THE DISTRIBUTION OF BIOLOGICAL ACTIVITY IN THE ANTERIOR PITUITARY OF THE OX

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(With Four Text-figures and One Plate.)

1. INTRODUCTION.

The stimulating influence of the anterior lobe pituitary upon growth and metamorphosis in amphibia has raised the question of the responsibility of one or more autacoids for these effects.

No active factor has been isolated so far and little chemical information is available, but meantime evidence and the circumstances relating to the production of the observed effects must be collected and examined for any basis of distinction between them.

Metamorphosis is in itself a growth process, but distinct in the readjustment, reorganisation and other changes required within the organism without necessarily any increase in size. The histological evidence showing the presence of different kinds of cells suggests more than one principle, although no explanation is forthcoming of the extent to which these cells indicate different secretory activities or definite phases of any one activity. Smith and Smith (1923) observed a central area of mainly basiphil cells in median sections of the bovine hypophysis with a surrounding outer area consisting chiefly of eosinophils and further found that the former advanced the rate of metamorphosis, but retarded growth, while the outer area produced the reverse effects when the two regions were separated and administered to tadpoles. They concluded that separate principles existed and that these were elaborated by specific types of cell. Extended observations (Spaul, 1929) upon the production of metamorphosis in axolotls and on the accelerated transformation of tadpoles by injections of 0.125 per cent. acetic acid extracts of the inner and outer regions of the lobe indicated that the response to the outer region was less, when successful, than that obtained with the extracts of the inner region, although occasional failures followed treatment with the latter. It was concluded therefore that, although no definite localisation existed, the active principle was more concentrated in the inner region and the results obtained depended upon the proportions of the different cells in the region used. Greater growth stimulation was apparent in the outer region, but, in the absence of an established specific test for the determination of the growth rate, equivalent to that for metamorphosis, and owing to our lack of knowledge concerning controlling or dependent facts,
no comparison, quantitative or otherwise, was possible. These results, whilst agreeing with those of Smith as regards the effects produced, suggest a more gradual transformation from one effect to the other between the two regions. Further, the threshold value for metamorphosis demands a fairly high concentration in the outer region and at the same time makes it doubtful whether the inner region is really the sole seat of metamorphic activity. The inner region here employed is only approximately equivalent to Smith’s area, since the latter varies in extent and considerable magnification and elaborate histological preparation are needed to reveal its more intricate ramifications. As a general rule somewhat more than this restricted region was inevitably included in the inner portion taken; the total amount used, however, was kept as constant as possible.

The results had not been correlated with histological observations and any reference to specificity so far as the cells were concerned was out of place, but in view of the findings it seemed desirable to reinvestigate the problem and thoroughly explore histologically the central region. Recent attempts to interpret biological activity by chemical means (Spaul and Myddleton, 1929, unpublished) gave further justification for this step.

Actually the division into the inner and outer portions, apart from the difficulties associated with complete dissection of Smith’s region, is unsatisfactory as the results are complicated to some extent by the inhibitory influence of diffused posterior lobe principles. Hence in these experiments the anterior lobe was divided into three regions—inner, middle, and outer.

2. EXPERIMENTAL OBSERVATIONS.

The frozen glands for these experiments were first split into two by a median sagittal cut and after separation of the anterior lobe portion divided into three parts, (a) inner—adjoining the cleft and pars intermedia, (b) middle—the intermediate part, (c) outer—a thick rind, least vascular to outward appearances. The parts were approximately equal and as far as possible similar in all the glands used. Portions of the central axis were included in each region (Figs. 1 and 2).

Three series of experiments were arranged for the administration of these parts to frog tadpoles.

(1) 0.1 c.c. of 20 per cent. extracts of each portion in 0.125 per cent. acetic acid was injected into tadpoles tri-weekly, the animals being kept in glass jars (25 per jar) placed on a white background in a good light at room temperature. The animals in each jar were about the same stage of development (hind limb buds just visible), and were given injections of the same portion of the gland on each occasion; they were not fed after the experiment commenced. The water was changed after each injection. The extracts were prepared by the standardised process (Spaul, 1925).

(2) Animals, selected and kept as in the first experiment, were fed once a week with the same portion of gland. Several small portions were dropped into each jar on these occasions and removed after 24 hours when the water was changed. Neither food was given during the period of the experiment.
Fig. 1. Approximately sagittal section of ox pituitary.

Fig. 2. Horizontal section of ox pituitary.

Fig. 3. Transverse section of ox pituitary about one-third from the posterior end.

Fig. 4. Transverse section of ox pituitary about two-thirds from the posterior end.

Figures drawn from preparations but diagrammatic.
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Small portions of gland tissue were grafted into tadpoles. The larvae were anaesthetised and a small slit made in the abdominal wall through which a small thawed portion of the gland was introduced into the abdominal cavity. A thin film of collodion in ether was applied over the slit as a protective skin during the healing of the wound. The animals were placed in running water and soon revived. There were some fatalities but the majority survived several days. No food was given at any time after the operation.

The length and breadth of the body and tail of each animal were measured once a week and a daily record kept of the progress of metamorphosis.

Results. The following summary indicates the results of treatment after 14 days.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of specimens taken</th>
<th>Mortality</th>
<th>No. completely transformed</th>
<th>No. with fore limbs</th>
<th>No. with hind limbs only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>8</td>
<td>6</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Fed, outer region</td>
<td>75</td>
<td>12</td>
<td>12</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>”, middle region</td>
<td>75</td>
<td>22 (majority transformed)</td>
<td>35</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>”, inner region</td>
<td>75</td>
<td>14 (many transformed)</td>
<td>24</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Injected, outer region</td>
<td>75</td>
<td>17</td>
<td>20</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>”, middle region</td>
<td>75</td>
<td>30 (nearly all transformed)</td>
<td>29</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>”, inner region</td>
<td>75</td>
<td>31 (several changed)</td>
<td>24</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Grafted, outer region</td>
<td>20</td>
<td>11</td>
<td>—</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>”, middle region</td>
<td>20</td>
<td>12 (some complete)</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>”, inner region</td>
<td>20</td>
<td>11 (two complete)</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

It will be seen from this table that the middle region of the anterior lobe of the gland induces the greatest acceleration whilst the outer is least active. Allowance must be made for an inhibitory influence of the posterior lobe in the inner region, but even so it is highly improbable that the activity of this region is as great as that of the middle region. The possible extent of the posterior lobe contamination can be gauged from the estimations of the threshold dose of melanophore stimulant in these regions: outer, 0.003; middle, 0.0012; inner, 0.00087; the actual quantity present being inversely proportional to these doses.

Similar results were obtained by injecting and feeding tadpoles at later stages of development.

It is noteworthy in the feeding experiments that conditions were obtained suitable for the absorption of sufficient quantities of the active principle for the maintenance of a concentration within the organism above the threshold value required to stimulate metamorphosis, in spite of the destructive influence of digestive enzymes (Spaul, 1929). Hitherto feeding has been shown to influence primarily growth and not metamorphosis and the injection of extracts is required.
to produce any acceleration, but the advanced stages of development of the animals selected and the quantity of substance given, particularly in the later phase when a loss of digestive power occurs, apart from any special susceptibility, have contributed undoubtedly to this result.

Tadpoles given the outer region appeared to grow slightly at first, but, as no food was supplied and metamorphosis and growth are not apparently stimulated at the same time (Spaul, 1929), shrinkage was inevitable. A gradual decrease was noted in the controls and, more definitely, in those treated with the other regions, especially the inner region. At the end of the period those given the outer region were slightly larger than the remainder but smaller than they were at the beginning of the experiment. The most marked growth effect was obtained with the grafts of the outer part of the lobe.

The distribution of the metamorphic activity here observed receives striking confirmation from the chemical tests applied to the extracts of these regions (Spaul and Myddleton, 1929, unpublished). The iodine precipitates were as follows: outer, 0.23; middle, 1.0; inner, 0.8 (vol. in c.c. with 10 c.c. of extract and 6 c.c. of N/10 iodine solution). The amount of phosphate in these precipitates is still more significant, as a more direct and exact indication of the activity is given through the apparent association of the phosphate content with the active factor.

Extracts prepared from these regions after exposure for a few hours show a loss but also equalisation of the activity, except in the outer region, where apparently autolytic effects cause greater loss. The melanophore and chemical tests give similar indications.

3. HISTOLOGICAL.

Introduction. In spite of the extensive studies of morphology and histology of the pituitary gland, doubt still exists as to the responsibility of the various types of cells for any specific activity. The attempts made so far, with one exception, have been concerned in associating certain histological features with pathological or normal symptoms of hyper- and hypo-activity of the gland, and hence to identify definite cells or types of cells as active or otherwise. As already mentioned Smith and Smith (1923) identified two areas in the anterior lobe, the basiphil and the oxyphil, which, they maintained, influenced growth and metamorphosis respectively. In the previous section of this paper the distribution of the biological activity in this portion of the gland has been studied by comparing the effects produced by the administration of different regions. Further, the iodine test supported these findings and hence an attempt has been made to apply this chemical reaction histologically, and so correlate the distribution of the cells with activity. With this in view a careful comparison has been made between sections treated in this manner and similar ones stained by established histological methods and the distribution of the various cells, with their staining reactions, determined in each case.

Few specific details of the histology of the posterior lobe of the ox pituitary have been published and therefore a short account of the more striking features of the pars intermedia and pars nervosa revealed by this comparison is also included.
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Methods. Whole glands were obtained at the abattoir and fixed as soon as possible after death, but, since approximately half an hour elapsed before the tissue was put into fixative, it was necessary that some indication of the probable course of the degenerative processes occurring during this period should be obtained. A series of glands was therefore exposed for three and six hours at 40° C. before fixation.

Glands were cut into three pieces in sagittal, horizontal, and transverse planes and fixed in the following: Bouin, Carnoy, Cajal’s uranium formal; Da Fano’s cobalt formol, Flemming without acetic, formol bichromate and Gilson. After dehydration they were cleared in cedar wood oil and sections cut throughout the depth of each piece.

The combinations of stains used were: haemalum and Scott’s Biebrich scarlet, Erhlich’s haematoxylin and Biebrich scarlet, Hastings’ Romanowsky, iron haematoxylin and van Gieson, Leishman, Mallory’s connective tissue stain, carbol methyl green pyronin and Erhlich’s acidophilous mixture, but it was found that haemalum, haematoxylin and Biebrich scarlet, Leishman and Mallory gave the most satisfactory differentiation. The Da Fano and Cajal material was treated with silver nitrate, etc., for preparations of Golgi bodies.

The iodonophil reaction found by Spaul and Myddleton (1929) was adapted to the study of the gland in the following manner.

Sections from absolute alcohol treated with tincture of iodine showed some differential absorption, but the colour contrast was insufficient for detailed study and the process was accordingly modified as follows:

Sections from absolute alcohol were treated with
1. 4 per cent. iodine in absolute alcohol (3–6 hours).
2. A mixture, prepared immediately before use, of 0.5 per cent. leuco-base of malachite green in absolute alcohol, 50 c.c.; distilled water, 20 c.c. (6–12 hours).

As a precipitate gradually forms in this solution, slides were placed therein, with the section facing downwards.
3. A saturated aqueous solution of potassium iodide, saturated with iodine and diluted to a deep claret colour with distilled water (24 hours).
4. 0.5 per cent. hydrochloric acid in 30 per cent. alcohol (5 minutes).

After wiping, sections were taken up the alcohols very rapidly to avoid excessive removal of the stain, cleared in origanum oil and mounted in origanum balsam.

The posterior lobe and the basiphil areas are stained pale green, while certain cells in the oxyphil area stand out as dark green or greenish brown ovals, with the nucleus as a clear space. Owing to the solubility of malachite green in alcohol, completely satisfactory preparations are difficult to obtain; the best results were obtained after Bouin or Gilson fixation (Pl. I, figs. 5, 6, 7).

Low-power examination. From a study of sagittal, horizontal, and transverse sections, the anterior lobe was seen to be divided into two distinct regions—basiphil and oxyphil. The former was found to extend from about the middle of the left opposite the cone of Wulzen towards the anterior end, forming a central axis with a cone-shaped expansion at the anterior and antero-dorsal end; in some
cases peripheral continuations round the gland were found. No gland was examined in which this region was absent, although it varied considerably in shape and extent. It consists of white fibrous tissue in which lie numerous sinus-like blood capillaries and nests of basiphil cells, with occasional small collections of oxyphil cells (Figs. 1-7).

This area presumably corresponds with the region of growth described by Portella (1924) in the human gland, and the central axis described but not figured by Smith and Smith (1923) in that of the ox. De Beer (1926) also figured and discussed a basiphil area which does not, however, coincide with that described above, unless the diagram shown is of a section through a lateral expansion and hence not in the median sagittal plane.

The remaining parts of the gland contain fewer blood vessels, the connective tissue trabeculae are much finer, and the cells predominantly oxyphil. In the glands examined, few colloid vesicles were found, those present being extremely small and largely confined to these regions.

High-power examination. The pars anterior contains the following types of cells: Firstly, there are both basiphil and oxyphil cells of a granular type, each group being subdivided into strongly and weakly staining categories. Secondly, there are both undifferentiated and small epithelial cells, which with the connective tissue cells are non-granular.

The basiphil reaction of Smith's central axis is due to the concentration of the majority of basiphil cells within that area. The cells are granular and are of two kinds—weakly and strongly chromaphil, the former being more numerous. The weakly chromaphil cells appear to correspond with the neutrophil cells described by Cooper (1925) in the human gland. They are large and generally oval (although not infrequently irregular in outline), with coarsely granular, faintly basiphil protoplasm; their nuclei are large, with one or two nucleoli. They line the walls of the spaces in the thick white fibrous tissue, frequently leaving a small central lumen, and at the posterior end, nearest the cleft, of Smith's axis form the greater proportion of the cellular constituents; towards the anterior end they are gradually replaced by strongly chromaphil basiphils. These latter are found in sizes varying from nuclei surrounded by a little deeply staining cytoplasm to large cells about 12 μ by 10 μ. The smaller forms are usually at the posterior end of Smith's axis, but there is an increase in size and number towards the anterior end, where whole nests of the largest size are found, frequently in close relationship with the capillaries. Large chromaphil basiphils are also found at the edges of the oxyphil areas, rarely among the oxyphil cells, and occasionally at the periphery of the gland. Using the iodine leuco-base reaction, Smith's area is stained a homogeneous light green, no cells, except a few groups of what are apparently oxyphil cells, being differentiated from the rest.

Apart from a few exceptions which occur within the central area, the oxyphil cells form the chief constituents of, and are confined to, the rest of the anterior lobe. They are ovoid in shape, approximately 12 μ by 10 μ, having large clear nuclei containing large central nucleoli; the protoplasm is granular. Near Smith's area
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and towards the cleft, they are especially numerous, clearly defined and containing fine granules, showing a strong and bright oxyphil reaction; towards the periphery an increasing number of cells is found containing coarser granules which are not quite so strongly oxyphil; at the periphery itself, the cells toward the anterior end become a damask red with Mallory, haematoxylin, haemalum and Biebrich scarlet, since the cytoplasm takes up basophil stain. The cells tend to form clumps, without definite cell boundaries, while those towards the posterior end assume a peculiar transparent appearance and have indented cell walls. This effect is increased by exposure of the glands at 40°C, before fixation, becoming particularly marked after approximately five hours. It has already been ascertained by the work upon the secretory action (Spaul, 1925, 1928) that autolysis occurs about this time at this temperature. It is highly probable, therefore, that this effect in the fresh gland indicates the beginning of autolysis occurring in the interval between death and fixation.

Weakly oxyphil cells, similar in shape and structure to the weakly basophil cells of Smith’s area, are found among the strongly oxyphil cells but in fewer numbers; they are most common at the periphery. Probably like the weakly basophil cells, they correspond to the neutrophils described by Cooper (1925). Occasionally, cells, apparently intermediate in structure and staining reaction between the weakly and strongly oxyphil cells, are found amongst them.

In these areas, where oxyphil cells predominate, it is remarkable that the iodine leuco-base technique shows the most intensive reaction and many deeply stained oval cells are found corresponding in number with, and showing similar distribution to, the strongly oxyphil cells. Like the oxyphil cells these show the greatest definition and depth of stain near Smith’s area, with a lessening intensity towards the periphery; here coalescent masses of similar cells and cells with indented edges are found, which with this technique are stained only slightly more deeply than the surrounding connective tissue.

It is interesting to note that in the glands exposed at 40°C the edge of the basophil area at first stains slightly more darkly, and then gradually tends to show an oxyphil reaction. The iodine leuco-base also shows this effect, the depth of stain in the oxyphil areas diminishing, while that in Smith’s area increases. At six hours considerable autolysis occurs around the periphery, a thick crust of degenerate cells with an oxyphil reaction being formed, which does not stain very deeply with this technique. Cells, intermediate in staining reaction between the strongly basophil and oxyphil, occur sparsely in the anterior lobe, and, in some glands, they may be seen in fair numbers lying among weakly oxyphil cells at the junction between the anterior lobe and the pars intermedia.

Undifferentiated epithelial cells are found in all parts of the lobe, but they are few in number and difficult to find; they have very large clear nuclei and are surrounded by clear unstained cytoplasm. The small epithelial cells are generally found near the connective tissue trabeculae and have small darkly staining nuclei, surrounded by a thin layer of non-granular oxyphil cytoplasm. These cells are fairly numerous.
Golgi preparations of the pars anterior gave little information. The apparatus itself took the form of a coarse network near or round one end of the nucleus. Rod-like mitochondria were found at the ends of most of the cells. No correlation could be found between either the shape of the Golgi bodies or their orientation and the nature of the cells and their relationship to blood vessels and colloid vesicles. With deep toning, the oxyphil cells showed a greyish, finely granular appearance and a few containing coarse black granules were also found.

The cleft. The cleft was widely open in all but two of the thirty-six glands examined and was lined with flattened cubical epithelium on both sides. In approximately half the glands it contained colloid material.

Pars intermedia. The main portion of the pars intermedia lying postero-dorsal to the cleft always formed a wide zone at least fifteen cells deep, generally with deeper projections into the pars nervosa in some regions. The cells are epithelial in type and more or less irregular in outline with a spherical nucleus containing a central nucleolus; their cytoplasm is faintly granular, staining slightly basiphil with haematoxylin and with Mallory, but faintly oxyphil with Leishman. Some of the nuclei were more darkly stained, especially near the pars nervosa, but no signs of mitosis were found. An occasional strongly basiphil cell, not unlike those of the pars anterior, was noticed. The Golgi bodies were spindle-shaped networks, lying at one end of the nucleus, and having, apparently, no regular orientation. No colloid vesicles were found in the pars intermedia of any gland examined.

Projecting from the pars intermedia, and separated from it by a strand of connective tissue, is the cone of Wulzen (Wulzen, 1914) (Figs. 1 and 2). This is formed of thickish connective tissue trabeculae, among which lie nests of weakly and of strongly oxyphil cells, the staining reactions of which are similar to those of the oxyphil cells of the pars anterior.

Pars nervosa. This consists of neuroglia and ependymal cells. In the glands examined, Herring’s “hyaline bodies” and the granular masses described by Kohn were both present in considerable numbers, the former being most numerous in the neighbourhood of the pars intermedia, and the latter at the posterior end. Occasional oval cells, with darkly staining nuclei and granular eosinophil cytoplasm, were also found.

The pars tuberalis was removed during excision of the gland and was not examined.

4. DISCUSSION.

The histological observations do not allow a decision to be made as to the relationship between the various types of cells in the anterior lobe, as only adult material of unknown age and sex was used. However the grading of the cytoplasmic staining of the strongly oxyphil cells, from the centre to the periphery, and the increase in size and numbers of the strongly basiphil cells, from the posterior to the anterior end of Smith’s axis, taken in conjunction with the great vascularity of that area, might indicate that this axis is, as Portella (1924) suggests, an area of active growth, giving rise to basiphil cells at its anterior end and later to oxyphil cells. This material does not indicate whether the strongly oxyphil cells
at the periphery are the earliest developed cells and those near Smith's area of recent development, or whether their apparent activity judged by their staining capacity is due to the proximity of numerous blood vessels; neither does it definitely show that the small basiphil cells are developing into large cells of the same type. Furthermore, no signs of cell division were found in any of the glands examined, although signs of probable degeneration appear at the periphery. The relationship of the weakly staining oxyphil cells is not at all evident.

The absence of cysts in the pars intermedia, which was also found by De Beer (1926), and the rarity of colloid vesicles in the pars anterior are also interesting facts from which nothing is at present deducible.

Coupled with the experimental evidence, the grading of the oxyphil cells is significant as it predominates in the middle and most active region.

Smith's axis runs through each region but the strongly basiphil cells predominate in its outer portion. The feature serving, however, to differentiate them is the intensity of the response of the oxyphil area to the iodine leuco-base technique contrasted with the seemingly similar response of the central axis and the posterior lobe. This area alone shows any definite reaction which is graded according to the staining of the cells and their distribution in a manner corresponding to the oxyphil affinity. Extracts of both the anterior and posterior lobes give an iodine precipitate, but they are distinguished by the great difference in the phosphate content. It may well be, therefore, that this phosphate content is associated with the reaction obtained in the iodine leuco-base technique, especially as a similar distribution of the phosphate content and the intensity of the reaction are apparent both in the fresh glands and after exposure. It would appear, therefore, that the strongly oxyphil cells are most concerned with the metamorphic activity and not the basiphil suggested by Smith.

The relation of growth stimulation to specific types of cells is more complicated and the information gathered contributes little towards its solution. If a separate secretion for growth exist, the basiphils of the central axis, largely confined to its anterior end, and the weakly staining oxyphil and basiphil cells are possibilities. Of these weakly staining cells only the oxyphil are located towards the periphery in any quantity. Until a specific test for growth has been established it is impossible to identify any of these types of cells with growth stimulation.

5. SUMMARY.

1. There is a graded distribution of metamorphic activity from the inside of the anterior pituitary to the periphery. This is similar to the distribution of the phosphate content of the iodine precipitate obtained with extracts of different regions of the gland.

2. A new technique based upon iodine absorption and the reaction between the absorbed iodine and the leuco-base of malachite green is described.

3. The distribution of the staining intensity with this technique corresponds to the oxyphil affinity and the biological activity.

4. The oxyphil cells appear to be mainly concerned with the metamorphic activity.
6. REFERENCES.


EXPLANATION OF PLATE I.

Fig. 5. Section showing the oxyphil area of the pars anterior (a) and the pars intermedia with the cleft between. Gilson. Leuco-base technique. ×60. Note the differentiated cells in the pars anterior and the uniformity of the pars intermedia.

Fig. 5 a. Section showing the oxyphil area of the pars anterior (a) and the pars intermedia with the cleft between. Gilson. Mallory. ×60. Note the oxyphil cells in the pars anterior (appearing as dark patches in the photograph) and the uniformity of the pars intermedia.

Fig. 6. Section through the basiphil area and the neighbouring oxyphil area. Gilson. Leuco-base technique. ×60. Note the differentiation of some of the cells in the oxyphil area (a) and the homogeneity of the basiphil area.

Fig. 6 a. Section in similar region to that in Fig. 6. Gilson. Mallory. ×60. Note the differentiation of the oxyphil cells (appearing black) outside the basiphil area.

Fig. 7. Oxyphil area. Gilson. Leuco-base technique. ×340. Showing the large cells differentiated by this method.

Fig. 7 a. Oxyphil area. Gilson. Mallory. ×340. The large dark cells are the oxyphils. Note their similarity in shape, size and distribution with the cells differentiated by the leuco-base technique.
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