The Separate Existence of the Pituitary Erythropoietic Hormone

By Donald C. Van Dyke, M. E. Simpson, A. N. Contopoulos and H. M. Evans

That erythropoiesis is controlled by one or more circulating substances seems well documented.1-11 There is adequate evidence that the anterior pituitary is in some way involved in erythropoiesis and that administration of anterior pituitary substance will stimulate erythropoiesis.5-9 The anterior pituitary may be the source of one of the circulating erythropoietins but since control of erythropoiesis is not abolished by hypophysectomy, it is apparent that the pituitary is not the only source of erythropoietic stimulation. Feigin and Gordon10 demonstrated that hypophysectomized animals respond to hypoxia with an increased erythropoiesis and more recently Fried et al.11 and Crafts and Meineke12 demonstrated the presence of circulating erythropoietin in the absence of the pituitary.

Considerable investigation has been devoted in the last few years to establish the role of the pituitary in control of red blood cell production, through a specific pituitary erythropoietic hormone, having as its target organ the erythropoietic portion of the marrow. It is apparent from the various experiments in which pituitary substance has been administered, as food or by injection, that none of the known pituitary hormones other than ACTH need be considered.5, 9, 12 Clarification of the role of the anterior pituitary in the control of erythropoiesis has been delayed by the failure to extract from this gland an erythropoietically active material which is distinct from ACTH. It should be emphasized that no pituitary extract has been prepared that will stimulate erythropoiesis which does not contain ACTH, and no ACTH has been found free of erythropoietic activity. However, when either hypophysectomized or normal rats were fed a diet of anterior pituitary, erythropoietic stimulation was obtained with no stimulation of the adrenal in many of the animals, and with minimal stimulation of others, suggesting that digestion and absorption might afford the best method of separation of erythropoietic activity from ACTH. For this reason a series of experiments were performed to determine whether erythropoietic activity might be separated from ACTH by digestion in vitro. Since these experiments were an attempt to imitate what occurs in the digestive tract, it was decided to start with the whole pituitary rather than purified products. Since the adrenocorti
trophic preparation in which erythropoietic activity was first observed had been subjected to boiling in the process of isolation and since the digested glands could not be injected for assay as such, due to their toxicity, boiling was used to

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check the digestion process and simultaneously free the preparation of much inert and toxic material.

**Materials and Methods**

Hypophysectomized female rats of the Long-Evans strain were used to assay the preparation throughout these studies. They were fed a complete diet* with a supplement of liquid diet every night and lettuce once a week. All animals were maintained for at least 48 days following hypophysectomy before being used for assay to allow time for them to develop the characteristic post-hypophysectomy anemia and thus provide a more sensitive assay animal.

Hypophysectomy was performed through the parapharyngeal approach and the completeness of hypophysectomy was determined by examination of the pituitary site at autopsy as well as by the condition of the pituitary target organs. All animals were hypophysectomized at 28 days of age. No data from animals found to be incompletely hypophysectomized were included in the results.

The total circulating red cell volumes were determined by the Fe* labeled cell dilution method. The method was as follows: The labeled cells were obtained from a Long-Evans donor rat previously injected with Fe*. The experimental animals were injected through the jugular vein with 0.1 ml. of donor blood containing approximately 0.03 μC Fe*. After allowing 6 minutes for the blood to mix, a sample of blood was drawn from the vena cava into a heparinized syringe. A known volume of this blood was pipetted into a vial and its activity counted directly in a scintillation counter. The total blood volume was calculated from the fraction of the injected activity recovered in this sample. The hematocrits were determined in Wintrobe tubes. The total blood volume thus calculated multiplied by the hematocrit gave the total circulating red cell volume. The total circulating red cell volumes were then corrected for the body weights of the animals and the results are presented in terms of ml. of red blood cells per 100 Gm. body weight.

The hemoglobin concentration was determined by the method of Turner and the results are presented as Gm. of hemoglobin per 100 ml.

At autopsy the adrenals and thymus and in some cases the thyroid and ovaries were carefully dissected and weighed. After weighing the adrenals were fixed in neutral-formalin, imbedded in gelatin, cut in frozen section and stained with Sudan Black for the demonstration of lipid distribution. The thyroids and ovaries were stained with hematoxylin and eosin for histological analysis.

Sheep pituitaries were collected soon after death, frozen in solid CO₂, wrapped in plastic tissue and frozen at -10 C. until used.

The effectiveness of boiling whole pituitary suspensions as a method of separation of the erythropoietic activity from the heat labile and heat precipitable substances was demonstrated as follows:

Two hundred Gm. of frozen sheep pituitaries were ground with 400 ml. of 0.9 per cent saline in a mechanical blender for 1 minute. The pH was adjusted to 5.5 by the addition of N/1 HCl. This material was put into a boiling water bath for 10 minutes, centrifuged and adjusted to pH 6.8 by the addition of N/1 NaOH. This yielded 400 ml. of clear solution, which was frozen until used for assay.

A dose of 1 ml. was injected intraperitoneally daily for 14 days into hypophysectomized rats. On the 15th day blood volume, hemoglobin and hematocrit determinations were made and the adrenal, thymus, thyroid and ovaries were dissected, weighed and examined histologically. Examples of the potency of the material at this stage of preparation are given as the first items in tables 1 to 3 and the relation between dose and response is given in table 4. A substantial increase in both the circulating red cell volume and adrenal weight resulted.

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* Diet I (modified from McCollum's formula) consists of 67.5% whole wheat, 15% casein, 10% whole milk powder, 0.75% NaCl, 1.5% CaCO₃, 5.25% hydrogenated vegetable oil, and a concentrate of fish oil in amount to give 19 U.S.P. units of Vitamin A and 2.5 A.O.A.C. chick units of Vitamin D per gram of diet.
VAN DYKE, SIMPSON, CONTOPULOS AND EVANS

from this procedure. From extensive experience it is known that an increase of 20 per cent in either is significant. The pituitary target organs demonstrated ACTH to be present in high concentration but no thyrotrophic, interstitial cell stimulating, follicle stimulating or growth hormone. Boiling therefore provides an extraction method which is consistent in activity from preparation to preparation and which is well tolerated by the animals.

Extracts of this type were prepared from sheep, beef, pig and horse pituitaries by this method to determine whether the ratio of erythropoietic and ACTH activities differed sufficiently to be suggestive of the separate nature of these effects. The increases in red cell volume and adrenal weight given by sheep, pig, beef and horse pituitary extracts were remarkably similar and no differences in the ratio of erythropoietic and ACTH activity were observed (table 1). Sheep pituitaries were used as the starting material for the remainder of the experiments.

Although both erythropoietic and ACTH activity withstand boiling in a water bath for 10 minutes, the effect of boiling for longer periods was investigated.

Table 1.—Comparison of the Erythropoietic and ACTH Activities of Boiled Extracts from Pituitaries of Various Animals

<table>
<thead>
<tr>
<th>Animal source</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hematocrit (%)</th>
<th>RCV/100 Gm.* (ml.)</th>
<th>Adrenal (mg.)</th>
<th>Thymus (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>12.4 ± 0.6†</td>
<td>39.4 ± 1.7</td>
<td>1.90 ± 0.10</td>
<td>14 ± 2</td>
<td>92 ± 14</td>
</tr>
<tr>
<td>Pig</td>
<td>11.5 ± 0.3</td>
<td>36.7 ± 0.4</td>
<td>1.90 ± 0.04</td>
<td>13 ± 1</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>Beef</td>
<td>11.2 ± 0.4</td>
<td>37.0 ± 1.2</td>
<td>1.86 ± 0.07</td>
<td>12 ± 1</td>
<td>98 ± 6</td>
</tr>
<tr>
<td>Horse</td>
<td>12.0 ± 0.3</td>
<td>35.7 ± 0.7</td>
<td>1.81 ± 0.05</td>
<td>12 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Uninjected control</td>
<td>9.2 ± 0.4</td>
<td>30.0 ± 1.4</td>
<td>1.42 ± 0.07</td>
<td>8 ± 1</td>
<td>122 ± 13</td>
</tr>
</tbody>
</table>

* Red cell volume per 100 Gm. body weight.
† Figures preceded by ± in tables are the standard errors of the means.

Table 2.—Lack of Effect of Prolongation of the Boiling Period on the Erythropoietic and ACTH Activities

<table>
<thead>
<tr>
<th>Conditions of boiling</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hematocrit (%)</th>
<th>RCV/100 Gm. (ml.)</th>
<th>Adrenal (mg.)</th>
<th>Thymus (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water bath; 10 min.</td>
<td>13.2 ± 1.4</td>
<td>39.4 ± 1.4</td>
<td>2.37 ± 0.08</td>
<td>12 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Open flame; 15 min.</td>
<td>12.5 ± 1.3</td>
<td>37.6 ± 1.5</td>
<td>2.16 ± 0.06</td>
<td>13 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Open flame; 1 hr.</td>
<td>14.7 ± 1.6</td>
<td>36.2 ± 1.5</td>
<td>2.19 ± 0.14</td>
<td>12 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Open flame; 3 hr.</td>
<td>13.5 ± 1.4</td>
<td>40.6 ± 1.2</td>
<td>2.35 ± 0.08</td>
<td>13 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Controls</td>
<td>9.2 ± 0.4</td>
<td>28.8 ± 1.4</td>
<td>1.65 ± 0.10</td>
<td>7 ± 0.4</td>
<td>100 ± 17</td>
</tr>
</tbody>
</table>

Table 3.—Effect of Length of Autolytic Digestion on Erythropoietic and ACTH Activities

<table>
<thead>
<tr>
<th>Length of digestion (Hours)</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hematocrit (%)</th>
<th>RCV/100 Gm. (ml.)</th>
<th>Adrenal (mg.)</th>
<th>Thymus (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.1 ± .4</td>
<td>40.5 ± 0.9</td>
<td>2.28 ± 0.07</td>
<td>12.0 ± 1.4</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>1</td>
<td>12.7 ± .4</td>
<td>37.0 ± 0.7</td>
<td>2.02 ± 0.08</td>
<td>11.0 ± 0.8</td>
<td>76 ± 15</td>
</tr>
<tr>
<td>3</td>
<td>13.7 ± .8</td>
<td>37.0 ± 1.2</td>
<td>2.06 ± 0.13</td>
<td>8.9 ± 0.5</td>
<td>102 ± 14</td>
</tr>
<tr>
<td>6</td>
<td>12.3 ± .7</td>
<td>34.8 ± 1.1</td>
<td>1.93 ± 0.06</td>
<td>8.8 ± 0.7</td>
<td>105 ± 12</td>
</tr>
<tr>
<td>Controls</td>
<td>10.1 ± .5</td>
<td>27.8 ± 0.7</td>
<td>1.51 ± 0.06</td>
<td>8.5 ± 0.5</td>
<td>92 ± 9</td>
</tr>
</tbody>
</table>
Variations from pH 6.8 to pH 3 were tried without alteration of the result; pH 5.5 was arbitrarily chosen for the remainder of the experiments.

**Table 4.**—Erythropoietic and ACTH Dose-Response Relationships of Undigested Extract

(6 H rats per group)

<table>
<thead>
<tr>
<th>Dose (mg./day/14 days)</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hematocrit (%)</th>
<th>RCV/100 Gm. (ml.)</th>
<th>Adrenal (mg.)</th>
<th>Thymus (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.5</td>
<td>14.0 ± .3</td>
<td>43.8 ± 1.5</td>
<td>2.17 ± .16</td>
<td>18 ± 2</td>
<td>&lt;50</td>
</tr>
<tr>
<td>15</td>
<td>14.2 ± .3</td>
<td>43.1 ± 0.9</td>
<td>2.13 ± .04</td>
<td>15 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>7.5</td>
<td>13.8 ± .6</td>
<td>43.6 ± 1.8</td>
<td>2.18 ± .15</td>
<td>13 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>3.3</td>
<td>13.7 ± .4</td>
<td>41.2 ± 1.4</td>
<td>1.92 ± .09</td>
<td>11 ± 1</td>
<td>59 ± 7</td>
</tr>
<tr>
<td>Controls</td>
<td>11.7 ± .6</td>
<td>32.5 ± 1.6</td>
<td>1.51 ± .05</td>
<td>7 ± 1</td>
<td>83 ± 13</td>
</tr>
</tbody>
</table>

Sheep pituitaries were ground with saline, the pH was adjusted to 5.5, and the preparation was placed in a boiling water bath for 10 minutes. An aliquot was removed for assay and the remainder was boiled over an open flame for 1 and 3 hours. The volume was maintained by the addition of distilled water. Aliquots removed at each interval were centrifuged, neutralized and tested in the usual way at doses of 1 ml. per day for 14 days. There was no change in either the erythropoietic or ACTH activity during 3 hours of boiling (table 2). As precipitation of inert and toxic material occurred immediately on boiling, the extracts thereafter were boiled over an open flame for 1 minute.

**AUTOLYTIC DIGESTION**

Since the object of these experiments was to determine whether proteolytic enzymes had a differential destructive action on erythropoietic and adrenocorticotropic activity, the action of enzymes present in the pituitary tissue itself was examined.

Four hundred Gm. of whole sheep pituitary were ground with 800 ml. of 0.9 per cent saline and an aliquot removed for assay. The remainder was immersed in a 37 C. water bath and after 1, 3 and 6 hours of digestion aliquots were removed. Each fraction was adjusted to pH 5.5*, boiled and prepared for assay at 1 ml. per day for 14 days.

Table 3 compares the resulting circulating red cell volumes and adrenal weights. Between 1 and 3 hours ACTH activity, judged by adrenal weight, decreased markedly whereas the greater part of the erythropoietic activity remained even after 6 hours of digestion. Histologically the adrenals obtained after injection of digested material showed barely detectable evidence of stimulation. Thymus weight was decreased by the undigested material, but no depletion from digested material occurred (table 3). Digestion for periods longer than 6 hours resulted in toxic preparations and in loss of both activities.

A comparison of the adrenocorticotropic and erythropoietic activities of highly purified ACTH, undigested boiled extracts, and autolytically digested pituitary material is shown in figure 1. The ACTH, prepared by Dr. C. H. Li* was a highly purified peptide fraction given in oil at a daily dose of 25 mg. for 10 days. The other two preparations are the boiled extract before and after 4 hours of autolysis as described above. At the doses given the three preparations gave equal erythropoietic responses (45% increase in circulating red cell volume) but as the figure shows, varied greatly in adrenocorticotropic activity.

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* Variations from pH 6.8 to pH 3 were tried without alteration of the result; pH 5.5 was arbitrarily chosen for the remainder of the experiments.
The digested material could be dialyzed 24 hours at 0°C against distilled water without loss of erythropoietic activity. The method adopted in subsequent experiments was as follows:

Four hundred Gm. whole sheep pituitary were ground in a mechanical blender with 800 ml. of 0.9 per cent saline for 1 minute, were placed in a 37°C. water bath for 4 hours with gentle stirring. At the end of the 4 hour digestion the pH was adjusted to 5.5 by the addition of N/1 HCl, boiled over an open flame with stirring for 1 minute, centrifuged, the precipitate discarded and the solution neutralized by the addition of N/1 NaOH. The clear supernatant (800 ml.) was dialyzed against distilled water for 24 hours at 0°C with stirring. The contents of the dialysis bag were lyophilized.

The apparently greater destruction of the adrenocorticotropic than of erythropoietic activity by autolysis could be interpreted as due to two biological effects of the same hormone having different dose-response relationships. Tables 4, 5 and 6 show the dose-response relationship in regard to adrenocorticotropic and erythropoietic activities of boiled material, before and after digestion, as compared with those of highly purified ACTH. In all three preparations adrenal weight increases with dose more abruptly than does red cell volume. However, one cannot duplicate the proportion of erythropoietic and ACTH activities obtained with digested material simply by reducing the dose of undigested material or "pure" ACTH.

Since adsorption on oxycellulose is an excellent method for concentration of ACTH and since ACTH and erythropoietic hormone behave similarly, concentration of erythropoietic activity was attempted by this method. It was found that by adsorbing autolyzed extracts with small amounts of oxycellulose, material high in ACTH and low in erythropoietic activity was obtained, indicating that oxycellulose is a better adsorbent of ACTH than of erythropoietic activity.
PITUITARY ERYTHROPOIETIC HORMONE

Table 5.—Erythropoietic and ACTH Dose-Response Relationships of Digested Extract

(6 H rats per group)

<table>
<thead>
<tr>
<th>Dose (mg./day/14 days)</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hematocrit (%)</th>
<th>RCV/100 Gm. (ml.)</th>
<th>Adrenal (mg.)</th>
<th>Thymus (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>13.7 ± .3</td>
<td>44.2 ± 0.7</td>
<td>2.38 ± .06</td>
<td>17 ± 1</td>
<td>&lt;50</td>
</tr>
<tr>
<td>36</td>
<td>13.8 ± .3</td>
<td>43.5 ± 1.1</td>
<td>2.26 ± .09</td>
<td>12 ± 0.2</td>
<td>53 ± 9</td>
</tr>
<tr>
<td>18</td>
<td>13.6 ± .9</td>
<td>39.8 ± 1.3</td>
<td>1.99 ± .07</td>
<td>9 ± 1</td>
<td>78 ± 16</td>
</tr>
<tr>
<td>4.5</td>
<td>11.3 ± .6</td>
<td>36.1 ± 1.0</td>
<td>1.90 ± .04</td>
<td>8 ± 0.3</td>
<td>124 ± 12</td>
</tr>
<tr>
<td>Controls</td>
<td>10.1 ± .6</td>
<td>30.0 ± 1.5</td>
<td>1.42 ± .07</td>
<td>8 ± 0.4</td>
<td>112 ± 10</td>
</tr>
</tbody>
</table>

Table 6.—ACTH and Erythropoietic Dose-Response Relationship of α-Corticotrophin (Li)

(6 H rats per group; injection in beeswax)

<table>
<thead>
<tr>
<th>Dose (pg./day/14 days)</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hematocrit (%)</th>
<th>RCV/100 Gm. (ml.)</th>
<th>Adrenal (mg.)</th>
<th>Thymus (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>11.7 ± .4</td>
<td>43.5 ± 1.3</td>
<td>2.11 ± .03</td>
<td>35.0 ± 2.0</td>
<td>&lt;50</td>
</tr>
<tr>
<td>25</td>
<td>13.7 ± .5</td>
<td>48.4 ± 2.1</td>
<td>2.18 ± .04</td>
<td>25.3 ± 3.0</td>
<td>&lt;50</td>
</tr>
<tr>
<td>10</td>
<td>11.8 ± .2</td>
<td>37.2 ± 1.0</td>
<td>1.88 ± .04</td>
<td>11.5 ± 0.9</td>
<td>&lt;50</td>
</tr>
<tr>
<td>5</td>
<td>9.6 ± .3</td>
<td>30.6 ± 1.3</td>
<td>1.52 ± .04</td>
<td>8.5 ± 1.0</td>
<td>82 ± 7</td>
</tr>
<tr>
<td>Control (beeswax)</td>
<td>9.0 ± .3</td>
<td>27.7 ± 1.2</td>
<td>1.43 ± .02</td>
<td>6.0 ± 3</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>Uninjected control</td>
<td>9.5 ± .3</td>
<td>28.4 ± 0.8</td>
<td>1.45 ± .04</td>
<td>5.7 ± 1</td>
<td>86 ± 4</td>
</tr>
</tbody>
</table>

Table 7.—Effect of Oxy cellulose Adsorption on Erythropoietic and ACTH Activities

(5 H rats per group)

<table>
<thead>
<tr>
<th>Dose (15 mg./day/14 days)</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hematocrit (%)</th>
<th>RCV/100 Gm. (ml.)</th>
<th>Adrenal (mg.)</th>
<th>Thymus (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorbed</td>
<td>12.4 ± .3</td>
<td>39.8 ± 1.1</td>
<td>1.94 ± .07</td>
<td>11.7 ± 1.2</td>
<td>75 ± 20</td>
</tr>
<tr>
<td>Non-adsorbed</td>
<td>10.7 ± .1</td>
<td>35.9 ± 0.5</td>
<td>1.95 ± .04</td>
<td>7.0 ± 0.4</td>
<td>138 ± 9</td>
</tr>
<tr>
<td>Uninjected controls</td>
<td>9.0 ± .3</td>
<td>26.1 ± 1.0</td>
<td>1.30 ± .04</td>
<td>6.0 ± 0.5</td>
<td>103 ± 7</td>
</tr>
</tbody>
</table>

Table 7 shows that the erythropoietic activity of adsorbed and non-adsorbed material was identical. The adsorbed material significantly increased adrenal weight and reduced thymus weight whereas the non-adsorbed material failed to influence adrenal or thymus weight though minimal evidence of stimulation was detected histologically.

This erythropoietically active fraction which appeared to be practically devoid of adrenal stimulating potency when administered in aqueous solution, was no more potent in adrenocorticotrophic or erythropoietic activity when administered in agents which delay absorption.

DISCUSSION

Since hypoxia, bleeding, increased hemolysis, cobalt, pituitary erythropoietic hormone and plasma erythropoietin are the only known stimulants of erythropoiesis it is probable that some relationship exists between them. Cobalt may interfere with oxidative mechanisms resulting in hypoxia, in response to which
erythropoietin is released. However, since hypophysectomized animals respond to hypoxia and to cobalt and since erythropoietin has been demonstrated in the plasma of hypophysectomized rats it is apparent that there is a source of erythropoietin other than the pituitary.

Hypoxia must result in the elaboration of at least two plasma erythropoietins, one from the pituitary and another from an as yet undetermined source (figure 2). A similar scheme has been suggested by Gley, who has demonstrated the presence in plasma of two erythropoietins. Such a scheme is compatible with the demonstration that although hypophysectomized rats respond to hypoxia with an increase in erythrocytes, the response is less than that shown by normal rats. It is also compatible with the data of Crafts and Meineke indicating that although plasma erythropoietin appears after bleeding of hypophysectomized rats, the activity is less than that obtained in animals possessing a pituitary.

Although it is recognized that a number of factors are involved in the mechanism controlling red blood cell production, the anterior pituitary apparently furnishes one factor of importance. This pituitary factor is apparently a hormone distinct from other trophic hormones. It is allied chemically only to ACTH and yet both biological and chemical evidence indicate that the pituitary erythropoietic hormone and ACTH are not identical:

1. Normal animals placed at 22,000 feet altitude develop a 40% increase in circulating red cell volume in 14 days without adrenal enlargement.
2. The adrenalectomized animal, following exposure to reduced barometric pressure, becomes polycytemic to the same degree as the normal animal.

![Diagram](image)

**Fig. 2**—Representative of the possible relationship of pituitary erythropoietic hormone and plasma erythropoietin.
3. The adrenalectomized animal has a several-fold increase in ACTH content of its blood but no increase in circulating red cells.\textsuperscript{8, 25}

4. Hypophysectomized, adrenalectomized or normal rats can be made polycythemic by feeding anterior pituitary. The process of digestion destroys all other pituitary hormones.\textsuperscript{8, 9}

5. Pituitary extracts, freed of everything but ACTH, are effective in stimulating erythropoiesis in the absence of the adrenals in either normal or hypophysectomized rats.\textsuperscript{8, 23}

6. In parabiotic cross transfusion experiments increased circulating ACTH due to adrenalectomy or to injection into one member produces little effect on the partner.\textsuperscript{26} When one member is subjected to hypoxia the erythropoietic effect is easily demonstrated in the partner.\textsuperscript{1} The most likely explanation for this difference is that the two hormones have different survival times in the circulation.

7. Autolytic digestion of whole sheep pituitary consistently reduces the ACTH activity, while erythropoietic activity is retained.

8. Oxycellulose is a better adsorbent of ACTH than of erythropoietic hormone.

**SUMMARY**

Although it is recognized that a number of factors are involved in the mechanism controlling red blood cell production, the anterior pituitary furnishes one factor of importance. This pituitary factor is apparently a hormone distinct from other trophic hormones. It is allied chemically only to ACTH and yet both biological and chemical evidence indicates that the pituitary erythropoietic hormone and ACTH are not the same. The major problem in further elucidating the role of the pituitary in the control of erythropoiesis resolves itself into developing a satisfactory method of preparation of erythropoietic hormone which will separate it from ACTH. It is true that no ACTH preparation has been made which does not possess some erythropoietic activity, and no pituitary erythropoietic hormone has been prepared which is entirely devoid of ACTH. Nevertheless, the proportion of these two activities in different preparations has varied so greatly as to assure their separate existence. Autolytic digestion consistently reduces the ACTH activity, while erythropoietic activity is retained. It has been found that oxycellulose is a better adsorbent of ACTH than of erythropoietic hormone. These observations add chemical evidence to the accumulated biological evidence establishing the separate existence of these two pituitary principles.

**SUMMARIO IN INTERLINGUA**

Il es recognoscite que un numero de factores es implicate in le mechanismo que regula le production de erythrocytos, e le glandula pituitari anterior forni un factor importante. Iste factor pituitari es apparentemente un hormon distincte ab altere hormones trophic. Chimicamente illo es affin solmente a ACTH, e tamen datos biologic e chimic indica que le erythropoietic hormon pituitari a ACTH non es identic. Le major problema in le elucidation ulterior del rolo del glandula pituitari in le regulation de erythropoiese se reduce al problema de disveloppar un metodo satisfacente del preparation del hormon erythropoietic que va separar lo ab ACTH. Il es ver que nulle preparato de ACTH ha essite
facile que non possede un certo grado de activitate erythropoietic, e nulle erythropoietic hormon pituitari ha essite preparate que es completamente libre de ACTH. Nonobstante, le proportion de iste duo activitates ha variate si considerabilemente in varie preparatos que le separate existentia del duo es indicate. Digestions autolytic regularmente reduce he activitate de ACTH, durante que he activitate erythropoietic es retinite. II ha essite constatate que oxycellulosa es un melior ahsorhemste de ACTH que de hormon erythropoietic. 1st-observationes adde datos chinssic a! accunsulate datos biologic que estabhi be existentia separate de iste duo principios pituitari.

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PITUITARY ERYTHROPOIETIC HORMONE


The Separate Existence of the Pituitary Erythropoietic Hormone

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