THE INCREASE OF CIRCULATING THYROTROPHIN AND THE ACTIVATION OF THE THYROID BY MEANS OF ELECTROSHOCK IN GUINEA PIGS

By

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The activation of the hypophysis and of the thyroid by means of repeated electroshocks to animals has been described by various authors (Verdozzi, Masini, Novelli & Biaggini, 1949, using adult dogs; Novelli & Masini, 1950, and Masini & Trasino, 1950, with young dogs; Giorgi, 1949, and Marzullo & Taparelli, 1950, using rabbits). These experimental data are in agreement with the numerous, though indirect, findings afforded by clinical experience (bibliography in Novelli & Biaggini, 1951). However, general histological criteria (fixation with formol, staining with hematoxylin-eosin) have essentially been used in these experiments, giving only a qualitative idea of the result. This is, probably, the reason for certain contradictory data, and for the impossibility of observing activation after a single electroshock (Giorgi, 1949), or after a few such shocks (Marzullo & Taparelli, 1950).

By means of a very sensitive and discriminating quantitative index of thyroid activity, namely the cytological coefficient of the guinea pig (bibliography in Del Conte, 1949 a), we have tried to demonstrate much more rapid reactions (reactions thirty minutes after a single application) in the hypophysis-thyroid system, since, as appears from the literature, the thyroids react secondarily to hypophyseal activation. To this end and with this method, a study, and a comparison with normal cases, has been made of the thyroids of electroshocked animals and also of the concentration of thyrotrophin in the blood; the investigation was completed by a cytological examination of the hypophyses, especially with regard to the beta cells, which take aldehyde fuchsin stain and which seem to be the source of thyrotrophin (Halmi, 1950, 1951, 1952; Purves & Griesbach, 1951).

1. This work was done at the Research Center of the Ministry of Public Health of Argentina (Dr. Estanislao Del Conte).
Material and Methods

Twenty-one guinea pigs, weighing between 150 and 200 gm., were used, in four groups: one of six and three others of five animals. The guinea pigs of the first group were submitted to electroshock; the precaution of obtaining a complete shock in each case was observed, for which it was necessary to apply an induction tension of between 120 and 160 volts for eight seconds. Thirty minutes later the guinea pigs which had been given the electroshock were sacrificed by means of a blow on the head, after extracting from each, by cardiac puncture, approximately 3 ml. of blood, from which a pool of 18 ml. was made. The thyroids were fixed in Heidenhain's Susa solution, and their sections stained with aniline blue-orange G, to determine their cytological coefficients by the simplified method (Del Conte & Vasena, 1951). The hypophyses were fixed in Helly solution and the sections stained by Mallory's trichromic method and with aldehyde fuchs in (Gomori, 1950). The animals of the second group, which were used as controls, were subjected to the same procedure with the exception of the electroshock.

Both pools were extracted by the Fellinger method (1936) so as to isolate the thyrotrophin from any other substances which might possibly act upon the thyroids; the procedure for this purpose was as described previously (Del Conte, 1947 and 1949). The extract from the guinea pigs which had received the electroshock was administered in individual doses equivalent to 2 ml. of blood, by intracardiac injections and under light ether anesthesia into the animals of the third group; likewise those in the fourth group received the extract from the control guinea pigs. Thirty minutes after the injection, the animals were sacrificed and the cytological coefficient of their thyroids determined by the simplified method.

Results

Table 1 shows the individual thyroid cytological coefficients, the average for each group, the differences between the averages and the corresponding standard errors. The data are presented graphically in Fig. 1. A statistically significant difference exists between the results for the group to which electroshock was applied and those for the control group, and similarly between the findings for the group injected with blood from the treated animals and those for the control group. No structural modification in the thyroids of the treated animals was observed, with the procedure used, beyond the relative number of intra-cellular colloid droplets quantitatively expressed in this manner.

In the sections of the hypophyses of electroshocked guinea pigs, stained by Mallory's method, a loss of specific granulations in the chromophilic cells of the anterior lobe was observed in contrast to the sections in the analogous planes of the normal cases. This phenomenon becomes more evident in the sections treated with aldehyde fuchs in, which allows them to be referred to the type of basophyllic cell believed to be responsible for the secretion of thyrotrophin. It is observed in effect, that in the treated animals the majority of the cells which take this stain do so in a faint and diffuse way; they show an almost
Table 1.

Summary of thyroid cytological coefficients in controls and experimental animals.

<table>
<thead>
<tr>
<th>Guinea pigs</th>
<th>Controls</th>
<th>Electroshocked</th>
<th>Injected with extract of 2 ml. of blood of</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Controls</td>
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<tr>
<td></td>
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</tr>
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<td>16</td>
<td>11</td>
</tr>
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<td>9</td>
</tr>
<tr>
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<td>26</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Averages</td>
<td>4 ± 0.4</td>
<td>25 ± 2.1</td>
<td>10 ± 1.6</td>
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<tr>
<td>and standard errors</td>
<td></td>
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<tr>
<td>Differences</td>
<td></td>
<td></td>
<td>19 ± 2.2</td>
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<td>between the averages</td>
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</tbody>
</table>

Fig. 1.

Graphic representation of the results obtained. The white circles represent the individual results; the black circles, the averages for each group.

Complete lack of the intensely stained granules and clumps such as characterize normal cases (Figs. 2 to 5).

10
Figs. 2–5

are photographic micrographs of sections of the hypophyses of guinea pigs stained with aldehyde fuchsin. Figs. 2 and 3 were obtained with an 8 × objective. Figs. 4 and 5 were obtained with a 90 × immersion lens.

Fig. 2: Topographic aspects of hypophysis in a normal guinea pig, stained with aldehyde fuchsin.

Fig. 3: Decrease in affinity for stain in the hypophysis of an electroshocked animal.

Fig. 4: Part of the field of Fig. 2, highly magnified. The great quantity of intracellular granulations and clumps with intense affinity for aldehyde fuchsin is observable.

Fig. 5: Part of the field of Fig. 3, highly magnified. Half an hour after one electroshock the cells have lost the greater part of their specific granulations.
DISCUSSION

The existence of a definite thyroid activation in the electroshocked animals is shown by the data obtained. This activation is somewhat greater than that produced under like conditions by the injection of 0.001 Junkmann-Schoeller units of thyrotrophin (Del Conte & Vasena, 1951). In this way the cytological method employed is shown to be much more useful than that of simple histological qualitative evaluation of the gland; in addition to yielding quantitative data, it makes it possible to reveal clearly the activation produced by a single electroshock in a half-hour period, a phenomenon not detected by other investigators (Giorgi; Marzullo & Taparelli). Indeed, this reaction has previously only become apparent, and then not consistently, after 15 electroshocks administered every other day (Verdozzi, Masini, Novelli & Biaggini).

It is also evident that there is an increase in the circulating thyrotrophin in the treated animals. The method used to determine the hormone combines the best available features, being a direct index of thyroid activity, which up to the present is the only known test for thyrotrophin. In order to obtain valid results it is therefore sufficient to eliminate the interference of other factors which might act upon the thyroid glands. For this purpose the blood was extracted by the Fellinger method, which eliminates a large amount of the proteins, and, especially, the substances which contain iodine.

The injection of an extract of 2 ml. of the electroshocked animals’ blood into other animals produces in the latter a thyroid activation which is greater than that obtained by the injection of 0.0001 Junkmann-Schoeller units of thyrotrophin (Del Conte & Stux, 1953), while the administration of the same quantity of extract from control animals causes a much less intense activation. The 2 ml. of blood used represent a little more than a tenth of the blood volume of the animals, hence the increase in circulating thyrotrophin (about 0.001 units in all) corresponds exactly to the thyroid activity found, this being analogous to that produced by the same quantity of hormone. This gives support to the assumption that the activation of the thyroid is secondary to a liberation of thyrotrophin by the hypophysis.

This point of view also finds support in the last of the observations made: the loss, in the treated animals, of specific granulations by the hypophyseal cells, which take the aldehyde fuchsin stain and which are held to be responsible for the secretion of thyrotrophin (Halmi; Purves & Griesbach). This phenomenon may be interpreted as the liberation, on the part of these cells, of their secretory product. There is actually only an apparent contradiction between this picture and those of irregular hypertrophy and hyperplasia observed by other investigators 48 hours after repeated electroshocks (Giorgi; Marzullo & Taparelli; Novelli & Biaggini). If the existence of a secretory cycle in these cells is borne in mind, it may be considered that the initial response to excita-
tion is the liberation of accumulated substances and that the repetition of this stimulus, followed by a period that allows for cellular recovery, brings about the hypertrophy and hyperplasia described.

**SUMMARY**

Six guinea pigs weighing between 150 and 200 gm. were given electroshocks with an induction tension of between 120 and 160 volts during 8 seconds. Thirty minutes later they were sacrificed, after approximately 3 ml. of blood had been removed from each, from which a pool for each group was made up. Their thyroid activity was then investigated by means of the cytological coefficient (simplified method) and the cytology of their hypophyses studied by means of Mallory's trichromic stain and Gomori's aldehyde fuchsin. Another group of 5 animals was used as a control. The blood pools from both groups were extracted by the Fellinger method and the respective extracts injected in individual doses corresponding to 2 ml. of blood into two other groups of 5 guinea pigs, which were sacrificed 30 minutes later to obtain the thyroid cytological coefficients.

The increase in the cytological coefficient of the treated animals (25 ± 2.1 as contrasted to 4 ± 0.4 in the controls) shows that electroshock produces, in half an hour, an activation of the thyroid which is somewhat greater than that obtained by the injection of 0.001 Junkmann-Schoeller units of thyrotrophin. The increase of circulating thyrotrophin within the same time period is also definite; the difference between the cytological coefficients of the groups which received the extracts of blood (17 ± 2.0 as opposed to 10 ± 1.6) shows that the increase in 2 ml. is of the order of 0.0001 units; if the blood volume of the animals is taken into account, then the increase in circulating thyrotrophin may be considered as being responsible for the thyroid activation noted. This point of view is also supported by the hypophyseal cytology, which shows, in the treated animals, a loss of specific granulations in the cells which take aldehyde fuchsin stain and which are considered to be the source of thyrotrophin.

**REFERENCES**

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