td>10</td>
<td>105–150</td>
<td>60</td>
<td>6.2</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

For explanation of the symbols see Table 2.
Table 4.
The effect of ACTH on the spread 60 min. after the intradermal injection of 0.05 ml. hemoglobin solution in rats, exposed to a stress by infection. The ACTH treatment began 24 hours before the test.

<table>
<thead>
<tr>
<th>Dose per 100 gm. body weight.</th>
<th>n</th>
<th>Body weight (range in gm.)</th>
<th>Spread M</th>
<th>Spread s²</th>
<th>Difference from (stressed) controls</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH doses refer to ACTH:1 as standard. (Intramuscular injections)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Intact rats.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Pneumonia (virus?) infection.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:2, 6.5 μg. divided in 3 doses with 4 hour intervals.</td>
<td>9</td>
<td>104—125</td>
<td>96</td>
<td>213.0</td>
<td>5</td>
<td>0.27</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>2. Controls with saline.</td>
<td>6</td>
<td>110—128</td>
<td>101</td>
<td>274.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Pneumonia (virus?) infection.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:2, 40.5 μg. divided in 3 doses with 3 hour intervals.</td>
<td>4</td>
<td>115—135</td>
<td>91</td>
<td>162.0</td>
<td>-1</td>
<td>0.007</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>2. Controls with saline.</td>
<td>4</td>
<td>113—151</td>
<td>90</td>
<td>133.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Adrenalectomized rats with pneumonia (virus?) infection.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. ACTH:2, 13 μg. divided in 6 doses with 4 hour intervals.</td>
<td>10</td>
<td>79—95</td>
<td>75</td>
<td>32.0</td>
<td>9</td>
<td>8.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2. Controls with saline.</td>
<td>14</td>
<td>83—126</td>
<td>84</td>
<td>58.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For explanation of the symbols see Table 2.
ACTH: 2 for 7 days. As is seen from Table 2, A, there is a significant reduction of the spread in the treated group.

**The effect of ACTH on spread in adrenalectomized rats.**

Similar experiments as those for intact rats were made in adrenalectomized animals in order to see if the adrenals were necessary for the inhibitory effect of ACTH on the spread. After adrenalectomy the control rats had smaller spreading areas than the intact ones. ACTH preparations, however, were still effective in reducing the spread.

One group of rats received two different doses of ACTH: 2 or an ACTH peptide mixture, (ACTH: 3), during 24 hours immediately after the adrenalectomy. The spread was then measured in living animals (Table 1, B). A significant inhibition of the spread was obtained of the same percentage magnitude as in intact animals given corresponding doses. The small effect of ACTH: 3 may be due to excretory conditions of the peptides.

Another group of adrenalectomized rats was injected with ACTH: 2 for 6 days (Table 2, B). There was a reduction of the spread of the same magnitude as that previously found in intact rats injected with the same amount of ACTH for 7 days (Table 2, A).

The results obtained with the ACTH preparations on the spread will be discussed later in connection with those following the injection of a posterior pituitary preparation.

**The effect of a posterior pituitary preparation on the spread.**

It has previously been suggested by Favilli (1939) that vasopressin inhibits the spread in living rabbits, when the dye is injected with hyaluronidase, but is ineffective without

---

1) The inhibition of spread after adrenalectomy seems to be independent of the time at which measurements are made after the adrenalectomy. Carstensen (unpubl.) has found that completely adrenalectomized rats tested 12 days after the operation had significantly lesser spread than intact controls or unilaterally adrenalectomized rats tested 12 days after the operation.
The following experiments were made in order to answer the question whether vasopressin in the amount present in the ACTH:2 preparation influenced the spread in the experiments reported.

For this purpose two different doses of a posterior lobe preparation (Hypadrin, Astra) were used. The lower dose, corresponding to 0.024 I.U. of vasopressin per 100 gm. body weight injected during a period of 24 hours, was about 200 times higher than the vasopressin content of the lowest dose of ACTH:2 given (6.5 µg. ACTH per 100 gm. body weight per 24 hours). The higher dose of Hypadrin corresponded to 0.24 I.U. of vasopressin per 100 gm. body weight per 24 hours.

As previously mentioned Hypadrin has a considerable adrenocorticotropic activity when tested according to the ascorbic acid depletion method of Sayers (45 µg. ACTH per I.U. of vasopressin). The amounts of ACTH contamination for various doses of Hypadrin are given in Table 3. The results in these experiments may be explained by the presence of ACTH activity in the posterior lobe hormone preparation.

The spread was measured the day after adrenalectomy and after an injection period of 24 hours. It is evident from Table 3, A, that both doses produced an inhibition of the spread, which was more marked for the higher dosage. However, an equally marked inhibition of the spread as found with the higher dose of Hypadrin was obtained with ACTH:2 in a dose which contained about the same amount of ACTH — 6.5 µg. instead of 10 — but about 2000 times less vasopressin (Table 1, B 2).

In other groups intact and adrenalectomized rats were treated during 6 days with three daily injections of Hypadrin. The spread was measured in the recently killed animals, Table 3, B. There was about the same reduction of the spread in both groups. The interpretation of the results is difficult owing to the ACTH contamination of the vasopressin preparation and will be discussed.
Relationship between the inhibition of the dermal spread in living rats in per cent of controls and log (dose of ACTH). No account is taken of the vasopressin content of the preparations. The treatment started 24 hours before the spreading test and the spread was measured 60 minutes after injection of the hemoglobin wheal.

- Intact rats given ACTH:1 or ACTH:2.
- Adrenalectomized rats given ACTH:2.
- Adrenalectomized rats given Hypadrin.

**Dose-response relationship.**

The ratio between the activity of ACTH and vasopressin varied in the different experimental groups. The inhibition of the spread in living rats treated during 24 hours before the test is considered in relation to the different doses of ACTH and vasopressin (Figs. 2 and 3). A rough direct proportionality between log (dose of ACTH) and percentage inhibition of the spread is suggested from Fig. 2 which shows this relationship for living intact and adrenalectomized animals. Fig. 3 demon-
Fig. 3.

Relationship between the inhibition of the dermal spread in living rats in per cent of controls and log (dose of vasopressin). No account is taken of the ACTH content of the preparations. The treatment started 24 hours before the spreading test and the spread was measured 60 minutes after injection of the hemoglobin wheal.

- Intact rats given ACTH:1 or ACTH:2.
- Adrenalectomized rats given ACTH:2.
- Adrenalectomized rats given Hypadrin.

strates the absence of any regular relation between the log (dose of vasopressin) and the inhibition of the spread.

Effect of some alarming stimuli.

Inhibition of the spread and low adrenal ascorbic acid content in rats with intestinal infection. In one group of rats showing signs of intestinal infection with diarrhoea the spreading areas were smaller than in the control groups (mean area
109 vs. 129 mm². P < 0.001), as found after 60 minutes spread in 8 intact living animals. In 9 other rats of the same group the adrenals were examined for ascorbic acid content. This was low, being 350 ± 26\(^1\) mg per 100 gm. fresh gland for the left adrenals. In 5 hypophysectomized rats of the same group the mean ascorbic acid content was also reduced when measured the day after the operation, being 355 (288—456) mg per 100 gm. fresh gland for the left adrenals. The corresponding value for 22 normal hypophysectomized rats was 504 ± 9.8\(^1\) mg per 100 gm. fresh gland.

The effect of ACTH in rats exposed to some other alarming stimuli.

The effect of ACTH on dermal spread was tested in two groups of intact rats, one of which was infected with a liver parasite,\(^2\) the other with an agent producing pneumonia (probably a virus).\(^3\) The injections of ACTH started before the test. The spread was measured after 60 minutes in living animals, both intact and adrenalectomized. In all the control groups in these experiments there was a more or less marked reduction of the spread as compared with that in healthy control rats. In the adrenalectomized rats 13 µg. ACTH: 2 per 100 gm. body weight produced a significant decrease of the spread as compared with the controls (Table 4, B) but the decrease was smaller than that obtained with a smaller dose (6.5 µg. ACTH: 2) in healthy adrenalectomized rats (Table 1, B). The intact rats with pneumonia infection showed an uncertain inhibition of the spread after 6.5 µg. ACTH: 2. The

\(^1\) Arithmetic mean ± standard error of the mean.

\(^2\) The livers of all these rats showed a small number of whitish nodules. Histological examination for the cause of infection is so far not conclusive. However, there was a capsule surrounded by connective tissue and eosinophile leucocytes.

\(^3\) Some of the rats in this group died spontaneously, while the rest appeared to be healthy. However, the mortality after adrenalectomy was unexpectedly high. Autopsy showed pneumonia. Only apparently healthy animals were used in the experiments.
group with liver parasite infection, in which the controls had about the same reduction in the spread as the rats given the high dose of 660 µg. ACTH: 1, showed no further reduction of the spread after 40.5 µg. ACTH: 2. The number of animals, however, is too small to exclude an effect in the intact animals.

DISCUSSION

The experiments reported here show that ACTH inhibits the spread in living animals 12—14 hours after the beginning of repeated intramuscular injections, and possibly even earlier, while in dead animals no inhibition was found until later. A significant reduction of the spread in recently killed rats was found after daily injections of ACTH during one week. The effects were similar after adrenalectomy, the completeness of which was confirmed in survival experiments.

If these results are pure ACTH effects they are, as far as we know, the first to be described which are (at least partially) independent of the adrenals. The possibility that some other factor present in the preparations might have interfered with the results requires careful consideration. The occurrence of vasopressin contamination of the ACTH preparations as well as ACTH contamination in the posterior hormone preparations have caused the greatest difficulties in this respect. Concerning other hormone contaminations of possible importance it should be mentioned that thyrotrophic and gonadotrophic hormones are claimed to be absent from all the ACTH preparations used.

Possible importance of vasopressin contamination.

Rats treated during 24 hours. Assuming vasopressin to be the only active factor, it is evident from the dose-response relationships of Fig. 3 that it is difficult to see any regular connection between the percentage inhibition of the spread and the dose of vasopressin given during 24 hours before the
spreading test. Furthermore, if the results represent part of a
dose-response curve one must assume that vasopressin is active
in amounts considerably smaller than the lowest dose given
in connection with ACTH: 2 (0.00013 I. U. per 100 gm. body
weight injected during a period of 24 hours). According to
Shannon (1942) 0.00016—0.00120 I. U. per 100 gm. body weight
per 24 hours is the amount of vasopressin physiologically se¬
creted and active on diuresis in a normal dog. The sensitivity
of the rat to vasopressin is probably similar. It is difficult to
believe that such low doses as those given with ACTH: 2 would
influence the dermal spread so markedly.

The possibility of a combined effect of ACTH and vas¬
opressin seems unlikely. A marked effect was obtained with
ACTH: 1 which is claimed to be free from vasopressin. Finally
the roughly linear relationship between log (dose of ACTH)
and percentage inhibition of the spread, also evident in the
case of the adrenalectomized rats (Fig. 2), is in favour of the
view that ACTH is the only active principle.

Rats treated during 6 days. It is difficult to exclude the
possibility that vasopressin may influence the spread after
treatment during one week. The low dose of vasopressin
(0.00079 I. U. per 100 gm. per 24 hours) given with ACTH to
intact and adrenalectomized rats (Table 2, A and B) is within
the range of amounts physiologically active on diuresis (Shan¬
on, 1942) and an increase in the dose of vasopressin of about
250 fold gives about the same inhibition of the spread as this
low dose (Table 3, B). At the same time the ACTH dosage
differed only by a factor of 4. As the doses varied too little to
make it possible to estimate the dose-response curves, the ex¬
periments are not conclusive. However, it does not seem im¬
probable that ACTH is the active factor. Although incomplete,
the results are of interest as they demonstrate that pituitary
hormones inhibit the spread in dead animals after 6 days
treatment with an ACTH preparation while no such effect was
obtained after only 24 hours treatment.
Mechanisms of inhibition of the spread.

The interesting difference between the effects on the spread of ACTH when tested in living and recently killed rats treated for various periods of time suggests that two different mechanisms may be present. An essential difference between living and dead animals with regard to the kinetics of the spread is the absence of the pulsations of the peripheral vessels in the latter. It is well known that the pulsations increase the spread (McMaster & Parsons, 1938, Parsons & McMaster, 1938). In the living animals treated for 24 hours with ACTH, a change of the pulsations may thus be a possible explanation of the inhibition of the spread. The absence of any inhibition of the spread in a corresponding group of rats killed immediately before the spreading test suggests that no change had occurred in the structure of the connective tissue or its resistance to spread.

In the groups treated with ACTH for 6—7 days there was an inhibition of the spread in dead animals suggesting a change in the connective tissue. Assuming the inhibition to be due to ACTH (and not to vasopressin) this change may be related to the influence of ACTH upon water and electrolyte metabolism (Ingle et al., 1947, Luft et al., 1950), as changes of the water content of the skin are claimed to influence the spread (Taylor & Sprunt, 1943). It is also possible that ACTH has a more specific effect on the ground substance of the connective tissue and in this way may influence the spread. Investigations by Baker et al. (1948) and Asboe-Hansen (1950) are suggestive in this connection.

Role of the adrenal cortex.

Using injection of cortical extract Opsahl (1949 a) found an inhibition of the dermal spread without hyaluronidase in recently killed mice. Similar results were obtained by Carstensen (unpubl.) after injection of cortisone to rats. The question arises to what extent is the inhibition of the spread in our experiments on intact rats after the injection of ACTH due to stimulation of the adrenal cortex and a direct inhibitory effect.
of its hormones. No effect was seen on the spread in recently killed intact rats given a moderate dose of ACTH (13 µg.) during 24 hours (Table 1, group A, II). In this group adrenocortical hormones could be expected to act similarly as in the experiments of Opsahl and of Carstensen. As no effect is obtained it is suggested that the dose of ACTH was too low to give a secretion of cortical hormones sufficient to decrease the spread. It is an open question whether this is also true for the results obtained in the living animals.

Adrenalectomized rats injected with saline showed a smaller spread than intact controls. It is difficult to know if this can be explained by an increased output of ACTH (or possibly vasopressin) from the pituitary which is said to occur after adrenalectomy (Sayers & Cheng, 1949, Birnie et al., 1949).

There are some reports in the literature of an increase of the spread after adrenalectomy (Opsahl, 1949 a, Opsahl et al., 1950). This was found only in living animals. The increase was counteracted by adrenal cortical hormones. It was most evident when the spread was studied in the presence of hyaluronidase. Using Indian ink without hyaluronidase the increase seems doubtful. Hyaluronidase has not been used in the present investigation, yet the results differ from those of Opsahl in so far as a generally restricted spread was found in adrenalectomized animals. The differences may be due to a different technique.

Inhibition of the spread after alarming stimuli.

The good agreement between the inhibition of the spread after ACTH and the inhibition after exposure to cold or other alarming stimuli (Linderholm, 1951) should be pointed out. In this investigation, we have found that ACTH either failed to reduce further or had a smaller effect in reducing the already restricted spread in rats exposed to stress by infection. The explanation of this may be that the organism is previously loaded with ACTH secreted under the stimulation of a stress. These findings confirm the view that the inhibition of the spread after different alarming stimuli is essentially due to the
increased secretion of ACTH in these conditions though they do not exclude the possibility that vasopressin may be active, as the secretion of this hormone appears to be increased in the general adaptation syndrome (for ref. see Selye, 1950).

SUMMARY

1. The effect of different ACTH preparations on the dermal spread was investigated. The preparations were assayed for adrenocorticotrophic and vasopressor activities. Special experiments were carried out to control possible effects of vasopressin.

2. After 24 hours' treatment with ACTH, an inhibition of the dermal spread was found in living intact as well as in adrenalectomized animals. No inhibition was found when the test was made in recently killed animals. The results indicate that this effect of ACTH is (at least partially) independent of the adrenals and may be connected with some effect on the peripheral circulation. ACTH is assumed to be the active factor because interfering effects of vasopressin can be excluded.

3. After treatment with ACTH for 6—7 days an inhibition of the spread was found in recently killed intact as well as adrenalectomized rats. In these groups vasopressin could not be excluded as an active factor, though some evidence supports the view that ACTH caused the inhibition of the spread. This inhibition may be connected with an effect of ACTH on the ground substance of the connective tissue.

4. Intact and adrenalectomized rats infected with an agent producing pneumonia, probably of viral origin, or with hepatic parasites had a reduced spread. After ACTH administration there was slight or no further inhibition of the spread.

5. A connection was observed between the reduction of the dermal spread and of the adrenal ascorbic acid content in animals with an intestinal infection.
Acknowledgements.

Pure ACTH protein was obtained by the courtesy of Professor Choh Hao Li, University of California, Berkeley. Cortrophin was generously supplied by Dr. med. F. Paulsen, Organon, Stockholm.

The investigation was aided by grants from the Medical Faculty in Uppsala.

REFERENCES