Chapter - 5

PITUITARY FUNCTION STUDIES
Introduction:

The human adenohypophysis secretes six distinct hormones which have definite functional significance. They are: (1) Corticotropin (ACTH), (2) Melanocyte stimulating hormone (MSH), (3) Thyrotropin (TSH), (4) Gonadotropic hormones (FSH and LH), (5) Prolactin and (6) Growth hormone (GH).

There are several methods of investigating pituitary functions. The present study is restricted to the functions which are related to thyroid and adrenal cortex.

The secretion of $T_4$ and $T_3$ is regulated by TSH, which is in turn controlled by hypothalamic TSH-releasing hormone (TRH) on the one hand and by the concentration of circulating $T_3$ and $T_4$ on the other. The evidence for the hypothalamic control of TSH secretion includes identification of TSH in the hypothalamus, portal blood and the increase in TSH secretion by the use of synthetic TRH. In fact, the availability of synthetic TRH for clinical investigation has helped considerably to investigate the interaction between TRH and thyroid hormones.
In order to distinguish between primary and secondary hypothyroidism, TSH assay is of fundamental importance. Evaluation of pituitary TSH response to TRH is also important because basal serum TSH levels often fail to provide adequate differentiation between normal subjects and those with a decreased TSH secretory capacity. There are many reports of TRH testing in patients with disorders of the hypothalamus or pituitary. These suggest that TRH testing is of particular value in the distinction between a hypothalamic lesion and one involving the pituitary.

Prolactin is unique amongst the anterior pituitary hormones in that its secretion is under tonic inhibitory control by the hypothalamus. There is also a short loop feedback inhibition. Dopamine is known to be a prolactin release inhibiting factor while serotonin is the prolactin releasing factor. After the isolation and use of TRH it was found that it released TSH as well as prolactin.

ACTH is a single chain peptide comprising of 39 amino acids. A portion of the chain has a common amino acid sequence to that of alpha MSH. There is also a common amino acid sequence between ACTH and beta MSH.
ACTH secretion is controlled by corticotropin releasing factor from the hypothalamus, circulating cortisol levels and different types of stresses.

With the introduction of radioimmunoassay, the estimation of circulating ACTH has become possible.

Beta and gamma lipotropin (LPH), alpha and beta MSH and ACTH contain a common heptapeptide core. All of these can cause dispersion of melanophores in frog skin, stimulate adrenal steroidogenesis and mobilize lipids from adipose tissues. Recent studies suggest that human beta MSH is an extraction artefact from beta LPH. Similarly alpha MSH is not found normally in human except during foetal life and pregnancy.

Materials:

As described in the chapter on thyroid function studies.
Methods:

TSH was estimated according to the method of Utiger\textsuperscript{10} with certain modifications. Prolactin was estimated according to the method of Jacobs\textsuperscript{11} with certain modifications. ACTH was estimated by ACTH immunoassay kit (IM.66) obtained from the Radiochemical Centre, Amersham, England.

Estimation of TSH -

Reagents -

1. 0.1 M Phosphate buffer.
   - \( \text{Na}_2\text{HPO}_4, 12\text{H}_2\text{O} \) - 71.64 gms/2 litres water
   - \( \text{NaH}_2\text{PO}_4, 2\text{H}_2\text{O} \) - 7.8 gms/500 ml water
   - 860 ml A + 140 ml B (PH 7.5) for 0.05 M Phosphate buffer 1:1 dilution was done.

2. 0.5 M Phosphate buffer.
   - 16.1 gms \( \text{Na}_2\text{HPO}_4, 12\text{H}_2\text{O} \) and 0.78 gm \( \text{NaHPO}_4, 2\text{H}_2\text{O} \) dissolved in water and the volume made upto 100 ml.

3. 0.1 M Barbitone buffer.
   - 35.9 gms sodium barbitone dissolved in about 1.5 litre water, pH adjusted to 8.6 with HCl and volume made upto 2 litres.

4. 0.01 M Phosphate-saline buffer.
   - 3.66 gms \( \text{Na}_2\text{HPO}_4, 2\text{H}_2\text{O} \) and 17.52 gms NaCl dissolved in about 1.5 litre water. 200 mg merthiolate and 1 gm bovine serum albumin were then dissolved. The volume was made upto 2 litres (pH 7.8).
Methods:

TSH was estimated according to the method of Utiger with certain modifications. Prolactin was estimated according to the method of Jacobs with certain modifications. ACTH was estimated by ACTH immunoassay kit (IM.66) obtained from the Radiochemical Centre, Amersham, England.

Estimation of TSH -

Reagents -

1. 0.1 M Phosphate buffer.
   A. Na₂HPO₄, 12H₂O - 71.64 gms/2 litres water
   B. NaH₂PO₄, 2H₂O - 7.8 gms/500 ml water
   860 ml A + 140 ml B (pH 7.5)
   for 0.05 M Phosphate buffer 1:1 dilution was done.

2. 0.5 M Phosphate buffer.
   16.1 gms Na₂HPO₄, 12H₂O and 0.78 gm NaH₂PO₄, 2H₂O dissolved in water and the volume made upto 100 ml.

3. 0.1 M Barbitone buffer.
   35.9 gms sodium barbitone dissolved in about 1.5 litre water, pH adjusted to 8.6 with HCl and volume made upto 2 litres.

4. 0.01 M Phosphate-saline buffer.
   3.66 gms Na₂HPO₄, 2H₂O and 17.52 gms NaCl dissolved in about 1.5 litre water. 200 mg merthiolate and 1 gm bovine serum albumin were then dissolved. The volume was made upto 2 litres (pH 7.8).
5. **0.01 M Phosphate - saline buffer + EDTA.**

The same buffer as above, but made 0.05 M with respect to EDTA (37.24 gms/2 lit).

6. **Standard TSH.** 0.1 ml aliquot contained 2.5 mU of standard TSH (kindly donated by Medical Research Council, UK) which was diluted to 2.5 ml with phosphate-saline buffer. This contained 1000 uU/ml. This was further diluted 1:10. Working standard: 100, 50, 25, 12.5, 6.25, 3.1, 1.55, 0.77, 0.39 uU/ml.

7. **Anti-TSH.** Obtained by courtesy of Dr. K. Mashiter, Royal Post Graduate Medical School, London. Stored at -20°C in 1 ml aliquots at a dilution of 1:1000. Guinea pig serum, stored at -20°C in aliquots of 2 ml (diluted to 1:10) was diluted to 1:75 with phosphate-saline buffer. 1 ml anti-TSH was added to 49 ml carrier guinea pig serum in phosphate saline buffer. 1 ml anti-TSH was added to 49 ml carrier guinea pig serum in phosphate saline (diluted to 1:50000).

8. **Second antibody:** Rabbit and guinea pig serum, stored at -20°C in 2 ml aliquots, diluted appropriately in phosphate saline buffer (by courtesy of Dr. K. Mashiter).

9. **125I - TSH preparation:**

Reagents - 0.5 M phosphate buffer, TSH (2.5 μg/5 μl) for iodination, Chloramine T (5 mg/10 ml 0.05 M phosphate buffer), sodium metabisulphite (30 mg/10 ml 0.05 M phosphate buffer), 100 mg bovine serum albumin/1 ml barbitone buffer, sephadex G-75 (pre swollen at room temperature in 0.1 M barbitone buffer), Na125I (Amersham, UK, 1 MS 30).
STANDARD CURVE FOR TSH

TSH (μU/l)

BOUND %

0.39 0.77 1.55 3.1 6.25 12.5 25.0 50.0 100
Method - The iodination was performed in TSH vial. The following reagents were added in rapid succession.

1. 20 ul 0.5 M phosphate buffer.
2. 1-1.5 mCi Na$^{125}$I.
3. 20 ug in 10 ul chloramine-T (10-12 seconds exposure).
4. 60 ug in 20 ul sodium metabisulphite.

About 0.5 ml barbitone buffer was used to transfer the mixture from the vial to the sephadex G-75 column. The column was eluted with barbitone. With the appearance of radioactivity, successive fractions (10 drops each were collected by a fraction collector. The combined fractions was diluted to about 12000 counts/100 seconds/0.1 ml.

Procedure -

Day 1.

A. I. (a) In a series of tubes, 0.2 ml serum samples were taken.
   (b) 0.2 ml phosphate-saline-EDTA buffer added.

II. (a) In a series of tubes, 0.2 ml each of the different standards were taken.
   (b) 0.2 ml $T_3$ - suppressed serum-TSH free, containing 0.05 M EDTA were added.

B. Anti-TSH, antiserum, 0.1 ml, was added to each tube except controls 1, 2.
Controls -

1. Six tubes containing no hormone but 0.2 ml buffer and 0.2 ml $T_3$ suppressed serum.

2. Three tubes with no anti-TSH but with 0.1 ml guinea pig serum diluted 1:75.

3. Two tubes anti-TSH, 0.1 ml, diluted 1:2000.

4. Aliquots of pooled serum, three levels, low, normal, high pools.

C. 0.1 ml $^{125}$I-TSH, suitably diluted, was added to each tube.

D. 0.1 ml $^{125}$I-TSH (Total radioactivity) alone in ten tubes.

Day 2.

0.1 ml second antibody, rabbit anti guinea pig precipitating serum, suitably diluted in phosphate-saline buffer was added to all tubes except total radioactivity tubes.

Day 3.

Tubes were centrifuged for 20 mins. at 2000 r.p.m. at 4°C. Supernatant was discarded and the precipitate was counted for 100 secs.

Rapid checks.

3 excess antibody tubes and 3 no hormone tubes were taken. They were handled in the same manner except that the
incubation times were different. Approximately 50% binding is expected in the no hormone tubes and 70% in the excess antibody tubes.

The values were calculated from the standard curve drawn on three-cycle semi-log graph paper.

**Estimation of prolactin:**

**Materials and reagents -**

1. **Standard.** Kindly donated by Medical Research Council, UK. This was made up in assay buffer at 100 ug/l and aliquots of 0.5 ml were kept at -20°C.

2. **Antiserum.** Kindly donated by Dr. K. Mashiter, Royal Post Graduate Medical School, London. Kept at -20°C at an initial dilution of 1:12000.

3. **Second antibody.** Donkey antirabbit serum, obtained from Welcome Reagents Ltd. Stored at 4°C. Diluted 1: 20 in assay buffer for use.

4. **Assay buffer.** 0.1 M Barbitone, pH 8.6, containing 2.5% bovine serum albumin (Sigma fraction V powder).

   Stock barbitone: 35.9 gms sodium barbitone \[2 \text{ litres}, \ 2.15 \text{ ml conc. HCl} \]

   12.5 gms of BSA dissolved in 500 ml barbitone for the assay (freshly prepared).
Method -

20 ul 0.5 M phosphate buffer was added to prolactin in annacup. 1-1.5 mCi Na$^{125}$I was then added. 10 ul chloramine T was added, followed by 20 ul sodium metabisulphite 20 secs. later. Mixed by bubbling. Mixture transferred to the G50 column with barbitone buffer. Eluted with barbitone buffer. With the appearance of radioactivity fractions (10 drops each) were collected in an automatic fraction collector. The fractions were pooled for $^{125}$I - prolactin and stored at 4°C.

Some of the $^{125}$I prolactin was applied to G100 column. Eluted with barbitone buffer. 20 x 20 drop fractions were collected on fraction collector. 5 ul aliquots of each fraction were counted in a gamma counter. Elution pattern was drawn. Second peak was used in the assay. Usable fraction was diluted in assay buffer to give approx. 10000 counts/100 secs.

Method of assay -

Day 1.

a) Standard prolactin solutions were prepared by diluting the stock over range 100-0.39 ug/l.
IODINATION OF PROLACTIN

NO OF TUBES

COUNTS PER 10 SECNS PER 10^1 x 10000

125 I prolactin

Na 125 I
PURIFICATION OF $^{125}$PROLACTIN

COUNTS PER 10 SECS PER 10μL x 10000

NO OF TUBES

DAMAGED HORMONE

125 Prolactin

NO OF TUBES
b) Tubes 1-6, 0.5 ml buffer + 0.05 ml buffer (0 standard)

7-33. Standard curve in triplicate. 0.5 ml buffer + 0.05 ml standard (each).

34-36. 0.5 ml buffer + 0.05 ml buffer (no antibody).

37-38. Nothing (excess antibody).

39, 40, 41, 42 etc. 0.5 ml buffer + 0.05 ml serum.

Three pools (low, medium and high) were incorporated.

c) 0.1 ml antiserum was added to each tube except 3 no antibody tubes and 2 excess antibody tubes. 0.6 ml antiserum was added to the excess antibody tubes.

d) To each tube 0.1 ml $^{125}$I-prolactin was added. 20 tubes were taken containing 0.1 ml $^{125}$I-prolactin only (Total counts). All the tubes were incubated at 4°C.

Day 4.

a) 0.1 ml nonimmune rabbit serum (1:100) was added to each tube except total count tubes.

b) 0.1 ml second antibody was added to each tube except total count tubes.

All the tubes were then incubated at 4°C.

Day 5.

a) The tubes were centrifuged at 4°C at 2000 r.p.m. for 15 mins. Supernatant was discarded, except for the total count tubes.

b) Counts were taken in an automatic gamma counter (Picker Auto Gamma Counter).
Standard curve was drawn on three-cycle semi-log graph paper plotting % bound against concentration of prolactin. The values of unknown samples were read from the curve.

Estimation of ACTH -

Blood samples were collected in heparinised syringes and centrifuged immediately to separate the plasma. Heparin was used at a concentration of 10 units per ml of blood. The blood was centrifuged for 15 mins. at 1000 g at 4°C. The supernatant was recentrifuged at 6000 g at 4°C for 10 mins. The 6000 g plasma was decanted and stored at -70°C until analysed.

Method. One of the vials of freeze-dried human serum (ACTH-free) was reconstituted with 12 ml distilled water, injected through the rubber cap, using a syringe. Mixing was done on rotary mixer for 20 mins to give a homogeneous solution and two 5 ml aliquots were dispensed into two of the extraction tubes. Solution A was added to the freeze-dried buffer component to reconstitute the buffer solution. The standard ACTH was reconstituted with the volume of buffer solution specified on the vial and 400 ul of the resulting solution was added to one of the 5 ml aliquots of ACTH-free serum. The extract of this solution was used to construct
the standard curve. The extract of the other 5 ml aliquot of
ACTH-free serum was used to provide the zeropoint of the standard
curve.

Extraction

Plasma was taken into the extraction tube containing
adsorbent glass. The tube-cap was replaced and mixed on a rotary
mixer for 30 mins. It was then centrifuged at 1000 g for 2 mins and
the supernatant was discarded. The glass was resuspended in 2 ml
distilled water and vortex mixed. It was again centrifuged and
the supernatant discarded. The glass was resuspended in 2 ml
NHCl by vortex mixing, centrifuged and supernatant discarded.
ACTH was desorbed from the glass by mixing on the rotary mixer for
20 mins with 1 ml 50% (v/v) acetone in water, centrifuged and the
supernatant was transferred to an evaporation tube without dis-
turbing the glass particles. The glass was washed by vortex
mixing with a further 0.5 ml aqueous acetone, centrifuged and the
supernatant was added to the first aliquot. The solution was
dried by immersing in a water bath at 55°C and passing a gentle
stream of nitrogen on to the surface of the liquid. 700 ul
buffer solution was added to the residues of the zero serum and
the ACTH standard serum. 200 ul buffer solution was added to each
of the residues from the unknowns. The tubes were vortex mixed and centrifuged at 1000 g for 2 mins. Supernatants were assayed for ACTH as follows.

Assay procedure

The dilutions of the ACTH-standard serum extract were made for the standard curve. Four doubling dilutions were prepared as follows: 300 ul aliquots of the buffer solution were dispensed into each of four evaporation tubes. 300 ul of the neat extract solution was added to the first tube, mixed thoroughly and 300 ul of this solution was transferred to the second tube. The operation was repeated with successive tubes. 100 ul of the neat extract solution and 100 ul aliquots of the doubling dilutions in duplicate were dispensed into the assay tubes (tubes 1-10). 100 ul aliquots of the ACTH-free serum extract solution in duplicate were dispensed for zero hormone blanks (tubes 11-12). Unknown plasma extract solutions were measured at 2 concentrations. 2 tubes were set up for each unknown. 100 ul of the extract solution was added to one assay tube and to the second assay tube 50 ul with 50 ul buffer solution was added. The antiserum was reconstituted with 6 ml buffer solution. 100 ul was added to all the tubes. Each tube was vortex-mixed and curved with parafilm to prevent evaporation. The tubes were incubated at 4°C for 20 hrs
1 part of labelled ACTH solution was added to 7 parts of buffer solution and mixed thoroughly. 100 ul was added to all the tubes and vortex mixed. The tubes were covered with parafilm and incubated at 4°C for 6 hours. Several hours before use, freeze-dried charcoal adsorbent was reconstituted in 25 ml ice-cold distilled water, stirred for 10 mins on a magnetic stirrer and stored at 4°C until required.

Separation of free and bound antigen. After the second incubation, 0.1 ml aliquots of the charcoal suspension (continuously stirring on a magnetic stirrer) was added into each assay tube. The tubes were vortex mixed and centrifuged immediately at 100 g for 5 mins. The supernatant was discarded.

Measurement of radioactivity counts - The charcoal residues were counted for 300 secs each in a gamma counter.

Calculation - The standard curve gave the concentration range 800, 400, 200, 100, 50 pg ACTH/ml plasma. A curve was plotted with counts vs. pg ACTH/ml plasma. Concentration of ACTH (pg/ml) was read off the curve against the number of counts.

\[
\text{pg ACTH/ml plasma} = C \times \frac{5}{V} \times \frac{v}{700}
\]

- \(C\) = interpolated concentration from standard curve
- \(V\) = Volume of unknown plasma sample (ml)
- \(v\) = volume of extract solution of unknown (ml).
Results:

Table 1. Levels of TSH, prolactin and ACTH in control subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>μu/l</td>
<td>2.2 - 4.0</td>
<td>3.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>6.0 - 20.0</td>
<td>13.2</td>
<td>0.34</td>
</tr>
<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>30.0 - 76.0</td>
<td>52.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Table 2. Levels of TSH, prolactin and ACTH in vitiligo

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>μu/l</td>
<td>1.0 - 6.0</td>
<td>3.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>7.4 - 23.2</td>
<td>15.2</td>
<td>0.41</td>
</tr>
<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>14.0 - 42.6</td>
<td>30.5*</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* P < 0.001

Levels of TSH, prolactin and ACTH in control subjects and vitiligo patients are shown in Tables 1 and 2 respectively. On statistical analysis it was found that only ACTH was significantly lower in vitiligo patients whereas TSH and prolactin showed no change.
Table 3. Levels of TSH, prolactin and ACTH in 50 male patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
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<tbody>
<tr>
<td>TSH</td>
<td>mu/l</td>
<td>1.4 - 6.0</td>
<td>3.9</td>
<td>0.22</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>7.4 - 21.8</td>
<td>14.7</td>
<td>0.58</td>
</tr>
<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>14.0 - 41.2</td>
<td>27.8</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Table 4. Levels of TSH, prolactin and ACTH in 50 female patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>mu/l</td>
<td>1.0 - 5.5</td>
<td>3.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>9.2 - 23.2</td>
<td>15.7</td>
<td>0.61</td>
</tr>
<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>16.0 - 42.6</td>
<td>33.2</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Table 3 and 4 show the levels of TSH, prolactin and ACTH in male and female vitiligo patients respectively. On statistical analysis no significant differences could be found between the two groups in all the three parameters.

The patients were divided into four groups according to the onset of the disease as described in the chapter on thyroid function studies. The results are summarized in Tables 5-8.
Table 5. Levels of TSE, prolactin and ACTH in group 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>μ/1</td>
<td>1.6 - 6.0</td>
<td>3.8</td>
<td>0.32</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>7.4 - 20.0</td>
<td>13.9</td>
<td>0.78</td>
</tr>
<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>16.0 - 41.0</td>
<td>28.7</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Table 6. Levels of TSH, prolactin and ACTH in group 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>μ/1</td>
<td>1.3 - 5.8</td>
<td>3.6</td>
<td>0.30</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>8.0 - 22.6</td>
<td>15.4</td>
<td>0.81</td>
</tr>
<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>15.0 - 39.0</td>
<td>29.6</td>
<td>1.62</td>
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</table>

Table 7. Levels of TSH, prolactin and ACTH in group 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>μ/1</td>
<td>1.2 - 5.9</td>
<td>3.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>7.8 - 23.0</td>
<td>14.9</td>
<td>0.79</td>
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<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>14.0 - 42.0</td>
<td>30.6</td>
<td>1.58</td>
</tr>
</tbody>
</table>

Table 8. Levels of TSH, prolactin and ACTH in group 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>μ/1</td>
<td>1.0 - 5.7</td>
<td>3.9</td>
<td>0.40</td>
</tr>
<tr>
<td>Prolactin</td>
<td>ng/ml</td>
<td>8.5 - 23.2</td>
<td>17.4</td>
<td>1.02</td>
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<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>19.0 - 42.6</td>
<td>34.3</td>
<td>1.92</td>
</tr>
</tbody>
</table>
Statistical analysis were carried out between groups 1 and 2, groups 1 and 3 and groups 1 and 4. No significant differences were observed in groups 2, 3 and 4 in comparison to group 1.

Since the availability of synthetic TRH, it has been used to evaluate the pituitary TSH response as a test of pituitary-hypothalamic function. When administered intravenously TRH causes a rapid increase in blood TSH which is maximum at 20 mins. In some cases a delayed response is observed at 60 mins.

From 50 male vitiligo patients, 10 cases were selected by taking every 5th patient and similarly from 50 female vitiligo patients, 10 were selected by taking every 5th patient. 10 male and 10 female normal, healthy subjects were selected in the same age groups respectively. Blood samples were obtained by venepuncture at 8 a.m. after overnight fasting. 200 ug of synthetic TRH (Armour Pharmaceuticals, USA) in 2 ml solution was administered intravenously to each subject. Blood samples were obtained by venepuncture at 20 mins and 60 mins after the administration of TRH. The separated sera were preserved at -20°C till the assay was done. Serum TSH and prolactin were estimated according to the methods described earlier.
Table 9. TSH response in male patients.

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Control</th>
<th>Vitiligo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>0</td>
<td>2.2-3.8</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>20</td>
<td>6.0-16.4</td>
<td>11.4±0.8</td>
</tr>
<tr>
<td>60</td>
<td>4.2-10.4</td>
<td>7.2±0.5</td>
</tr>
</tbody>
</table>

* P < 0.001

Table 10. TSH response in female patients.

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Control</th>
<th>Vitiligo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>0</td>
<td>2.0-3.7</td>
<td>3.0±0.18</td>
</tr>
<tr>
<td>20</td>
<td>7.2-21.6</td>
<td>14.8±1.24</td>
</tr>
<tr>
<td>60</td>
<td>4.8-16.1</td>
<td>11.2±0.91</td>
</tr>
</tbody>
</table>

* P < 0.001

Table 11. Prolactin response in male patients.

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Control</th>
<th>Vitiligo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>0</td>
<td>6.0-18.2</td>
<td>12.4±1.1</td>
</tr>
<tr>
<td>20</td>
<td>22.4-31.6</td>
<td>26.2±0.83</td>
</tr>
<tr>
<td>60</td>
<td>10.6-21.8</td>
<td>16.5±1.2</td>
</tr>
</tbody>
</table>

* P < 0.001
Table 12. Prolactin response in female patients.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Vitiligo</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SEM</td>
<td>Range</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>0</td>
<td>9.0-20.0</td>
<td>14.1 ± 1.2</td>
<td>10.8-23.2</td>
<td>16.6 ± 1.1</td>
</tr>
<tr>
<td>20</td>
<td>41.4-58.6</td>
<td>49.6 ± 1.5</td>
<td>60.3-128.6</td>
<td>91.2 ± 6.7 *</td>
</tr>
<tr>
<td>60</td>
<td>15.5-28.6</td>
<td>21.8 ± 1.3</td>
<td>68.0-148.8</td>
<td>106.4 ± 7.4 *</td>
</tr>
</tbody>
</table>

* P < 0.001

From Tables 9 and 10 it is evident that TSH levels are significantly higher in vitiligo patients, both in males and females, at 20 mins and 60 mins as compared to controls. It is also observed that in vitiligo patients the TSH levels at 60 mins are somewhat higher than those at 20 mins. This is in contrast to the normal pattern where the peak is reached at 20 mins and the level gradually decreases subsequently.

From Tables 11 and 12 it may be seen that prolactin levels are significantly higher in both male and female vitiligo patients at 20 mins and 60 mins as compared to controls. Prolactin response was higher at 60 mins than at 20 mins. The pattern is similar to that of TSH.
Discussion:

From various experimental studies it has been proved that melanogenesis is influenced by certain polypeptide hormones of the pituitary. Highly purified ACTH produces melanogenesis in goldfish. In a study of xanthic goldfish, Chavin\textsuperscript{12} found that implantation of carp or goldfish pituitaries or the injection of mammalian ACTH would induce melanin synthesis. Egami et al\textsuperscript{13} also concluded that stimulation of ACTH secretion in the goldfish induces melanogenesis. That ACTH is directly responsible for melanogenesis was supported by experiments in which extracts of adrenal cortical tissues failed to produce melanin formation. Later on it was shown that ACTH and MSH induce pigment cell formation in goldfish tailfin cultures\textsuperscript{14}.

The dramatic colour changes produced in tadpoles and frogs following hypophysectomy or after injection of pituitary extracts were observed long ago\textsuperscript{15}. Experimental studies on frogs also show that MSH and ACTH have darkening effects. Bitensky and Burstein\textsuperscript{16} found that MSH and ACTH increase adenylyl cyclase activity in vitro in fish and mouse melanomas. Abe et al\textsuperscript{17} found that in skin from Rana pipiens MSH and ACTH increased cyclic AMP levels with the same relative potencies at which they stimulated darkening.
The roles of MSH and ACTH on melanogenesis were also established from various clinical studies. Lerner et al.\textsuperscript{18} reported that intramuscular injection of crude extract of hog pituitary into human produced darkening of skin and nevi. Later on Lerner and McGuire\textsuperscript{19} showed that daily injection of 8 mg of alpha or beta MSH or 20 mg of ACTH to men brought about pronounced darkening in 7 to 12 days. Snell\textsuperscript{20} observed initiation of melanogenesis in the skin of mammals on administration of alpha MSH. Injection of ACTH into a negro woman caused hyperpigmentation of skin and buccal mucous membrane\textsuperscript{21}. In a recent study\textsuperscript{22}, 6 out of 20 patients with extensive vitiligo treated with ACTH experienced repigmentation at the end of 15 weeks. However depigmentation recurred when the treatment was stopped.

There are evidences that the pituitaries of several mammalian species secrete a number of peptides which are related structurally to ACTH. These include big ACTH\textsuperscript{23}, ACTH, alpha MSH, beta MSH, corticotrophin-like intermediate lobe peptide (CLIP)\textsuperscript{24}, beta lipotrophin (LPH)\textsuperscript{25} and gamma lipotrophin (LPH)\textsuperscript{26}. Elucidation of the exact role of these peptides which share a common heptapeptide sequence, is the subject of intensive investigation.

The development of specific radioimmunoassay for alpha MSH led to the discovery that this peptide could not be detected
either in the pituitary or circulation of the human although it is detected during fetal life and pregnancy. The enzymes necessary to cleave ACTH for the formations of alpha MSH and CLIP are located only in the pars intermedia which explains their absence in the human who lacks a pars intermedia. The finding of beta MSH in the human pituitary and circulation appears to support the view that it is secreted by the pituitary gland. This view is also supported by the finding that circulating levels of this peptide parallels those of ACTH in the cause of hyperpigmentation in Addison's disease and Nelson's Syndrome. Recent studies indicate that beta MSH is an extraction artefact and the material secreted by human pituitary is in fact beta LPH. These studies would indicate that in human, MSH is not really the melanocyte stimulating hormone, as was thought earlier. Beta LPH and ACTH are now thought to be involved in melanogenesis in addition to their target gland actions.

The finding of low ACTH levels in the vitiligo patients in the present study is in agreement with the theories put forward in favour of ACTH about its role in melanogenesis. This is also in agreement with the observations that administration of ACTH causes hyperpigmentation in normal human and repigmentation in vitiligo patients. Generalized hyperpigmentation, as seen in Addison's disease, is also found in some patients with Cushing's
disease who undergo bilateral adrenalectomy. Hyperpigmentation is also found in ectopic ACTH syndrome. It may be presumed that, since excess of ACTH causes hyperpigmentation, low levels of ACTH may cause hypopigmentation as is seen in vitiligo. A question may be raised as to why this depigmentation is localized rather than generalized. In man the melanocytes of the skin have overlapping as well as distinctive features. For each type of melanocyte there would be variations in responsiveness depending on location. For example the melanocytes at the epidermal-dermal junction in the eyelids may react to hormones more readily than the melanocytes of the cheeks which in turn are more reactive than those of the thighs. It is difficult to explain the cause of low ACTH levels in the present study. But the probable causes would be glandular insufficiency or raised cortisol level or some derangement in the hypothalamo-pituitary axis.

From thyroid function studies it was observed that the patients in the present study were hypothyroids without any clinical manifestations. By TSH stimulation test it was shown that the hypothyroidism was not primary. TRH test was carried out subsequently in order to study whether this hypothyroidism was due to some defect at the hypophyseal level or hypothalamic level. Normal
basal TSH levels and a positive TSH response to TRH indicate that the pituitary is functioning. Delayed and exaggerated response of TSH to TRH suggests hypothalamic hypothyroidism.

Other factors producing alterations in TRH responsiveness include the effects of low circulating $T_3$.  

In mammals there is good evidence that thyroid hormones modulate peripheral actions of prolactin. TRH acts directly on the pituitary to release both TSH and prolactin. But the significance of prolactin response to TRH is still uncertain. Some hypothyroid patients have elevated prolactin levels but most do not. Most of the hypothyroid patients show exaggerated prolactin responses to TRH. In the present study prolactin levels were normal in vitiligo patients but the response to TRH was delayed and exaggerated. Although the patients in the present study were not primary hypothyroids. Probably low levels of $T_3$ and $T_4$ were responsible for such abnormal response.
References:


