A STUDY RELATING TO A SUPPOSED PRESSOR SUBSTANCE AND OF THE VASO-MOTOR REFLEXES AFTER PARATHYROIDECTOMY.

by

A.M. Lands, B.S., 1926
West Tennessee State Teachers College, Memphis, Tenn.

Submitted to the Department of Physiology and Pharmacology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.

Approved by:

O.P. Toland
Instructor in charge

Chaiman of Department

8/3/1929
INTRODUCTORY.

Carlson and Jacobson (1) observed that the arterial blood pressure is relatively high in parathyroid tetany. They note that the intravenous injection of calcium lactate slows the heart and lowers the arterial pressure, but not sufficiently to greatly diminish the blood flow through the brain the decrease being fairly insignificant. Vincent and Thompson (5) in a recently published account, believe the pressor response obtained upon stimulation of intestine, muscles or skin is due to the liberation of an autacoid substance having a pressor action. They further state that after thyroparathyroidectomy stimulation liberates large quantities which accounts for the increased pressor response obtained.

In this study, we have attempted to determine this pressor substance and study further the vaso-motor responses immediately following thyroparathyroidectomy.
THE VASO-MOTOR SYSTEM AND ITS NORMAL RESPONSES.

In beginning this study, it is well to give a brief review of the literature on the vaso-motor system and its normal responses. Bayliss (6) has given evidence showing that vaso-motor reflexes involve both excitation of a pressor centre and the inhibition of a depressor centre in pressor responses and the reverse in depressor responses. He also finds that they show a different degree of susceptibility to strychnine, first stimulating the vaso-constrictor centre then paralyzing it, the dilator effect then making itself evident. He states that the action of strychnine is to convert the inhibitory phase of all vascular reflexes into an excitation, so that; "the depressor nerves produce a rise of blood pressure under full dose of the alkaloid. It does this by exciting the constrictor centre by the same mechanism which normally inhibits it. Various parts (synapses) of the reflex arc are differently sensitive to the alkaloid with the constrictor centre being the first to show paralysis as the dose is increased."

Langley (9), working with spinal animals, has noted that stimulation of the central end of any limb or trunk nerve gives a trifling rise of blood pressure which was increased upon the injection of strychnine.

Anrep and Starling (7) have shown that blood pressure is controlled by both centrally and reflexly acting impulses. They note that a rise of pressure in the aorta causes a reflex general vaso-dilatation. It is further noted that a rise of pressure in the brain causes general
vasodilatation while a fall of pressure results in immediate general vaso-constriction. McDowall (10), working with cats, has demonstrated what he believes to be a pressor impulse passing up the vagus to the vaso-motor centre from the thoracic vena cava near the heart. He believes it to be stimulated by a fall of the venous pressure thus tending to maintain a constant level of blood pressure. Wright (8) in his study of the depressor reflexes believes the vaso-motor reflexes in many respects resemble those obtained with skeletal reflexes. It is suggested they offer evidence that the processes of "recruitment" and "after discharge" may take place in the vaso-motor centre.

Vincent and Thompson (4) believe that two antagonistic factors are requisitioned to influence the general blood pressure; a vaso-motor reflex of a depressor nature, and the liberation of an autacoid substance having a pressor action. They state further; "The response to stimulation of various tissues in any one individual may be pressor or depressor according to the general condition and nutrition of the part stimulated and other factors not determined, and that the vaso-motor depressor effect is in harmony with the results obtained by stimulation of nerve trunks." Bazett and Quinby (11) by crossed circulation experiments, have shown that under conditions of urethane anaesthesia, the pressor response to sciatic stimulation is almost entirely nervous in origin, and that chemical or mechanical factors play an entirely subsidiary and almost negligible part.
THYREOPARATHYROIDECTOMY AND THE VASO-MOTOR REFLEXES.

There has been little work reported on the effects of extirpation of the thyroid and parathyroid glands on the vaso-motor responses and the role they normally play in these. Carlson and Jacobson (1) believe that parathyroidectomy leads, either directly by the absence of a secretion or indirectly by the presence of toxic substances in the blood, to an increased excitability of the entire central nervous system. Felsa and Kahn (2), Frankl-Hochwirt (3) and others have postulated hyperexcitability of the vaso-motor responses after parathyroidectomy.

Vincent and Thompson (5) have recently published a study in which they have undertaken to determine the relation of the thyroid and parathyroid glands to the vaso-motor responses. They conclude that in intestinal, muscular and skin stimulation two antagonistic factors are requisitioned to influence the general blood pressure; a vaso-motor reflex of a depressor nature, and the liberation of an autacoid substance having a pressor action. They report that decerebrate cats in which the thyroid and parathyroid glands are removed, show after a certain lapse of time an abnormally large pressor response upon stimulation of skin, muscle or intestine. Since they find the abnormality combated successfully by the injection of parathyroid extract and also by double adrenalectomy they conclude the increased pressor response is due to liberation of large quantities of autacoid substance and is not owing to any depression of the vaso-motor reflex.
It is furthermore concluded that the secretion of this autacoid substance is stimulated by some substance liberated by the adrenal glands and one of the functions of the parathyroid glands (and possibly the thyroid glands also) is to limit the secretion of autacoid pressor substance by control of the aforesaid stimulating agent.
METHODS.

The animals employed were the cat and dog, anaesthetised with ether or by the intravenous injection of amytal (sodium-isoamyl-ethyl-barbiturate). The latter appears to be an ideal anesthetic for a study of the vaso-motor reflexes since it gives a constant and uniform anesthesia which, as shown in controls under local, does not interfere with the normal vaso-motor reflexes. In Series A, transfusion was done under ether anesthesia. In Series B and C amytal anesthesia was used.

Transfusion was done by direct injection of the blood from the study animals and by defibrination and subsequent injection. The animals were in every case bled from the femoral artery and injections made into the femoral vein, the blood pressure being recorded in the usual manner. The quantity injected varied from 50 c.c. to 300 c.c. in controls and thyreoparathyroidectomized to normal animals.

In Series B dogs were given amytal intravenously (about 30 mgms. per minute) until a deep sleep was produced. The amount given was between 60 to 80 mgms. per kilo of body weight. The carotid blood pressure was recorded in the usual manner, a tracheal cannula being inserted to attach apparatus for recording respiration. Usually, the gastrocnemius in one leg was exposed and dissected away from its insertion to its origin. In some the sciatic was dissected out in the popliteal space and stimulated by a du Bois inductorium.
through shielded electrodes.

The same methods were used in Series C, on cats, except the rate of injection of amytal was longer. The cat was first anesthetized with ether and fastened to the board. After exposure of the femoral vein, the animal was allowed to come out from the effects of the ether and then the injection of amytal begun with a solution containing 25 mgms. per cubic centimeter, the rate of injection being about one cubic centimeter per minute. The amount varied from 60 to 90 mgms. per kilo body weight.
RESULTS.

SERIES A.

I. Transfusion.

Animals put under ether anesthesia. The blood of dogs that had been allowed to develop tetany after thyreoparathyroidectomy was injected, directly or after defibrination, into normal animals. Amounts injected varied from 50 c.c. to 300 c.c., sometimes after bleeding the same amount to prevent volume effects and at other times when small amounts were used, without bleeding. Ten experiments with three controls gave;

A slight rise noted in transfusion but controls do not show any rise beyond experimental error when the quantity transfused, the temperature of the transfused liquid and the rate of injection are all carefully controlled.

II. Transfusion.

An attempt to determine if any early pressor substance is liberated into the blood stream was made. Two normal animals were placed under ether anesthesia and in one the thyroids and parathyroids removed. At regular intervals over a period of about ten hours transfusions were made, usually by the direct method. Seven experiments and control experiments gave;

No response that could not be duplicated in the controls.
SERIES B.

In this series dogs were used. Twelve animals were run giving the following results:

|--------|-----------------|------------|-------------------------------------|

1. Response to Scratching the Skin.

Number 4 6 mm. dep. 6 in. dep. (No change of response noted during 6 hours.)

" 6* Slight depression throughout.

" 7 Slight increase of depression after 5 hours.

" 8* Depression of 6 mm. throughout.

" 9 Slight pressor About 6 mm. dep. 3\(\frac{1}{2}\) hrs. Response.

Animal died of respiratory failure soon afterwards terminating the experiment.

" 10 4 mm. dep. 8 mm. dep. 6\(\frac{1}{2}\) hrs.

" 11 Very slight dep. 10 mm. dep. 7-10 hrs.

" 12 " " 22 mm. dep. 6 hrs.

In those animals showing a change in the normal response, lost the irritability fairly rapidly after reaching the maximum found within 5 to 7 hours.

* - Those so marked were controls.


" 4 10 mm. dep. 16-20 mm. dep. 5 hrs.

" 6* Response about 8-10 mm. throughout.

" 7 Very slight 18 mm. dep. 5\(\frac{1}{2}\) hrs.

" 8* A depression of 6-8 mm. throughout an 8 hr. observation period.

" 9 6 mm. pressor 8 mm. dep. 4 hrs.

" 10 6-8 mm. dep. 10 mm. pressor 6\(\frac{3}{4}\) hrs.
10.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; 11</td>
<td>4 mm. pressor</td>
<td>16 mm. dep.</td>
<td>5 hrs.</td>
</tr>
<tr>
<td>&quot; 12</td>
<td>none. (?)</td>
<td>6-8 mm. dep.</td>
<td>6 hrs.</td>
</tr>
<tr>
<td>&quot; 13</td>
<td>Depression of about 6 mm. throughout.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Massage of the Exposed Gastrocnemius.

| " 12             | Dep. 1-2 mm.    | Dep. 18-20 mm. | 3 hrs. |
| " 13             | Dep. 6-8 mm.    | Dep. 12-14 mm. | 3 hrs. |

4. Responses to Massage of the Abdominal Viscera.

| " 6*             | Tendency to a rise followed by a fall of 10 to 12 mm. Hg. |
| " 7              | Dep. 6 mm.      | Dep. 12 mm.    | 5 hrs. |
| " 8 *            | Slight depression throughout. |
| " 9              | Pressor 10 mm.  | Dep. 18 mm.    | 3½ hrs. |
| " 10             | Slight dep.(?)  | Pressor 20 mm. | 6½ hrs. |
| " 11             | Dep. 6 mm.      | Dep. 30-35 mm. | 8 hrs. |
| " 12             | Slight dep.     | Dep. 16-18 mm. | 4 hrs. |

* Controls.

Clamping the femoral and popliteal veins in no way altered the onset or extent of response to massage of the gastrocnemius muscle.

5. Stimulation of a Mixed Nerve Trunk.

| " 11             | (Used femoral nerve in this experiment.) Dep. 8-10 mm. | Dep. 20 mm. | 3 hrs. |
| " 12             | Slight dep.    | Dep. 10 mm.    | 4½ hrs. |

Throughout this series there has been a constant tendency toward an increase in the depressor response coming on usually 5-7 hours after removal of the thrycid and parathyroid glands. Number ten showed a tendency toward a positive or pressor effect to most responses. Repeated
stimulation of the tissues in this case in no way diminished the response. Clamping the popliteal and femoral veins did not alter the response from massage of the gastrocnemius muscle. There was nothing to denote a hormone or autacoid response in this or other parts of the experiments. If this response is nervous, cutting the nervous supply to a part would eliminate any vaso-motor response. The gastrocnemius in two dogs was denervated with a complete abolition of all response on stimulation. Massage of the exposed gastrocnemius brought on a response of maximal value earlier than other forms of stimulation except the nerve trunk. This probably offers a more effective means of stimulation so that more depressor afferents are stimulated at one time. This influx of a greater number of stimuli at the depressor centre results in a maximum response sooner but the response does not show a diminution until the same is seen for all other kinds of stimuli.

SERIES C.

The same stimuli were applied to a series of cats under amytal anesthesia. The results are in general the same. They are as follows:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Responses to Scratching the Skin.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number 3</td>
<td>Dep. 3 mm.</td>
<td>Dep. 12-14 mm.</td>
<td>3 hrs.</td>
</tr>
<tr>
<td>&quot; 6</td>
<td>Dep. 5-6 mm.</td>
<td>Dep. 18-20 mm.</td>
<td>4½ hrs.</td>
</tr>
<tr>
<td>&quot; 7</td>
<td>Dep. 5 mm.</td>
<td>Dep. 12-14 mm.</td>
<td>3 hrs.</td>
</tr>
<tr>
<td>&quot; 8</td>
<td>Dep. slight only</td>
<td>Dep. 18 mm.</td>
<td>4 hrs.</td>
</tr>
</tbody>
</table>

Number 9 (control) No change in responses during period of observation.

2. Massage of the Exposed Gastrocnemius.

"  3  Dep. 10 mm. A series of Traube waves initiated on 3½ hrs.

"  4  --- Pressor 22 mm. 5 hrs.

"  8  Dep. 6-8 mm. Slight fall followed a rise of 18-20 mm. 3-4 hrs.


"  3 Pressor Pressor 2 hrs.

4 mm. 6 mm.
Condition of animal prevented further readings.

"  8 Press. 8 mm. A fall followed by 4 hrs.

a rise of about 22 mm. from depressor level.

4. In one cat the gut (exposed) was kneaded by hand. There was an increase in the height or rise and the duration of the rise after the thyroids and parathyroids had been removed.

Normal Pressor 10 mm. After removal. Pres. 22 mm. Time
Duration 10 sec. Duration 18-20 sec. 6½ hrs.

The veins (popliteal and femoral) were clamped and readings made after stimulation of the exposed gastrocnemius. The responses were in no way modified by this. In cats we see both excitation of pressors and depressors, the pressor predominating in those in which the exposed gastrocnemius or popliteal nerve was stimulated. In other respects the results coincide with those obtained in dogs.
Following is a typical experiment in Series C.

Cat--# 8.

Female-wt. 3 kilos. 7/15/29

10:30 a.m. Animal put under ether anesthesia and femoral vein bared in the right leg.

11.00 a.m. Began amytal anesthesia. Animal showed respiratory arrest during the administration of amytal and was given artificial respiration for a few minutes after which respiration was regular and deep.

11:30 a.m. Injection of amytal intravenously completed. Amount injected was 150 mgm. or 50 mgm./kilo. Dissection made and carotid cannula inserted.

12:00 Noon 2 cc. 0.1% atropine injected.

1' - Sharp fall of 8 mm. followed by a sharp rise of 8 mm. which returned to normal in about 9 sec.

1 - Same type as above but slightly less marked.

2 - Hardly and detectable change. Maybe a slight depression.

Sc. (induct. at 11cm. Duration 10 seconds.)

Sharp rise of 12 mm. in height lasting about 25 sec. before again reaching normal level.

With a very weak current there was a slight rise with a tendency to an after fall.

12:50 p.m. Thyreoparathyroidectomy begun.

1:00 p.m. Thyreoparathyroidectomy completed.

4:00 p.m. Thirty mgms. amytal (10 mgms./kilo) injected.

1' - Sharp fall of 10 mm. followed by a sharp rise of 16 mm. which lasted about 20 sec.

1 - Sharp fall followed by a slight rise.

2 - A sharp fall of about 18 mm. lasting about 2 sec.

Sc. - Very weak stimulus now gives a rise of 16-18 mm. followed by an after fall of a few mm. which only very slowly returns to normal. Duration of depression about 25 seconds.

Stimulation with inductorium at 11 cm. gave
about the same response. Apparently the vaso-motor centre has undergone a change of irritability. There was less tendency to an after fall, being in this case very slight if at all.

5:30 p.m. 1'-Fall with a tendency to an after rise.
2 -Marked fall.

10:00 p.m. Blood pressure low. Responses hard to determine.
1' -A fall of 8-10 mm. which lasted about 3 sec.
2 -A fall of about 12 mm. and of 2-3 seconds duration.

Gastrocnemius exposed in the other leg. Gave similar results upon stimulation.

Sc. Inductorium at 6 cm. gave a rise of 14 mm. after which there was a fall reaching a level 10 mm. below the normal level and returning to normal in about 40 seconds followed by a mend above normal of about 4 or 5 mm.

10:30 p.m. Animal killed.
Since that ergotamine blocks all pressor action, it was thought that the injection of this would offer a means of checking the presence of an autacoid which would stimulate the smooth muscle of the blood vessels. After producing an ergotamine block the responses obtained upon stimulation of the exposed gastrocnemius were depressor, as would be expected. After allowing three hours to elapse, the muscle was again stimulated. Now the normal pressor response was obtained. If this autacoid, like pituitrin, acted upon the smooth muscle there should not have been a complete block as shown by this experiment.

Again, in animal that gave a decided pressor response five hours after removal of the thyroids and parathyroids, repeated stimulation was tried. Five successive stimuli following closely upon each other gave in each case the same response, showing neither a diminution of response or any delay in onset.
Fig. 7. Massage of the Gastrocnemius gives a pressor response. At L five successive stimuli were given. Thyro-parathyroidectomy five hours previously. (Cat).

Fig. 8. At A the Gastrocnemius was massaged with the veins open. At B the same was repeated with the femoral and popliteal veins clamped. Five hours after removal thyroids and parotis.

Fig. 9. At L' is the normal response to massage of the Gastrocnemius. The muscle was then denervated and the massage repeated, with no response. The central end of the cut popliteal was stimulated with the usual response.
DISCUSSION OF RESULTS.

No pressor substance could be detected in the general circulation of animals in tetania thyreoparathyropriva, using the transfusion method. There is no indication of a pressor or depressor substance liberated into the general circulation that can thus be detected during the first ten hours after the removal of the thyroid and parathyroids.

Our work has shown that there is an increase in the irritability of the vaso-motor centre giving an augmented pressor or depressor response depending upon the part to which the stimulus was applied and to a small extent upon variations in the animal. There was no sign of either the first or second crisis reported by Vincent or Thompson (5), the animals usually maintaining a good condition throughout the experiment. There was a gradual increase of irritability up to a maximum after which the responses were diminished, tending toward the normal again. We are not able to confirm the results they obtained upon clamping the venous return from the muscle stimulated as we find that this in no way affected the results normally elicited and that the denervation completely eliminated a response. The injection of ergotamine blocks all pressor responses normally obtained so that now the responses usually elicited are depressor in nature. After allowing a sufficient time for recovery from the effects of the drug the normal pressor response may be obtained. (massage of the gastrocnemius.)
The increased irritability is not to the pressor side only, but applies to the depressors as well. In some instances a depressor response was obtained followed by the pressor reaction. The reaction which predominates appears to depend usually upon the number of afferents of each kind which supply the part stimulated. Stimulation of the exposed gut was found to be predominatingly pressor while scratching the skin was predominatingly depressor. Vincent and Thompson (5) have noted that the injection of calcium in no way effected the responses obtained but the injection of parathormone (Collip) prevented the onset of the pressor rise in its entirety. It is probable that the absence of this secretion results in early changes which are easily demonstrated in the change of irritability of the vaso-motor centre. It is probable that there are other reflexes which also would show the increased irritability.

Our conclusion is that there are early changes which occur after removal of the parathyroid glands (and possibly the thyroids also) but that the responses obtained are not due to a pressor autacoid substance liberated from the tissues stimulated as suggested by Vincent and Thompson.
S U M M A R Y.

1. There is no pressor or depressor substance in the general circulation of dogs in tetanic thyreoparathyropriva which can be demonstrated by transfusion into normal animals.

2. In cats and dogs under amytal anaesthesia, the removal of the thyroid and parathyroid glands, after a certain lapse of time, results in an augmentation of the vaso-motor responses.

3. We find no evidence in these experiments which would substantiate the claims of Vincent and Thompson to a pressor autacoid substance liberated upon stimulation of the tissues in abnormally large amounts after removal of the thyroids and parathyroids. Such evidence as here presented indicates that this is not the case.

This problem was suggested by Dr. O. O. Stoland whose helpful advice and unfailing patience during the progress of the work were a constant source of encouragement.

I also wish to wish to acknowledge the assistance given by Mr. Robert Woodbury and to extend my grateful appreciation to all who have so willingly cooperated in this work.
Bibliography.

5. Vincent and Thompson; Ibid., 1928, lxvi, 449.
10. McDowall, R.J.S.; Ibid., 1924, lix, 41.