IDENTIFICATION OF THE ADRENOCORTICOTROPHIN-PRODUCING CELLS IN THE RAT HYPOPHYSIS BY AUTORADIOGRAPHY

ELEANOR R. SIPERSTEIN, Ph.D.

From the Department of Anatomy, The University of Texas Southwestern Medical School, Dallas

ABSTRACT

The relative rates of protein (hormone) synthesis and secretion by the various cell types in the anterior hypophysis of the rat have been studied by means of autoradiography. Normal and adrenalectomized male rats were injected with tritiated glycine and their hypophyses removed and fixed at 20, 40, and 90 minutes and 15 hours after injection. Autoradiograms of the hypophysial sections were prepared and autoradiographic grains were counted in the film overlying the cytoplasm of individual cells. With the aid of this method, a unique cell type was identified in the hypophyses of adrenalectomized rats. This cell is morphologically distinct from "gonadectomy cells," "thyroidectomy cells," and from previously described normal cell types, and is therefore designated as the "adrenalectomy cell." Among the 7 cell types differentiated in this study, the "adrenalectomy cell" had the highest tritium content and, in addition, at the time intervals studied this cell had the fastest rate of appearance and disappearance of protein tritium. This autoradiographic evidence of rapid protein (or polypeptide) turnover following adrenalectomy indicates that the "adrenalectomy cell" is the site of adrenocorticotrophin production in the adrenalectomized rat. Further autoradiographic and cytological evidence is presented which suggests that the "adrenalectomy cells" may be derived from chromophobes, and that a portion of the "large chromophobes" as defined in this study may be the site of adrenocorticotrophin production in the normal rat.

INTRODUCTION

Although an increasingly large body of evidence has established the localization in specific cell types of five of the six known hormones of the anterior hypophysis, the cell responsible for production of the adrenocorticotrophic hormone (ACTH) is as yet unidentified. The results of the numerous studies which have been concerned with the cellular localization of ACTH are contradictory to one another or inconclusive (1–21). The possibilities for the localization of ACTH production which would fit within the present framework of pituitary cytophysiology appear logically to be the following: (*a*) ACTH is synthesized by one (or more) of the presently identified cell types in addition to the hormone currently assigned to it; (*b*) An additional subtype of cell is present among the cells of one of the major chromophil groups, or among the chromophobes, and is yet to be differentiated and described.

In view of the limitations of the procedures which depend on correlating stored hormone with pituitary content of stainable granules and in view of the lack of a specific histochemical method for staining ACTH, it appeared to be desirable to find a new approach to the problem of hormone localization and particularly to find an approach that could provide information about the functional state of individual pituitary cells. For these reasons, the present study was undertaken in an attempt to apply the method of autoradiography to the problem of the identity of the ACTH-producing cell in the rat hypophysis. By means of high resolution autoradiographic technics it is possible to localize the incorporation of radioactive precursors into cells or parts of cells. Tritium-labeled compounds are particularly useful since the soft beta particle of tritium makes possible an autoradiographic resolution of less than one micron (22). Therefore, it should be possible to visualize the sites of incorporation of a tritiated amino acid into the polypeptide, ACTH, as well as into the protein hormones of the hypophysis. The present experiment is based on the findings that, following adrenalectomy, while the pituitary content of ACTH is only one and one-half times normal (a difference which may not be detectable by staining methods), the *turnover* of ACTH in the pituitary is increased thirtyfold (23). It should be possible to visualize this large difference in turnover on a pituitary autoradiogram either as greatly increased activity over the cells responsible for ACTH production or as expansion of the ACTH-producing compartment.

By means of this approach, it was found that a morphologically distinct and characteristic large chromophobe of a type rarely seen in normal rats was present in adrenalectomized animals. This chromophobic cell had the highest tritium content and the fastest rate of both tritium incorporation and loss of all the hypophysial cell types. It is concluded that this cell, designated "adrenalectomy cell," is the source of ACTH, and evidence is presented which suggests that the "adrenalectomy cell" might be derived from a portion of the normal chromophobes of the anterior hypophysis.

MATERIALS AND METHODS

Preparation of Animals

Male rats of the Long-Evans strain were obtained from the commercial supplier at 5 weeks of age. They were maintained at 25°C, and supplied with Simonsen white diet (Simonsen Laboratories, Gilroy, California) and tap water. When the rats were 6½ weeks of age, 32 of them were selected at random and adrenalectomized by the dorsal approach while under ether anesthesia. On the day of operation the body weights of the operated animals ranged from 143 to 187 gm, with an average of 166 gm. Beginning on the day of operation, adrenalectomized animals were provided with 0.9 per cent sodium chloride solution in drinking bottles, in addition to the Simonsen white diet and tap water. Three of the operated rats died within 2 days following adrenalectomy. On the 6th postoperative day the remaining 29 adrenalectomized rats were weighed, and 8 of them which had not gained more than 4 gm nor lost more than 12 gm since the day of operation were selected as the experimental animals. These experimental rats ranged in body weight from 154 to 180 gm, with an average of 167 gm. The average postoperative weight loss was 4 gm.

Eight normal control rats ranged in body weight from 148 to 183 gm, with an average of 170 gm, on the day after the operated rats were adrenalectomized. Five days later, the weights of the normal rats ranged from 180 to 218 gm, with an average of 204 gm. The average weight gain during this period was 34 gm.

Administration of Labeled Amino Acid

Glycine was used as the labeled amino acid for these studies. It is known that glycine is found in all the protein and polypeptide hormones of the anterior hypophysis. Glycine makes up 7.0 per cent, 6.2 per cent, and 7.7 per cent of the amino acid residues of growth hormone, lactogenic hormone, and adrenocorticotrophic hormone, respectively (24, 25). One of the thirteen amino acids of alpha-MSH (intermedin) is glycine (25). Another means of incorporation of glycine into proteins is *via* its ready transformation into serine (26). Glycine is not only a precursor of protein but also of nucleic acids, porphyrins, creatine, glutathione, and glycogen (27). Of these, creatine and glutathione are water-soluble and would be removed from the tissue during washing. For practical purposes, any tritium incorporated into porphyrins in the sections would be in red blood cells and, therefore, would not interfere with the observations. Glycogen is demonstrable only in cells of the pars tuberalis in the hypophysis (28). Ribonucleic acid was removed by digestion of sections by ribonuclease.

The tritiated glycine (glycine-2-H^3, New England Nuclear Corp., Boston, specific activity 44.2 mc/mmole) was dissolved in sterile normal saline solution to give an activity of 1.0 mc per 0.5 ml.

On the 7th day after adrenalectomy the 8 ad-

renalectomized and 8 normal rats were injected intraperitoneally with 1 mc of tritiated glycine. Two normal rats and 2 adrenalectomized rats were killed at each of the following postinjection time intervals: 20, 40, and 90 minutes and 15 hours. The animals were sacrificed by decapitation, and their pituitary glands were dissected out and fixed in Bouin's fluid which was buffered to pH 4.0 with sodium acetate. It was found in preliminary studies that use of this fixative did not produce chemical artifacts in the autoradiographic emulsion and that buffering to pH 4.0 improved the cytological preservation. After 1 to 2 days of fixation the glands were washed in four changes of distilled water for a total of 1 hour, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin. Pituitaries of non-injected normal and adrenalectomized rats were processed in the same manner to serve as unlabeled controls in the autoradiographic procedure.

Preparation of Autoradiograms

The labeled and unlabeled pituitaries were sectioned in the horizontal plane at thicknesses of 2 and 4 μ. Glass slides were "subbed" by dipping in an aqueous solution of 0.5 per cent gelatin and 0.1 per cent chrome alum. Ribbons of sections were placed in serial order on each of six slides for each gland; three to five different levels of the gland were represented on each slide. Sections were decerated with xylene, hydrated in descending grades of ethanol, and washed 1 hour in running tap water and 30 minutes in distilled water. Every other serial slide was submitted to the action of 0.02 per cent ribonuclease in distilled water for 90 minutes at 37° to 44°C. The ribonuclease solution had previously been heated in a water bath for 10 minutes at 80° to 85°C in order to destroy any possible proteolytic activity (29). After digestion by ribonuclease the slides were washed 30 minutes in running tap water then placed in distilled water. The ribonuclease-treated slides and the alternate slides bearing serially adjacent, non-digested sections were carried through the rest of the procedure simultaneously. The sections were then stained by the aqueous periodic acid–Schiff method ("traditional" method of Lillie, 30) for demonstration of mucoproteins of the pituitary basophil cells, following which they were washed in running tap water 1 hour, placed in distilled water, and transferred to the darkroom. Slides were covered with AR-10 Autoradiographic Stripping Film (Kodak, Ltd., London), dried 20 to 25 minutes in a light-tight drying box equipped with a blower, and placed in black Bakelite slide boxes containing Drierite wrapped in lens paper. The slide boxes were sealed with black Kodak binding tape and placed in the refrigerator during exposure of the film for 4, 6, and 18 weeks. At the end of each exposure time a set of autoradiograms was processed at 17° to 18°C according to the following schedule: Kodak D-19 developer, 5 minutes; distilled water, 30 seconds; 15 per cent sodium thiosulfate, 5 minutes; three distilled water rinses, totaling 4 to 5 minutes. After the last rinse, the films were air-dried in the drying box.

Staining the Autoradiographic Preparations

In order to identify and characterize properly the cell types associated with the developed autoradiographic image, it was necessary to devise a means of staining the pituitary sections through the overlying film. As mentioned in the previous paragraph, the sections were stained by the periodic acid–Schiff method (PAS) before application of the stripping film. This procedure will not cause chemical artifacts on the film, as was demonstrated in preliminary experiments and during the course of this study; and, furthermore, the stained structures were not faded or discolored during the subsequent developing procedure. Thus, at the time the autoradiograms are ready for further staining, the glycoprotein granules of the basophil cells and of the intermediate lobe cells are already stained pink, as are the other PAS-positive structures, *viz.*, collagen fibers, basement membranes, neurosecretory material and Herring bodies of the neural lobe, and glycogen in cells of pars tuberalis. The other structures which must be stained for identification and characterization of pituitary cell types, besides glycoprotein granules, are acidophil cell granules and nucleic acids. The method developed for staining these structures through the stripping film is based on optimum adjustment of the pH of the staining solution. Incidental staining of the gelatin of the film is minimized by application of the basic dye at a pH below, and of the acid dye at a pH above, the isoelectric point of gelatin, which is 4.9. Staining solutions of the appropriate pH values were prepared using McIlvaine buffers according to Lillie (30). Bergeron (31) reported the use of pH control to stain autoradiographic sections with basic dyes. For the present study, the pH values and dye concentrations were found which would result in negligible staining of gelatin, while providing specificity in staining acidophil granules and nucleic acids. This staining procedure was as follows:

1. Soak the autoradiograms in pH 9.1 buffer	30 minutes
2. Stain acidophils in 0.025 per cent metanil yellow at pH 9.1	20 minutes
3. Rinse films in pH 9.1 buffer	2 seconds
4. Soak films in pH 3.6 buffer	5 minutes

5. Stain nucleic acids in 0.025 per cent toluidine blue at pH 3.6 — 20 minutes
6. Rinse films in pH 3.6 buffer — 2 seconds
7. Drain and air dry by fan
8. Mount cover glasses with Permount

Quantitation of the Autoradiograms

Of the 16 rats injected for this study, 2 were found to have no radioactivity in their pituitary glands due, apparently, to faulty injection. The grain development in the film overlying sections from these two glands was the same as background, even after 18 weeks' exposure. Grain counts were done on 8 of the 14 successfully injected rats comprising a complete time series, 1 normal and 1 adrenalectomized rat at each postinjection time interval.

Comparison of the autoradiograms exposed for 4 weeks with those exposed for 6 weeks indicated that those exposed for 4 weeks were preferable for counting grains. While the additional grain production in the autoradiograms exposed 6 weeks was advantageous in rendering more outstanding the differences in labeling between cells, it was considered disadvantageous for the purpose of counting grains since, in the more heavily labeled areas, the accuracy of the counts would have been impaired by the close crowding.

The sections which were digested with ribonuclease prior to application of the stripping film were used for the grain counts in order to avoid counting any grains representing tritium which may have been incorporated into cytoplasmic ribonucleic acid.

The sections cut at 2 μ were clearly superior to those 4 μ thick for use in the grain counting procedure. The delineation of the cell borders was more readily discerned in the thinner sections, thus making the localization of grains over the individual cells more readily apparent. Furthermore, it is known that the use of thinner sections improves autoradiographic resolution. In the case of tritium there would be no appreciable loss of activity impinging on the film from use of 2 μ thick sections as opposed to 4 μ thick sections since it is known that 90 per cent of the beta particles emanating from the decay of tritium are absorbed by 1.2 μ of tissue (22).

Horizontal sections from the approximate center of the gland were chosen for counting. Individual silver grains were counted in autoradiograms of the ribonuclease pretreated sections after exposure of the film for 4 weeks. The number of grains overlying the nucleus and cytoplasm of the cell were recorded separately. For the counting procedure a 90 × oil immersion objective lens (N.A. 1.40) and 10 × eyepieces were employed. A Whipple disc was placed in one of the eyepieces to demarcate a field of 3600 μ^2 within which the cells and overlying grains were counted. The sampling method was similar to that described by Mellgren (32) for differential cell counts of human pituitaries. Beginning at one lateral pole of the section, the slide was moved across the field of view in successive rows parallel to the short dimension of the section. Every third field of every third row, and therefore one-ninth of the entire section of the anterior lobe, was included in the quantitation. In every ninth field of every ninth row and therefore approximately one-ninth of the total fields utilized in the counting procedure, each cell present was recorded according to its type, and the number of grains associated with it were counted. In the remaining fields all cells except acidophils and small chromophobes, the most frequently occurring cells, were counted in order to make possible the counting of a relatively larger number of the less frequent cells and thereby improve the accuracy of the resulting calculated average grain counts. Percentage of total cells for the various cell types so counted were calculated according to the procedure of Mellgren (32).

Background grains were counted in areas of film overlying the blank portion of the glass slide surrounding each of the quantitated autoradiograms. From ten to seventeen fields of 3600 μ^2 were counted for each film. The background count was found to be a fraction of a grain per 36 μ^2 of film (from 0.5 to 0.8 grains/36 μ^2), corresponding roughly to the area of cytoplasm of the largest acidophils and approximately twice the area of most acidophils. The cytoplasm of most basophils would be about twice this area. The largest error which could be introduced by omitting the background counts from the calculations for average grain count per cell would be roughly one and one-half background grains. Since the results of this study are based on large differences in mean grain count, no attempt was made to measure cytoplasmic areas for the purpose of subtracting background counts from the final total counts.

The autoradiograms of unlabeled control sections were examined after 6 weeks' exposure to the film and found to produce no artifactual reduction of the emulsion.

OBSERVATIONS

Effect of Adrenalectomy on the Hypophysial Cell Types

In the pituitaries of the adrenalectomized rats acidophils were somewhat reduced in size and numbers compared with the normal animals. On the other hand, no obvious changes were noted in the number, size, or morphology of basophils following adrenalectomy. Both the thyrotrophs and the gonadotrophs, the two types

of basophils differentiated by Purves and Griesbach (33), maintained a normal appearance in respect to their size, shape, staining reactions, and distribution at 1 week after adrenalectomy.

The primary cytologic difference between the pituitaries of intact and adrenalectomized animals was the presence in the adrenalectomized animals of a number of cells which had outstandingly large nuclei, peculiar and polymorphous cell outlines, and cytoplasm which was devoid of either PAS-positive or acidophilic granules. These cells, however, gave a strong autoradiographic reaction. For these reasons the cells of this particular appearance were tentatively designated as "adrenalectomy cells" in a terminology analogous to that of "thyroidectomy cells" and "gonadectomy cells" for those cells which appear characteristically following thyroidectomy and gonadectomy, respectively (34). "Adrenalectomy cells" were present in all 8 of the injected adrenalectomized rats and in 2 adrenalectomized rats used as unlabeled controls. Examples of "adrenalectomy cells" are illustrated in Figs. 1 to 3, 6 to 10, and 12.

Definition of Cell Types as Designated in This Study

The preliminary observations led to a subdivision of the anterior hypophysial cells into seven types. Certain well defined criteria, some of them arbitrary, were formulated as requisite for an individual cell to be assigned to a particular group in order to provide as much objectivity and consistency as possible in differentiating the cell types during the grain-counting procedure. Because of the apparent significance of the chromophobic "adrenalectomy cell," the chromophobes were divided into three groups: cells with a profile characteristic of "adrenalectomy cells" composed one group; the chromophobes of minimal size were recorded in a separate group as "small chromophobes;" and all of those chromophobes which were larger than "small chromophobes" but lacked the peculiar outlines of "adrenalectomy cells" were designated "large chromophobes." For the purposes of the present study the basophils, or PAS-positive cells, were subdivided into three groups. A PAS-positive cell was assigned to the category of "gonadotrophs" or of "thyrotrophs" only if it presented a *typical* appearance for that particular cell type according to criteria defined below. PAS-positive cells which did not meet the morphological criteria (*vide infra*) for classification as typical gonadotrophs or thyrotrophs were enumerated under a third category designated here as "unclassified PAS-positive cells." Clearly, many of the cells necessarily assigned to this category may be functionally either gonadotrophs or thyrotrophs which were rendered atypical in appearance by the geometry of sectioning or, particularly in the case of the thyrotrophs, by a partial degranulation. The provision of a third category of basophils was made in order to ensure that, if a type of basophil should exist which is concerned with ACTH production, it might not be overlooked in the counting procedure. This provision was considered important because of the fact that degranulated basophils have been reported in the hypophysis after adrenalectomy (10, 17, 18). These degranulated basophil cells may be similar in appearance to the "abnormal basophil transitional cell" described by Crooke and Russell (35) in human adrenal hypofunction, and it has been suggested that such cells may be involved in ACTH production (10, 36–38).

The seven cell types are listed below with the cytologic criteria which were used in assigning an individual cell to one of the seven groups according to their appearance in the sections pretreated with ribonuclease.

1. "ADRENALECTOMY CELLS": A cell whose cytoplasm lacked acidophilic and PAS-positive granules, *i.e.* a chromophobe, was placed in this category only if the outline of the cell was irregular in such a way that cytoplasmic projections cupped around at least one other neighboring cell.

2. LARGE CHROMOPHOBES: Cells belonging to the second of three categories of chromophobic cells were differentiated from the "adrenalectomy cells," on the one hand, by lacking the particular morphologic feature of the cell outline which was a necessary criterion for the "adrenalectomy cells," namely, extension of the cytoplasm around neighboring cells; and, on the other hand, they were distinguished from a third group of chromophobes, the small chromophobes, on the somewhat arbitrary criterion of cell size. In order to be classified as a large chromophobe the cell had to have a quantity of cytoplasm extending from the nuclear membrane to the cell border a distance of $2\frac{1}{2}$ μ, or more, at one pole of the cell or the sum of this extent of cytoplasm at opposite poles

of the cell. It was not required that a cell have irregular outlines. The shapes of the cells in this group varied, with gradations, all the way from highly tortuous outlines through various polygonal or elongated shapes to more or less oval and rounded forms.

3. SMALL CHROMOPHOBES: The third group of chromophobes consisted of those cells which had a barely discernible, colorless rim of cytoplasm around the nucleus. The dividing line between small and large chromophobes was set at 2½ μ of cytoplasm from the nuclear membrane to the cell border. A cell with less than this amount of cytoplasm was classified as a small chromophobe. Due to the extremely small quantity of cytoplasm in these cells by definition, these cells were necessarily of a round or ovoid shape.

4. ACIDOPHILS: The single criterion used for assigning a cell to the category of acidophils was the presence of the staining reaction characteristic of acidophilic granules. The staining method utilized in this study resulted in a greenish-yellow coloration of the cytoplasm of acidophils, which was easily distinguished from the non-specific, dull, yellow or buff "background" color seen in cytoplasmic areas of PAS-positive cells between granulated areas and in chromophobes.

5. GONADOTROPHS: The cells recorded in this group were those PAS-positive cells having rounded or ovoid cell outlines. The degree of granulation was not important. While some cells were more fully packed with granules than others, the staining reaction of the gonadotrophs was always of a pink or rose color.

6. THYROTROPHS: PAS-positive cells having angular, irregular outlines were counted as thyrotrophs in this experiment only if the color of the cytoplasmic granules was a characteristic and distinct magenta. It was found that, with the staining method used in this study, the well granulated thyrotrophs had a typical deep magenta color and a characteristic cytoplasmic texture. The finely granulated magenta cytoplasm was punctuated by a pale buff or yellowish background giving a fenestrated appearance to the cytoplasm.

7. "UNCLASSIFIED" PAS-POSITIVE CELLS: Cells were placed in this group if they lacked the specific criteria defined above for classification as gonadotrophs or thyrotrophs. This means that the cells in this group were those which had a variable degree of PAS-positive granulation of a *pink* color but which did not present the clearly rounded outlines required for classification as a gonadotroph. In many cases the angular outlines of the cells were similar to the outlines of thyrotrophs, but if the cytoplasm was pink instead of magenta it was placed in this non-specific category instead of with the thyrotrophs.

Further Morphological and Tinctorial Characteristics of the Cell Types

In addition to the above cytologic criteria which were used for differentiating the cell types in performing the grain and cell counts, certain details of the morphological and tinctorial characteristics of the cells should be noted.

The "adrenalectomy cell" typically had a conspicuously large, hypochromatic nucleus and often displayed one or more large, intensely stained nucleoli. In the ribonuclease-digested preparations the nucleoli appeared as pale discs surrounded by a deep blue ring of heterochromatin (Fig. 8). The cytoplasm, devoid of specific granules of either the PAS-positive or acidophilic type, displayed an extremely fine, dust-like dispersion of ribonucleic acid (RNA), which tended to be somewhat more concentrated in areas adjacent to the nucleus and sometimes also in more peripheral regions of the cytoplasm. Removal of RNA in the digested sections resulted in a colorless, non-granular cytoplasm which can be described as having a water-clear appearance in the majority of these cells. In occasional cells there appeared to be some extremely fine, faint pink strands, similar in form and staining quality to the PAS-positive material seen in the Golgi region of acidophils in these preparations. The "adrenalectomy cells" varied widely in size; some of them fell within the size range of large chromophobes, while some approximated but did not quite equal the cytoplasmic area of the largest PAS-positive cells. A striking characteristic of the "adrenalectomy cell" was its tendency to insinuate projections of its cytoplasm between neighboring cells in an apparent attempt to maintain contact with the sinusoids along which the neighboring cells were aligned (Figs. 1 and 10). The "adrenalectomy cells" were not localized or concentrated preferentially in any part of the anterior lobe but were scattered singly throughout.

The large chromophobes were very variable in shape. Some large chromophobes were relatively rounded or ellipsoidal, while many of these cells

were triangular or irregularly polygonal. Large chromophobes were often very elongated. The size varied from that of small chromophobes, by definition, up to the size of some of the larger "adrenalectomy cells." Indeed, because of the morphological criterion which was used to differentiate "adrenalectomy cells" from large chromophobes for this study, it is likely that some "adrenalectomy cells" were necessarily placed in the category of large chromophobes when they did not show the phenomenon of cupping around a neighboring cell in that particular 2 μ thick slice of cell present in the section. The nucleus of large chromophobes was generally vesicular and eccentrically located in the cell. A well stained nucleolus was often present. The cytoplasm was usually rich in RNA. Some cells were completely filled with a diffuse, deep-blue stain while in some the juxtanuclear area of cytoplasm was clear. In many large chromophobes RNA was located in the area between the nucleus and the nearest border of the cell and, in addition, occupied the peripheral margins of the cytoplasm to varying extents. In the sections pretreated with ribonuclease the cytoplasm was colorless. Examples of large chromophobes are illustrated in Figs. 1 to 6, 8, 13 to 15, and 17.

Acidophils varied in appearance from very small round cells which consisted of a thin rim of cytoplasm around the nucleus, to ellipsoidal or elongated cells with an eccentric nucleus. The cytoplasmic RNA, when present, had a typically compact disposition and appeared as a thin, intensely stained formation intimately applied to the nuclear membrane or as a compact, crescentic plaque applied against, or parallel to, the cell border at the apical pole of the cell. Where the section demonstrated the orientation of the acidophil on a sinusoid, the nucleus was in a basal position, the negative Golgi image was supranuclear, and the apical crescentic plaque of RNA partially surrounded the Golgi area. The apical RNA formation was sometimes immediately adjacent to the Golgi region and sometimes was separated from it by a yellow-stained, granulated portion of cytoplasm. In some cells the RNA formations were present both around the nucleus and peripherally. In the Golgi area of some acidophils there could be discerned a pale pink material in the form of very thin strands or fibrils which sometimes appeared to be arranged in a reticulum (Figs. 4, 9, and 17). A PAS-positive component of the Golgi apparatus was not identified in the basophils; if present, it was obscured by the predominance of the PAS-positive specific secretory granules throughout the cytoplasm.

In ribonuclease-digested sections the PAS-positive basement membrane surrounding acidophils could be discerned as an exquisitely fine pink line against the yellow cytoplasm (for example, see Figs. 1 and 2). In undigested sections the dense blue peripheral RNA tended to obscure this delicate structure.

Gonadotrophs appeared to be very poor in stainable RNA. When it was present, the RNA appeared as a fine, dust-like deposition of stain in a variable zone around the nucleus or, in some cases, as a faint bluish wash over the pink cytoplasm. The nuclei of most gonadotrophs were very pale, with fine, dust-like chromatin and blurred or indistinct nuclear membranes. Some gonadotrophs had kidney-shaped nuclei (Figs. 6, 8, and 10). Nucleoli were sometimes seen (Figs. 5, 13, and 17).

The cytoplasmic RNA of the thyrotrophs often appeared as diffuse, dust-like staining more or less concentrated in areas adjacent to the nucleus and in other areas of the well granulated cytoplasm. In addition, some thyrotrophs possessed discrete, compact, and densely stained formations of irregular shape located within the body of the cytoplasm. The nuclei were vesicular, with crisp nuclear membranes, and they often displayed prominent nucleoli. Examples of thyrotrophs may be seen in Figs. 1, 3, 6, 11 to 13, and 17.

The "unclassified" PAS-positive cells were either very poor in stainable RNA or had a dust-like staining reaction localized near the nucleus or elsewhere in the cytoplasm. The nuclei resembled those of either thyrotrophs or gonadotrophs. Some "unclassified" PAS-positive cells are illustrated in Figs. 6 to 9, 11 to 13, 15, and 16.

The small chromophobes often occurred in groups of cells closely compacted together (Figs. 10 and 15) resembling the "Kernhaufen" (aggregates of nuclei with shared cytoplasm) of undifferentiated cells which Romeis described (39). Indeed, in the preparations containing RNA it was difficult to discern the cell borders since the diffuse blue stain tended to obscure them. In the ribonuclease-digested sections, however, it was possible to determine the individuality of the cells.

FIGURES 1 TO 17

Photomicrographs of autoradiograms of hypophyses from rats injected with tritiated glycine. The sections were cut at 2 μ, digested by ribonuclease, and stained by periodic acid–Schiff, metanil yellow, and toluidine blue according to procedure given in text. All the autoradiograms illustrated here were exposed for 6 weeks but were otherwise identically processed, and prepared from the same glands, as those autoradiograms exposed for 4 weeks which were quantitated in the study. The photomicrographs were taken by means of the 63 × dry objective lens, at a magnification of 1,140. Where cells are indicated in the photomicrographs by letters, the appropriate letters were placed over the nuclei of the cells.

FIGURE 1

From adrenalectomized rat, 90 minutes after injection with glycine-H^3. Two "adrenalectomy cells" (*A*) whose cytoplasm produced many autoradiographic grains, apposed to and partially encompassing some acidophils (*a*); the latter are arranged in frequently-seen palisade formation along a sinusoid. The cytoplasm of the "adrenalectomy cells" appears in places to be extending toward the sinusoid. Two other acidophils (*a'*) display fine pink (PAS-positive) basement membranes. Gonadotrophs (*g*) and a thyrotroph (*t*) are present, as well as 2 highly labeled large chromophobes (*c*). Some small chromophobes (*s*) are grouped adjacent to one adrenalectomy cell.

FIGURE 2

Adrenalectomized rat, 20 minutes postinjection. An "adrenalectomy cell" (*A*) capped on one side by an acidophil (*a*) and indented by a small chromophobe (*s*) in such a manner that it appears in a bicuspid shape. A number of acidophils may be seen to have pink basement membranes. Two large chromophobes (*c*) with well labeled cytoplasm and an elongated shape are parallel and adjacent to each other. A third large chromophobe (*c*) at lower right having a rounded shape is highly labeled. A gonadotroph (*g*), poorly labeled, shows a prominent negative Golgi image above the nucleus.

FIGURE 3

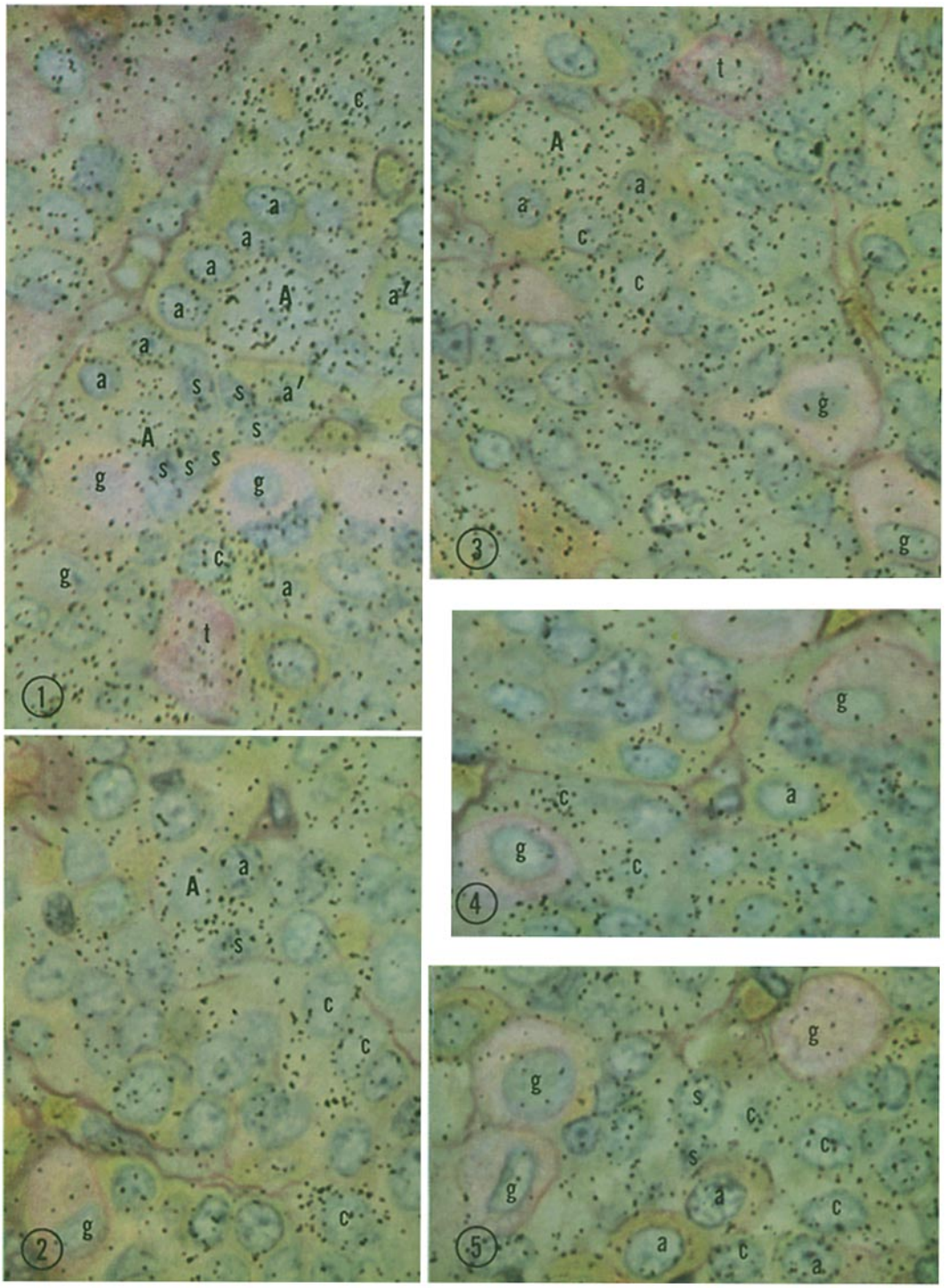
Adrenalectomized rat, 90 minutes postinjection. "Adrenalectomy cell" (*A*) with typically pale nucleus and clear cytoplasm producing many autoradiographic grains, here abuts directly on a sinusoid, and sinusoidal lining cell immediately above nucleus of (*A*). Two large chromophobes (*c*) give a strong autoradiographic reaction. A thyrotroph (*t*) has a characteristically normal appearance, a polyhedral shape, and granules which give a magenta staining reaction interspersed with buff-colored areas of background cytoplasm. Two typical, pale pink, rounded gonadotrophs (*g*) show the usual slight autoradiographic reaction. A number of acidophils (*a*) (yellow cytoplasm) are present in variable sizes and degrees of labeling.

FIGURE 4

Normal rat, 20 minutes after injection. Two large chromophobes (*c*) produced many autoradiographic grains. Two typical gonadotrophs (*g*) by contrast poorly labeled despite greater cytoplasmic area. This figure also illustrates an acidophil (*a*) in which the labeled protein is localized in the Golgi area; the fine PAS+ (pink) strands present in this region do not show clearly in the reproduction.

FIGURE 5

Normal rat, 15 hours after injection. Several large chromophobes (*c*) give a comparatively strong autoradiographic reaction. Three gonadotrophs (*g*); the nucleus of one does not appear in the section. Acidophils (*a*) of variable sizes, some relatively large. Some small chromophobes (*s*). Nucleoli may be found among all the cell types present. Shrinkage of this section has rendered the individual cells more clearly separate.


1
2
3
4
5

Results of Simple Inspection of the Autoradiograms

By simple inspection certain large differences in the labeling of the cells were apparent. At the first three postinjection intervals "adrenalectomy cells" in the adrenalectomized rats and chromophobes in both adrenalectomized and normal animals were the most heavily labeled cells in the hypophysis while, at 15 hours after injection, the tritium content of these cells was greatly reduced. Grain production by all cell types was obviously greater at 40 minutes and 90 minutes postinjection than at 20 minutes. Careful comparison of the autoradiogram used in the grain counts with that of the duplicate gland for each time interval showed that the autoradiographic results were very similar for the complete series of normal animals. The duplicate hypophyses from adrenalectomized rats, which were obtained only for the 20-minute and 15-hour intervals, corresponded closely to those used in the counts. The labeling of "adrenalectomy cells" at 15 hours was likewise greatly diminished compared to the labeling at 20 minutes in both the counted and the duplicate glands.

In a number of acidophils in hypophyses taken at the first three time intervals, the grain produc-

FIGURE 6

Adrenalectomized rat, 40 minutes after injection. Two strongly labeled "adrenalectomy cells"; their nuclei (*A*) are not easily visible in the photomicrograph. Acidophils (*a*), small chromophobes (*s*), thyrotrophs (*t*, lacking nuclei), "unclassified" PAS+ cells (*u*) surround the "adrenalectomy cells" and, on the sides opposite their nuclei, indent the cytoplasm to some extent. Two large chromophobes (*c*) display autoradiographic grains closely crowded in their relatively scant cytoplasm.

FIGURE 7

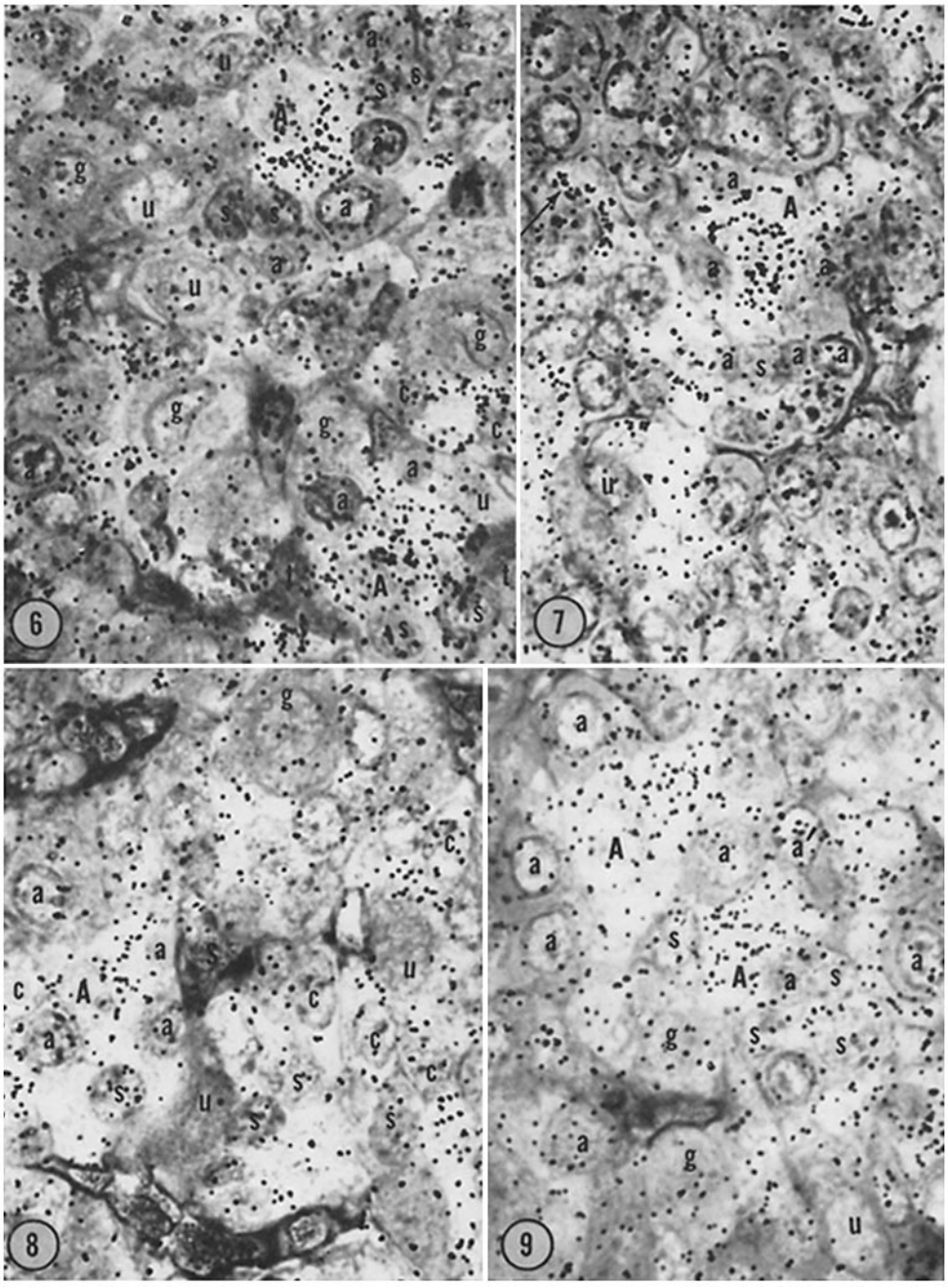
Adrenalectomized rat, 20 minutes postinjection. An "adrenalectomy cell" with a very pale nucleus (*A*) has a roughly L-shaped, densely labeled cytoplasm indented by several acidophils (*a*). An equally densely labeled crescent-shaped strip of chromophobic cytoplasm (arrow) the nucleus of which is not in the section. Well labeled segments of chromophobic cytoplasm are seen throughout these preparations, wedged between other cells, and, presumably, belong to large chromophobes or "adrenalectomy cells" whose nuclei lie in adjacent sections. An "unclassified" PAS+ cell (*u*) has a prominent nucleolus and the usual sparse autoradiographic grains. A small chromophobe (*s*) lies against the lower border of *A*.

FIGURE 8

Adrenalectomized rat, 20 minutes after injection. An "adrenalectomy cell" (*A*) with a very large nucleolus, illustrating the pale center and dark rim of nucleoli following ribonuclease digestion. This nucleolus also has an autoradiographic grain over its center. The cytoplasm of the "adrenalectomy cell" is indented by a small acidophil (*a*) around which it extends to impinge on a sinusoid (*S*) on its right border.
Several elongated adjacent large chromophobes (*c*); also, above, right, a large chromophobe (*c*) with a prominent nucleolus. Two "unclassified" PAS+ cells (*u*) almost devoid of autoradiographic grains. At upper center, a gonadotroph (*g*) with a kidney-shaped nucleus sometimes seen in this cell type, and nucleolus adjacent the nuclear membrane. Some small chromophobes (*s*) and acidophils (*a*) are lettered to clarify the shapes and position of neighboring cells.

FIGURE 9

Adrenalectomized rat, 20 minutes after injection. Two "adrenalectomy cells" (*A*). The PAS+ basement membranes of a number of acidophils (*a*) appear as thin dark lines surrounding the lighter (gray) cytoplasm of these cells. In the cytoplasm of one acidophil (*a'*) the PAS+ material associated with the Golgi region can be seen as a darker configuration in that area of the cytoplasm. Two gonadotrophs (*g*) and an "unclassified" PAS+ cell (*u*) are also present.


6
7
8
9

tion over the cytoplasm appeared to be concentrated over the Golgi region (Figs. 4, 16, and 17). At 15 hours, however, the reverse pattern was seen in many acidophils in which the Golgi region had few or no grains overlying it while many grains were concentrated over the granule-rich portion of the cytoplasm.

While the ribonuclease-digested sections were used in the grain-counting procedure in order to ensure against counting tritium that may have been incorporated into ribonucleic acid, no obvious difference in grain production could be noted by visual, microscopic comparison between the digested and non-digested sections of each hypophysis. Therefore, it appears that the incorporation of tritiated glycine into ribonucleic acid, if any, was not significant compared to its incorporation into protein.

An indication of the high degree of resolution known to be obtained by the use of tritium for autoradiography was seen in the labeling of nucleoli in digested, as well as non-digested, sections. It is known that amino acids and precursors of nucleic acids are incorporated by nucleoli in the chromatin associated with the nucleolus and also by the periphery of the nucleolus itself, causing a localization of autoradiographic grains on the periphery, or annulus, of the nucleolus in thin sections (40). This precise localization was seen in many nucleoli in the present study (for example, Fig. 17).

Cell Percentages

Table I presents the per cent of total cells constituted by each of the seven cell types in the pituitary sections of the 4 normal and 4 adrenalectomized rats. Examination of Table I indicates that, following adrenalectomy, the small chromophobes are increased in numbers, the acidophils and large chromophobes are re-

FIGURE 10

Adrenalectomized rat, 40 minutes after injection. Two irregularly star-shaped "adrenalectomy cells" (*A*); each has an "arm" of cytoplasm extending to contact the sinusoid which runs diagonally across the picture.
Several gonadotrophs (*g*); one at lower left has a kidney-shaped nucleus. A group of small chromophobes (*K*) resembling "Kernhaufen" of Romeis. Acidophils (*a*) show varying degrees of labeling by tritium; some have visible basement membranes.

FIGURE 11

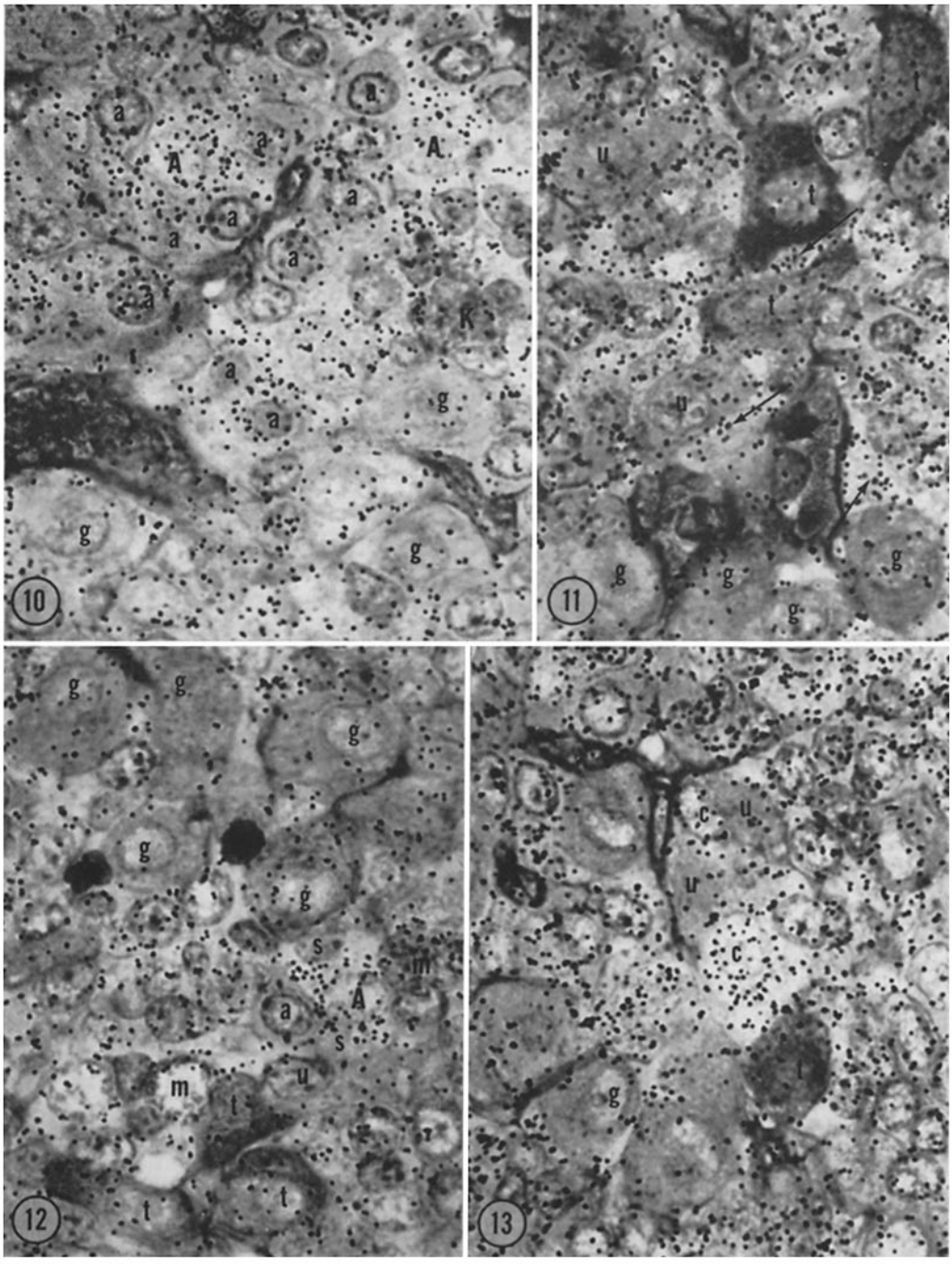
Adrenalectomized rat, 90 minutes after injection. Three thyrotrophs (*t*) illustrating the usual normal morphology and staining reaction and sparse autoradiographic reaction. Also some "unclassified" PAS+ cells (*u*) and gonadotrophs (*g*) are not heavily labeled. By contrast, segments of chromophobic cytoplasm (arrows) interposed between chromophilic cells give a dense autoradiographic reaction; this cytoplasm is considered to represent extensions of large chromophobes or "adrenalectomy cells" whose nuclei are not in the section.

FIGURE 12

Adrenalectomized rat, 20 minutes postinjection. An "adrenalectomy cell" (*A*) having a large nucleolus, indented by a small chromophobe (*s*) and acidophil (*a*) has a relatively small extent of cytoplasm in the section. Two mitotic figures (*m*) are present in chromophobes. Normal-appearing gonadotrophs (*g*), thyrotrophs (*t*), and "unclassified" PAS+ cells (*u*).

FIGURE 13

Adrenalectomized rat, 90 minutes after injection. A large chromophobe (*c*) of roughly triangular shape and considerable grain production. Its extensive cytoplasm and large, very pale nucleus gives this cell an appearance resembling "adrenalectomy cells" with which it may be transitional as it lacks only indentation by a neighboring cell for classification as an "adrenalectomy cell." Another large chromophobe (*c*) of small size is lodged between 2 "unclassified" PAS+ cells (*u*). A gonadotroph (*g*) at lower left has prominent nucleolus.


10
11
12
13

duced, while each of the three categories of PAS-positive cells is unchanged. At 1 week after adrenalectomy the "adrenalectomy cells" constituted from 1.3 to 1.8 per cent of the cells. While, in the normal rats, "adrenalectomy cells" were calculated to compose from 0.04 to 0.19 per cent of the total, the actual number of cells counted (from one to five cells in each case) was too few to give an accurate result. The presence of a few "adrenalectomy cells" in normal glands does not diminish the significance of the "adrenalectomy cells" as a specific response to adrenalectomy, when they become considerably increased in numbers. This situation is analogous to the presence of a few basophils of either the castration or thyroidectomy type commonly seen in normal pituitaries in small numbers but greatly increased by castration or thyroidectomy, respectively.

Occurrence of Mitotic Figures

Mitotic figures which appeared within the microscopic field during the grain counting procedure were noted. A total of 18 mitotic figures were counted in the normal pituitaries, and these were present in the following cells: small chromophobes, 10; acidophils, 5; and large chromophobes, 3. In the hypophyses of the adrenalectomized rats a total of 14 mitoses were counted, distributed as follows: small chromophobes, 11; acidophils, 1; and large chromophobes, 2.

Results of the Autoradiographic Grain Counts

The results of the grain counts are presented in Table II and represented graphically in Figs. 18 and 19. Inspection of the results shows that, following adrenalectomy, the "adrenalectomy cell" incorporated more tritiated glycine into protein and had a faster *rate* of accumulation and discharge of protein tritium than any other hypophysial cell type. The postinjection time intervals bracketed the rising and falling slopes of the plotted grain counts. The validity of the curves as representing relative rates of tritium incorpora-

FIGURE 14

Normal rat, 40 minutes after injection. Showing a number of well labeled large chromophobes (*c*). Many acidophils (*a*), some having prominent nucleoli; in some, the annulus, or outer shell, of nucleolus is labeled with 2 or more autoradiographic grains (*a'*). The size of acidophils, on the whole, appears greater than in adrenalectomized rats. Some gonadotrophs (*g*) also present.

FIGURE 15

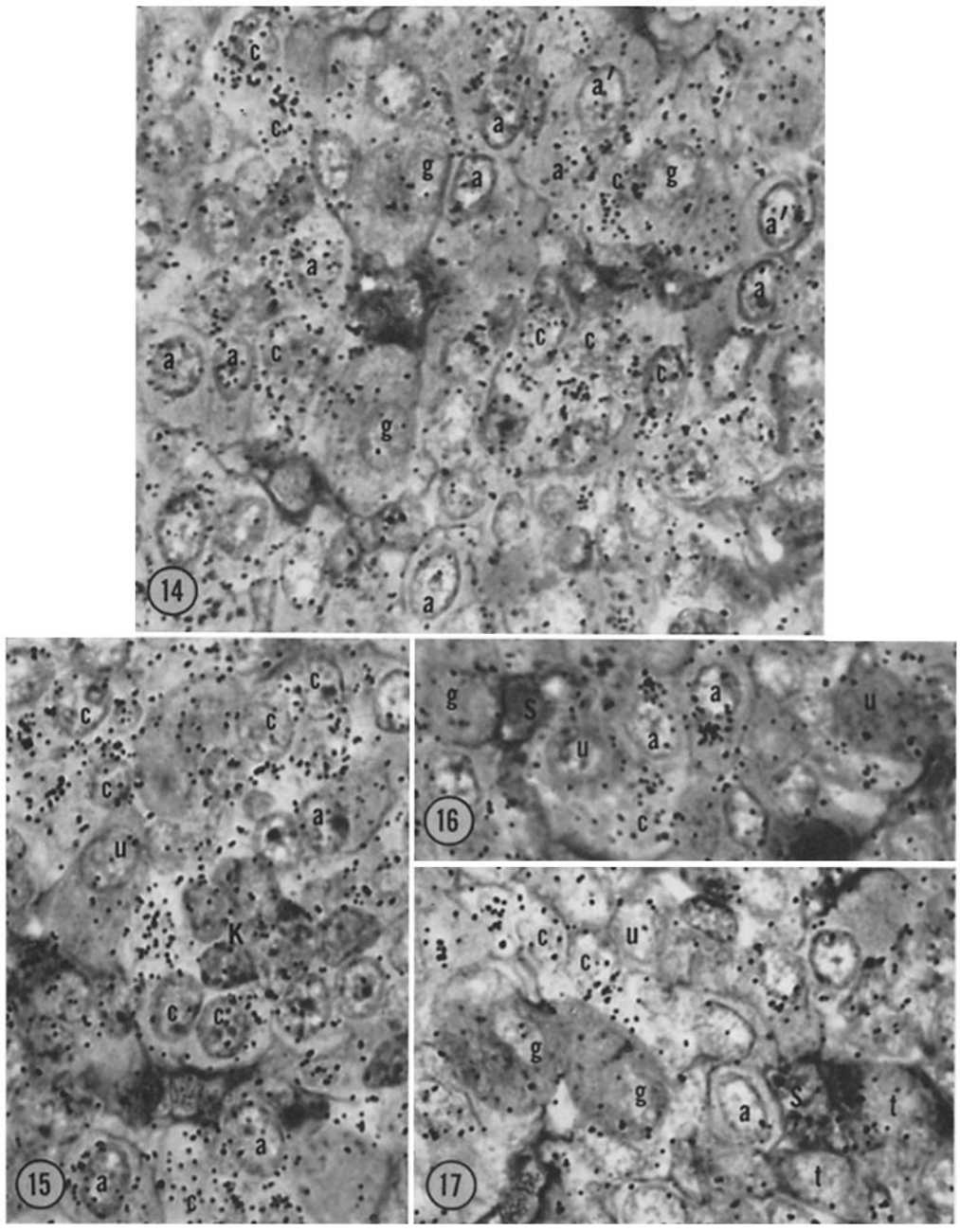
Normal rat, 40 minutes after injection. An "unclassified" PAS+ cell (*u*) poorly labeled, adjacent to a strongly labeled large chromophobe (*c*) of comparable size. Several other large chromophobes (*c*), acidophils (*a*) of variable degrees of labeling, and a group of small chromophobes (*K*) resembling "Kernhaufen" of Romeis.

FIGURE 16

Normal rat, 40 minutes postinjection. Showing 2 acidophils (*a*) in which the incorporated tritium is concentrated in the Golgi area, as sometimes seen in the earlier intervals after injection of the tritiated glycine. Large chromophobe (*c*) (nucleus not in section), "unclassified" PAS+ cells (*u*), a gonadotroph (*g*) on a sinusoid (*S*).

FIGURE 17

Normal rat, 20 minutes after injection. Two large chromophobes (*c*) have dense concentrations of autoradiographic grains over cytoplasm. An acidophil (*a*) has a reticulum of PAS+ material in Golgi area (just below nucleus); the only 2 autoradiographic grains over cytoplasm of this cell are localized in the Golgi region. This acidophil and 2 thyrotrophs (*t*) border on a sinusoid (*S*). There are two gonadotrophs (*g*). The one on the right has a single large nucleolus with appearance typical after removal of RNA; *i.e.*, a pale center and dark shell or annulus. The gonadotroph on its left has 2 nucleoli, one of which is labeled by 2 autoradiographic grains precisely localized on the annulus, a type of label often seen in these preparations. An "unclassified" PAS+ cell (*u*).


14
15
16
17

tion is based in part on the facts that the absorption of glycine injected intraperitoneally is undoubtedly extremely fast (41) and that injected glycine disappears from the blood into the tissues very rapidly; *i.e.*, within 10 to 15 minutes (42, 43).

Another significant finding is that the large chromophobes, a group of cells generally regarded as inactive in respect to hormone synthesis, had, after the "adrenalectomy cells," the largest incorporation of tritium and the sharpest rise and fall in tritium content. On the other hand, all the cell types other than "adrenalectomy cells" and large chromophobes had decay slopes remarkably similar to one another.

eyepiece net micrometer and divided by the number of nuclei in the field to give average grain counts per cell. From four to six fields were counted for each hypophysis. From the results in Table III it appears that adrenalectomy was not followed by an increase in amino acid incorporation by the intermediate lobe cells. The average number of cells per field was somewhat greater in the adrenalectomized animals; it may be inferred from this observation that adrenalectomy did not provoke the intermediate lobe cells to undergo hypertrophy 1 week postoperatively. As it is known that the intermediate lobe is the site of intermedin (MSH) production

TABLE I

Effect of Adrenalectomy on the Frequency of the Cell Types of the Anterior Hypophysis of the Rat

	Per cent of total cells							
	Normal rats				Adrenalectomized rats			
Cell type	20 min.*	40 min.	90 min.	15 hrs.	20 min.	40 min.	90 min.	15 hrs.
"Adrenalectomy cells"	0.16	0.19	0.14	0.04	1.6	1.3	1.8	1.5
Large chromophobes	6.6	8.4	9.2	7.9	4.1	3.4	5.1	3.2
Small chromophobes	40.8	31.4	43.7	40.4	48.5	60.4	49.1	48.5
Acidophils	40.3	40.0	35.4	37.2	34.7	21.4	30.2	35.9
Gonadotrophs	3.7	6.4	3.8	4.5	4.1	5.0	3.6	4.1
Thyrotrophs	1.9	2.3	0.84	1.8	0.67	1.7	1.9	1.1
"Unclassified" PAS + cells	6.4	13.5	7.0	8.2	6.3	6.8	8.4	5.7

* Time interval between injection of tritiated glycine and sacrifice. Each time interval represents 1 rat.

Comparison of the grain counts obtained for any one type of cell in the normal rats with that for the same type of cell in the adrenalectomized rats does not reveal any effect of adrenalectomy on tritium incorporation or turnover in any of the cell types.

It may be noted that the average grain counts for the small chromophobes, although small, are definitely above background. The cytoplasmic area of these cells is, at most, a few square microns; the background for this area of film was a small fraction of one grain.

Table III summarizes the results for grain counts in the autoradiograms of ribonuclease-digested sections of intermediate lobes of hypophyses of normal and adrenalectomized rats. Because of the indistinctness of the cell borders in this lobe of the gland, and because the cells are of one type, namely, well granulated PAS-positive cells, the grains overlying cytoplasm were counted within the field delimited by the

in the rat, the finding in the present study that adrenalectomy had no effect on the mean grain count per cell in the absence of hypertrophy of the intermediate lobe indicates that MSH synthesis was not stimulated by adrenalectomy of 1 week's duration.

DISCUSSION

The methods which have previously been successfully applied to the demonstration of hormone production by individual pituitary cell types can be classed into four general categories: (*a*) Observation of characteristic cytological response to alteration of hormone production; (*b*) correlation of the numbers and secretory granule content of a particular cell type with known hormone content of the gland; (*c*) use of specific histochemical techniques; (*d*) studies of cell types by means of the electron microscope.

It has been shown by these methods that the group of cells designated as "basophils," the

TABLE II

Incorporation of Tritiated Glycine into Cytoplasm of Cells of the Anterior Hypophysis in Normal and Adrenalectomized Rats

Mean No. of autoradiographic grains per cell and total number of cells counted for each of seven cell types in ribonuclease-digested sections after intraperitoneal injection of tritiated glycine.

Time between injection and sacrifice	"Adrenalectomy cells"			Large chromophobes			Small chromophobes		Acidophils			Gonadotrophs			Thyrotrophs			"Unclassified" PAS+cells		
	Mean*	SD‡	No. of cells	Mean	SD	No. of cells	Mean	No. of cells	Mean	SD	No. of cells	Mean	SD	No. of cells	Mean	SD	No. of Cells	Mean	SD	No. of cells
Normal rats																				
20 min.	22.2	±11.1	5	10.7	±5.9	202	1.5	169	3.4	±2.8	167	6.5	±3.6	114	4.7	±2.9	58	5.3	±3.4	200
40 min.	36.8	±14.5	4	17.1	±8.2	179	2.1	84	5.6	±3.7	107	7.1	±3.6	136	7.3	±4.5	50	6.8	±4.5	289
90 min.	38.8	±4.7	4	18.5	±8.0	263	2.8	153	6.3	±3.7	124	7.6	±4.1	108	6.2	±3.4	24	7.5	±4.6	200
15 hrs.	15	—	1	10.4	±5.6	204	2.2	125	5.8	±4.0	115	6.8	±3.5	117	8.2	±4.3	47	6.5	±4.0	213
Adrenalectomized rats																				
20 min.	31.3	±11.6	75	11.8	±7.3	189	1.3	273	2.7	±2.2	195	4.6	±3.3	191	5.4	±4.0	31	5.1	±3.8	288
40 min.	46.7	±14.7	53	18.4	±11.2	141	2.3	313	6.1	±4.2	111	7.7	±4.4	209	8.4	±6.0	72	8.2	±3.8	285
90 min.	45.9	±17.1	51	19.7	±11.1	142	2.9	174	5.9	±3.4	107	9.7	±5.0	99	13.4	±6.4	52	11.3	±6.9	232
15 hrs.	20.1	±7.2	61	9.6	±4.9	125	1.9	227	4.4	±2.6	168	8.1	±4.4	161	8.9	±6.2	43	7.9	±4.3	227

* Mean No. of grains per cell overlying cytoplasm.

‡ Standard deviation of the distribution.

granules of which are periodic acid–Schiff positive, is the site of production of the three glycoprotein hormones, *viz.*, follicle stimulating hormone (FSH), luteinizing hormone (LH), and thyrotrophin (TSH) (reviewed by Pearse, 44). The acidophil cells, on the other hand, are known to produce lactogenic hormone and growth hormone (44, 45).

be responsible for the production of one of five protein hormones of the hypophysis.

The third major group of cells, the chromophobes, has generally been regarded as representing the depleted or resting stage of cells belonging to one of the two major chromophil groups (53, 44, 54).

Some of the more recent studies attempting

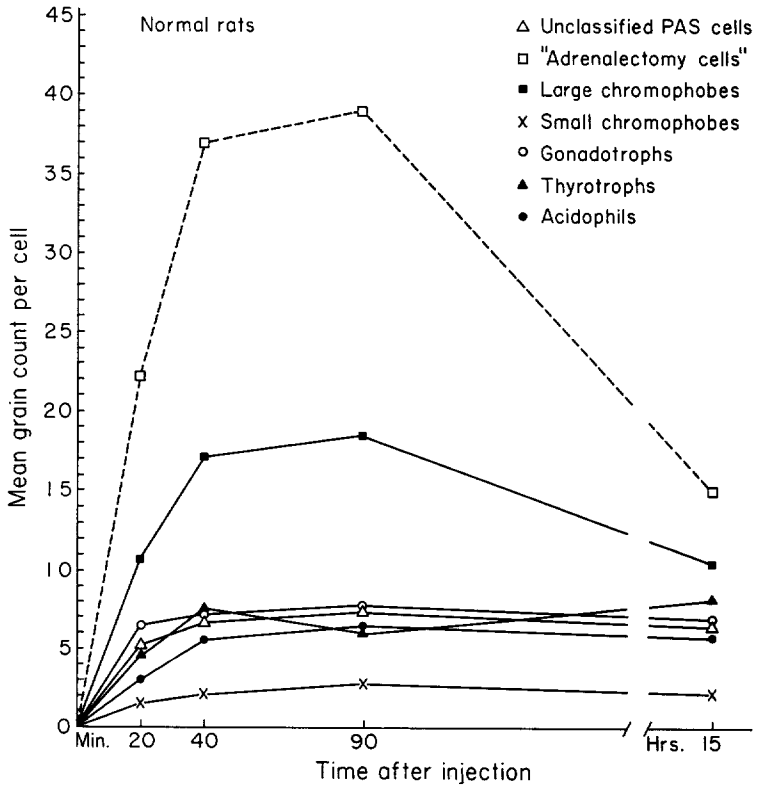


FIGURE 18

Rates of incorporation and loss of tritium by cells of anterior hypophysis after intraperitoneal injection of tritiated glycine in normal rats. The values for the "adrenalectomy cells" are connected by a dashed line because these values represent only one to five cells at each time interval.

The basophils have, in turn, been subdivided into three morphologically distinct subtypes in the rat hypophysis, each of which is considered to be responsible for the production of one of the three glycoprotein hormones (33, 46–50). In rats two types of acidophils are recognized which are believed to be the sites of production of growth hormone and lactogenic hormone, respectively (51, 52). Thus, we have at present five different types of chromophil cells (three types of basophils and two types of acidophils), each considered to

to correlate cell population and staining with hypophysial ACTH content conclude that ACTH is associated with the acidophils (1–7). Other investigators have noted a correlation between ACTH activity and basophil population or cytology (8–14). Marshall (15) noted the localization of the fluorescent antibody to ACTH in basophil cells of pig hypophysis, but the specificity of this reaction was questioned by a later study (16). Several studies have confirmed the diminution in numbers and staining reaction of both

types of chromophils following adrenalectomy without drawing conclusions as to the site of ACTH production (17–19). Halmi and Bogdanove (20, 21) demonstrated a striking lack of correlation between pituitary content of ACTH and granulation of both acidophils and basophils and concluded that the site of production and storage of ACTH could not be convincingly possible by development of a procedure for staining the pituitary sections through the autoradiographic stripping film, thus allowing simultaneous evaluation of grain production in the film and identification of the underlying cell type. By the use of this method, it became readily apparent that at 1 week following adrenalectomy a unique cell type is present which synthesizes

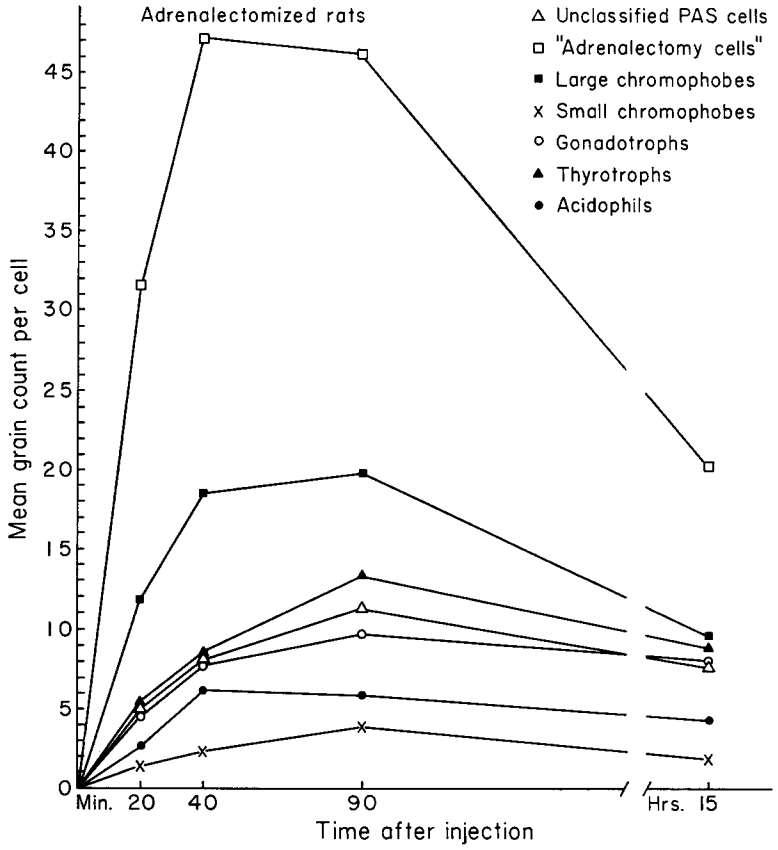


FIGURE 19

Rates of incorporation and loss of tritium by cells of anterior hypophysis after intraperitoneal injection of tritiated glycine in adrenalectomized rats.

established with present histophysiologic technics. Using the electron microscope, Farquhar (55) observed changes in colloid-containing microfollicles in response to cortisone administration or partial adrenalectomy and on this basis suggested that the cells lining the follicles may be related to ACTH production.

The application of the autoradiographic method to the problem of localization and identification of the ACTH-producing cells was made and secretes protein at a rate greater than that of any cell type in either the normal or adrenalectomized animal. As it is well known that adrenalectomy provokes an increase in the rate of ACTH synthesis and release, which is almost 30 times normal at one week following operation (23), it is concluded that the "adrenalectomy cell" described in this study is involved in ACTH production in the adrenalectomized rat. The answer to the question as to why this "adrenal-

ectomy cell" appears not to have been identified in previous studies of the rat hypophysis following adrenalectomy may be found in the peculiar morphologic and tinctorial characteristics of this cell. Not only is the cytoplasm chromophobic (except in respect to specific RNA-staining), but it tends to be compressed between and indented by neighboring cells in such a way as to render it inconspicuous. Indeed, the unique advantage of the autoradiographic approach to this problem, aside from demonstrating the high protein synthetic activity of these cells, was in calling the investigator's attention to the existence of these cells by virtue of the denser concentration of autoradiographic grains over these cells as compared to surrounding cells. At magnifications of less than 1,000 the delineation of the cell borders is very difficult to determine. At lower magnifications the only striking characteristic of the cell, in the absence of the autoradiogram, is the very large, hypochromatic nucleus.

TABLE III

Effect of Adrenalectomy on the Incorporation of Tritiated Glycine into Cytoplasm and on Cell Size in the Intermediate Lobe of the Rat Hypophysis

	Normal rats				Adrenalectomized rats			
	20 min.*	40 min.	90 min.	15 hrs.	20 min.	40 min.	90 min.	15 hrs.
Average No. of grains per cell	4.5	8.9	10.0	8.3	4.4	7.4	7.9	5.9
Total No. of cells counted	187	176	163	199	179	139	224	189
Average No. of cells per field of 3600 μ^2	37.4	29.3	27.0	33.2	35.8	34.8	37.2	37.8

* Time interval between injection of tritiated glycine and sacrifice.

It is not possible to ascertain the relationship of the "adrenalectomy cell" described in this experiment to the hypertrophic amphophils which Mellgren (10) reported in increased numbers in the hypophyses of adrenalectomized rats. The morphologic characteristics of the hypertrophic amphophils are not described in detail in his paper, other than to indicate that they are chromophobes by the eosin-aniline blue staining method used and are differentiated from the other chromophobes by having nuclear diameters greater than 7.6 × 7.6 μ, or 10.2 × 5.1 μ. Mellgren concluded that the basophils were responsible for ACTH production and that the hypertrophic amphophils were derived by further degranulation of large, poorly granulated basophils; the dividing line between them was indistinct as early as 24 days postoperatively. One reason for the difficulty in determining the relationship between Mellgren's hypertrophic amphophils and the "adrenalectomy cells" of the present study is that basophils stained by aniline blue are not exactly equivalent to "basophils" which give a positive reaction in the application of the PAS method. In particular, as Pearse (56) observed, many poorly granulated PAS-positive basophils are chromophobic in aniline blue–stained preparations. Thus, it is not possible to know whether the hypertrophic amphophils would be wholly, or in part, poorly granulated PAS-positive cells or truly chromophobic by the PAS method. In the present experiment it was observed that nuclear size, alone, was not a sufficient criterion for differentiating "adrenalectomy cells" from PAS-positive cells; a number of "unclassified" PAS-positive cells, some of which were poorly granulated, in addition to some gonadotrophs, had nuclei in the size range of nuclei of "adrenalectomy cells."

A description of a cell type more closely approaching that of the "adrenalectomy cell" is to be found in a paper by Reese, Koneff, and Akimoto (17). These authors observed, in pituitaries taken from adrenalectomized rats and stained by Koneff's Mallory-azan method, numerous cells having a large, light, vesicular nucleus and a tendency to occupy the space between neighboring cells and often appearing partially to engulf them. Cytologically, these cells appeared stimulated and had an extensive, elaborate Golgi network. These cells were chro-

mophobes in respect to aniline blue staining but were interpreted by the authors to be basophils in the last stages of granular depletion and to represent an attempt to compensate for the degenerative changes seen in many basophils. In a later paper, Koneff, Holmes, and Reese (57) reported that maintenance of the adrenalectomized rats on 1 per cent sodium chloride in drinking water prevented this degranulation due to excessive activity of basophil cells.

The present observations do not seem to confirm the suggestion (55) that the "corticotrophs" of the rat pituitary may be the cells lining colloid cysts or microfollicles and which were seen by electron microscopic observations to be characterized by a paucity of formed elements (secretory granules, mitochondria, Golgi material, endoplasmic reticulum). The "adrenalectomy cells" were not localized on colloid cysts or follicles of sufficient size to be detected in the light microscope; the cells lining such follicles, whether small chromophobes, large chromophobes, or acidophils, generally were poorly labeled in both normal and adrenalectomized animals.

The conclusion that the "adrenalectomy cell" described in the present study is involved in ACTH production is strengthened by the observation that no other cell type was stimulated to increased protein turnover in response to adrenalectomy coupled with the fact that ACTH is the only hormone of the anterior pituitary known to be stimulated by removal of the adrenals. Indeed, it is well known that, at longer postoperative intervals, growth is retarded, thyroid and accessory sex organs show histological signs of atrophy and are lighter (10), and pituitary stores of thyrotrophin and gonadotrophins are lowered (13). Rats, cats, and dogs maintained in *chronic* adrenal insufficiency undergo cessation of growth and depression of reproductive and thyroid activity, deficiencies shown to be secondary to pituitary insufficiency as they were relieved by injection of hypophysial extracts but not by adrenal cortical hormone therapy (58).

Apart from the discovery of the "adrenalectomy cell" perhaps the most important result of the application of the autoradiographic method to hypophysial cytology is the demonstration that the cells designated here as large chromophobes are more active in the synthesis and secretion of protein than either acidophils or basophils. This finding contradicts the classical view that acidophils and basophils are the active secretory elements of the pituitary while the chromophobes are the resting, reserve, or depleted stages of the two main chromophil cell types and that the chromophobes do not produce a physiological secretion (53). In addition to the autoradiographic results presented here, further evidence that the large chromophobes are functionally active cells is derived from the rich cytoplasmic RNA content noted in these cells in the present study. It is well known from the work of Brachet (59), Caspersson (60), and others, that the amount of RNA in the cytoplasm of various types of tissues correlates with the protein synthetic activity of the tissue. Ficq and Brachet (61) have demonstrated this correlation using the autoradiographic approach. The instrumental role of the endoplasmic reticulum, represented at the light microscope level by cytoplasmic RNA, in protein synthesis has been demonstrated (62). Utilizing specific staining methods for RNA, Desclin (63), in a report on the hypophyses of rats and guinea pigs; Herlant (64), studying the human pituitary; Tuchmann-Duplessis and Labat (65), in a study of mouse pituitaries; and Wolfe (66), employing rats, reported that acidophils and chromophobes were richest in RNA while basophils stained only weakly or not at all. All but one of these authors considered that in normal pituitaries the acidophils contained the greatest quantities of RNA. Herlant reported that RNA was most abundant in the cytoplasm of chromophobes and somewhat less in acidophils. In conditions which provoke an intense stimulation of secretion of lactogenic hormone—namely, pregnancy, lactation, and estrone treatment—the cytoplasmic basophilic formations of acidophils and particularly of chromophobes are greatly increased (63, 67). Wolfe (66) in his careful cytological analysis of the response to estrogen injection presented evidence that both acidophils and some chromophobes participate in prolactin synthesis. Thus an unassessable number of the chromophobes may be functionally equivalent to acidophils.

This conclusion is consonant with, though not identical with, Severinghaus's subdivision of the chromophobes into two groups, one belonging to the acidophil series and one to the basophil series on the basis of the resemblance of the Golgi apparatus to that typical of either acidophils

or basophils (68). Severinghaus postulated that the chromophobe represents a reserve or "mother" cell which, when stimulated, acquires either basophilic or acidophilic granules. The granules are regarded as representing hormone precursors. The granulated cells are assumed then to function as active hormone-secreting cells through concomitant discharge and elaboration of granules. On occasion, it is said, all the granules are discharged and the cell returns to the chromophobic state where it remains as a reserve cell until it begins a new accumulation of granules of the type specific for that particular cell (53). The existence among the chromophobes of cells belonging to either the acidophil or basophil series is confirmed by the observations of Farquhar and Rinehart (48). Using the electron microscope, these authors observed that there are very few true chromophobes; that most cells which would be classed as chromophobes by light microscopy had variable numbers of granules of the size characteristic of either acidophils (350 mμ) or basophils (140 to 200 mμ).

The results of the present autoradiographic and cytochemical study suggest that there may be yet another type of chromophobe, the one concerned with ACTH production. If this postulation is correct, it appears that the "large chromophobes" may be composed of three main types of cells: those chromophobes related to acidophils, those related to basophils, and a third group which is responsible for ACTH production in the normal animal. To what extent the chromophobes of minimal size, designated in this study as "small chromophobes," are likewise subdivided cannot be surmised; that is, it is not known whether a portion of the small chromophobes may be truly undifferentiated cells in the adult animal, or whether their potentiality to enlarge and become one or the other specific cell type is already fixed. The increase in number of small chromophobes following adrenalectomy in the present experiment in the absence of pycnotic or dying cells took place at the expense of acidophils and of large chromophobes. Therefore the small chromophobes in the adrenalectomized animals may be constituted in part of cells which had previously been differentiated acidophils and large chromophobes and had undergone atrophy.

The observation of a new type of cell, the "adrenalectomy cell," which appeared in response to adrenalectomy, raises the question of the origin of this cell; *i.e.*, the question of which cell type is responsible for ACTH production in the normal rat. "Adrenalectomy cells" were scarce in normal pituitaries but at one week following adrenalectomy constituted approximately 1.5 per cent of total cells, a tenfold increase over the numbers found in the normal. As mitotic figures were not seen in "adrenalectomy cells," they probably arose by transformation of one of the cell types of the normal gland. It is proposed here, for the following reasons, that the most likely possibility is that a portion of the large chromophobes gives rise to the "adrenalectomy cells" and is the site of ACTH secretion in the normal animal:

1. The "adrenalectomy cells" and many of the larger chromophobes are identical with respect to tinctorial and morphologic characteristics except for the criterion more or less arbitrarily set up for the purpose of discriminating the "adrenalectomy cells" from the large chromophobes as a group, namely, a cell outline sufficiently irregular and extensive as to encompass neighboring cells. That is to say, there is a gradual transition between large chromophobes and "adrenalectomy cells." In many cases it would only be necessary for a large chromophobe to enlarge its already irregular outline in such a way as to interpose some cytoplasm between neighboring cells in order to qualify for classification as an "adrenalectomy cell."

2. At 1 week after adrenalectomy transitional types between "adrenalectomy cells" and any cell type other than large chromophobes are not seen in important numbers. The only other cell types which show irregular cell outlines, and occasionally can be seen to engulf partially a neighboring cell, are the thyrotrophs and those cells grouped in this study as "unclassified" PAS-positive cells. On a morphological basis one can visualize these cells becoming transformed into "adrenalectomy cells" by complete degranulation, loss of PAS positivity, and increased accumulation of cytoplasmic RNA. However, the observations, while not disproving it, argue against the likelihood that this actually happens. While in a few instances it was observed that very poorly granulated PAS-positive cells with moderate amounts of RNA showed cell outlines indented by neighboring cells, these cells were not well labeled with tritium. If PAS-positive

cells are normally involved in ACTH production, then, barring a complete degranulation of *all* the cells involved prior to 1 week postoperatively (which is unlikely in view of the fact that at this postoperative interval the increased ACTH production in response to adrenalectomy is not fully developed), one should see a strong autoradiographic reaction over some of those PAS-positive cells. On the contrary, the autoradiographic pattern of the PAS-positive cells was not affected by adrenalectomy; furthermore, their morphology and relative numbers remained unchanged.

3. Acidophils are clearly not the site of ACTH production. Their response to adrenalectomy was a diminution in size and numbers while the average grain count per cell was not affected.

4. The decrease in numbers of large chromophobes after adrenalectomy is consistent with the possibility that they give rise to "adrenalectomy cells." Although the major part of their decreased numbers may be due to the reversion of chromophobes of the acidophil series into the ranks of small chromophobes, some of the loss may be due to transformation of "ACTH chromophobes" into "adrenalectomy cells." One can only speculate as to whether or not a segment of the small chromophobes may have been stimulated to develop into ACTH-producing large chromophobes.

5. In light of evidence that ACTH has the highest rate of turnover in the normal animal of all the hypophysial hormones so far studied, the high protein synthetic activity of the large chromophobes in the *normal* animals provides support for the suggestion that they may be the site of ACTH production. The half-life of growth hormone in the plasma has been estimated to be about 9 hours (69); the estimated half-life of FSH is 2 hours, and that of TSH is 1 hour (70); while the half-life of ACTH has been found to be about 5 minutes (23). Since, in a state of endocrine balance, the half-life of a hormone in the plasma is related to the rate of formation and secretion in the pituitary, it would be expected that the site of ACTH production in the pituitary would be associated with the highest rate of uptake and loss of tritiated amino acid. The results of the grain count for the normal glands indicate that this site is the large chromophobe. How large a portion of the large chromophobes is constituted by the ACTH-producing cells is a problem which must await development of methods for distinguishing between the individual chromophobes as to their actual functional type.

While it is clear from the results of this study that the "adrenalectomy cells" are a site of production of ACTH, the possibility remains that, in the adrenalectomized animals, ACTH production may also be carried on by a portion of the large chromophobes. In addition to the fact that there is a gradual morphologic transition between "adrenalectomy cells" and large chromophobes, it is known that ACTH (Armour Chemical Div., Armour and Co., Chicago LA-1-A equivalent) constitutes about 13 per cent of the dry weight of the pituitary of the rat at 1 week after adrenalectomy (23). As the "adrenalectomy cells" constitute only about 1.5 per cent of total cells at 1 week after adrenalectomy, their numbers, alone, do not seem sufficient to account for all of the ACTH present in the gland.

The observation in the present study that acidophils were diminished in size and numbers following adrenalectomy is in accord with a number of previous reports (71, 17, 11, 19). However, the finding of no obvious changes in the number, size, or morphology of basophils following adrenalectomy is in contrast to the results of several other studies in which the number and morphology of basophils were affected by adrenalectomy (58, 17, 10, 11, 18, 13). It is to be noted that the latter studies were based on longer postoperative intervals than the present experiment.

This investigation was supported by research grant A-4041 from the United States Public Health Service, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and by an institutional grant from the Southwestern Medical Foundation-Dreyfuss Estate Fund to the University of Texas Southwestern Medical School.

Received for publication, August 14, 1962.

BIBLIOGRAPHY

1. Heinbecker, P., and Rolf, D., Hypophyseal eosinophil cell and insulin sensitivity, *Am. J. Physiol.*, 1944, **141**, 566.
2. Finerty, J. C., and Briseno-Castrejon, B., Quantitative studies of cell types in the rat hypophysis following unilateral adrenalectomy, *Endocrinology*, 1949, **44**, 293.
3. Brokaw, R., Briseno-Castrejon, B., and

FINERTY, J. C., Quantitative studies of cell types in the rat hypophysis following prolonged periods of unilateral adrenalectomy, *Texas Rep. Biol. and Med.*, 1950, **8**, 312.

4. FINERTY, J. C., HESS, N., and BINHAMMER, R., Pituitary cytological manifestations of heightened adrenocorticotrophic activity, *Anat. Rec.*, 1952, **114**, 115.
5. SOULAIRAC, A., DESCLAUX, P., SOULAIRAC, M. L., and TEYSSEYRE, J., Action de la corticostimuline sur le comportement sexuel du rat mâle normal et étude concomitante des modifications de système nerveux central et de l'hypophyse antérieure, *J. Physiol. (Paris)*, 1953, **45**, 527.
6. DESCLAUX, P., SOULAIRAC, A., and CHANÉAC, H., Origine de l'hormone corticotrope à partir des cellules éosinophiles de l'hypophyse, *Compt. rend. Soc. biol.*, 1953, **147**, 44.
7. RENNELS, E. G., The effects of a severe scald on pituitary cytology in the albino rat, *Acta Endocrinol., Suppl.* **51**, 1960, 53.
8. SMELSER, G. K., Differential concentration of hormones in the central and peripheral zones of the bovine anterior pituitary gland, *Endocrinology*, 1944, **34**, 39.
9. GIROUD, A., and MARTINET, M., Sur l'origine de l'hormone corticotrope et de l'hormone gonadotrope, *Compt. rend. Soc. biol.*, 1948, **142**, 734.
10. MELLGREN, J., Experimental investigation into the genesis of the adrenogenital syndromes by analysis of the morphology of the pituitary in hyper- and hypofunction of the adrenal cortex in rats, *Acta Path. et Microbiol. Scand.*, 1948, **25**, 284.
11. TUCHMANN-DUPLESSIS, H., Structural changes in the anterior pituitary with special reference to the adrenal cortex. *Ciba Found. Colloq. Endocrinol.*, 1952, **4**, 33.
12. KALLMAN, R. F., and GORDON, A. S., Morphologic effects produced by the implantation of steroid hormone pellets near the hypophysis, *Anat. Rec.*, 1954, **118**, 185.
13. KNIGGE, K. M., Cytology and hormone content of rat pituitary glands following adrenalectomy, *Proc. Soc. Exp. Biol. and Med.*, 1957, **94**, 640.
14. ROCHEFORT, G. J., and SAFFRAN, M., Distribution of adrenocorticotrophic hormone in the pituitary gland, *Canad. J. Biochem.*, 1957, **35**, 471.
15. MARSHALL, J. M., Localization of adrenocorticotrophic hormone by histochemical and immunochemical methods, *J. Exp. Med.*, 1951, **94**, 21.
16. CRUICKSHANK, B., and CURRIE, A. R., Localization of tissue antigens with the fluorescent antibody technique: application to human anterior pituitary hormones, *Immunology*, 1958, **1**, 13.
17. REESE, J. D., KONEFF, A. A., and AKIMOTO, M. B., Anterior pituitary changes following adrenalectomy in the rat, *Anat. Rec.*, 1939, **75**, 373.
18. BACHRACH, D., KOVACS, K., DAVID, M., HORVATH, E., and KORPASSY, B., Morphology of the anterior pituitary in increased ACTH production in the rat, *Acta Morphol. Acad. Sc. Hung.*, 1954, **4**, 429.
19. FIELD, E. J., The effects of adrenalectomy on the anterior lobe of the pituitary gland of the rat, *J. Anat.*, 1958, **92**, 137.
20. HALMI, N. S., and BOGDANOVE, E. M., Effect of thyroidectomy on ACTH content of rat adenohypophysis, *Proc. Soc. Exp. Biol. and Med.*, 1951, **77**, 518.
21. HALMI, N. S., and BOGDANOVE, E. M., Effect of estrogen-treatment and castration on ACTH content of rat adenohypophysis, *Proc. Soc. Exp. Biol. and Med.*, 1951, **78**, 95.
22. FITZGERALD, P. J., EIDINOFF, M. L., KNOLL, J. E., and SIMMEL, E. B., Tritium in radioautography, *Science*, 1951, **114**, 494.
23. GEMZELL, C. A., VAN DYKE, D. C., TOBIAS, C. A., and EVANS, H. M., Increase in the formation and secretion of ACTH following adrenalectomy, *Endocrinology*, 1951, **49**, 325.
24. HAYS, E. E., and STEELMAN, S. L., Chemistry of the anterior pituitary hormones, *in* The Hormones, (G. Pincus and K. V. Thimann, editors), New York, Academic Press, Inc., 1955, **3**, 201.
25. LI, C. H., The relation of chemical structure to the biologic activity of pituitary hormones, *Lab. Inv.*, 1959, **8**, 574.
26. GOLDSWORTHY, P. D., WINNICK, T., and GREENBERG, D. M., Distribution of C^{14} in glycine and serine of liver protein following the administration of labeled glycine, *J. Biol. Chem.*, 1949, **180**, 341.
27. ARNSTEIN, H. R. V., The metabolism of glycine, *Adv. Protein Chem.*, 1954, **9**, 1.
28. SIPERSTEIN, E. R., Histochemical demonstration of glycogen in the mouse pituitary. *Proc. Soc. Exp. Biol. and Med.*, 1955, **88**, 296.
29. PEARSE, A. G. E., Histochemistry, Theoretical and Applied, Boston, Little, Brown and Company, 2nd edition, 1960.
30. LILLIE, R. D., Histopathologic Technic and Practical Histochemistry, New York, Blakiston Division, McGraw-Hill Book Company, Inc., 2nd edition, 1954.

31. BERGERON, J. A., Controlled staining of autoradiographs, *Stain Technol.*, 1958, **33**, 221.
32. MELLGREN, J., The anterior pituitary in hyperfunction of the adrenal cortex, *Acta Path. et Microbiol. Scand.*, 1945, Suppl., 60.
33. PURVES, H. D., and GRIESBACH, W. E., The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus-Hotchkiss staining for glycoprotein, *Endocrinology*, 1951, **49**, 244.
34. ZECKWER, I. T., Morphological changes in the pituitaries of rats resulting from combined thyroidectomy and gonadectomy, *Am. J. Path.*, 1937, **13**, 985.
35. CROOKE, A. G., and RUSSELL, D. S., The pituitary gland in Addison's disease, *J. Path. and Bact.*, 1935, **40**, 255.
36. LAQUEUR, G. L., The human hypophysis in diseases of the adrenal cortex, *Stanford Med. Bull.*, 1951, **9**, 75.
37. ADAMS, C. W. M., and PEARSE, A. G. E., Classification of the mucoid (basophil) cells in the normal and pathological human adenohypophysis, *J. Endocrinol.*, 1959, **18**, 147.
38. EZRIN, C., SWANSON, H. E., HUMPHREY, J. G., DAWSON, J. W., and HILL, F. N., Beta and delta cells of the human adenohypophysis: their response to adrenocortical disorders, *J. Clin. Endocrinol. and Metab.*, 1959, **19**, 621.
39. ROMEIS, B., Hypophyse, *in* Handbuch der mikroskopischen Anatomie des Menschen, (W. von Mollendorff, editor), Berlin, Julius Springer, 1940, **6**, pt. 3, 80.
40. SIRLIN, J. L., Autoradiography of nucleolar contents, *Exp. Cell Research*, 1958, **14**, 447.
41. ARNSTEIN, H. R. V., and NEUBERGER, A., Hippuric acid synthesis in the rat, *Biochem. J.*, 1951, **50**, 154.
42. BORSOOK, H., DEASY, C. L., HAAGEN-SMIT, A. J., KEIGHLEY, G., and LOWY, P. H., Metabolism of C^{14}-labeled glycine, L-histidine, L-leucine, and L-lysine, *J. Biol. Chem.*, 1950, **187**, 839.
43. FRIEDBERG, F., and GREENBERG, D. M., Partition of intravenously administered amino acids in blood and tissues, *J. Biol. Chem.*, 1947, **168**, 411.
44. PEARSE, A. G. E., Cytochemical localization of the protein hormones of the anterior hypophysis, *Ciba Found. Colloq. Endocrinol.*, 1952, **4**, 1.
45. JUBB, K. V., and MCENTEE, K., Observations on the bovine pituitary gland. I. Review of literature on the general problem of adenohypophyseal functional cytology, *Cornell Vet.*, 1955, **45**, 576.
46. PURVES, H. D., and GRIESBACH, W. E., The site of follicle stimulating and luteinising hormone production in the rat pituitary, *Endocrinology*, 1954, **55**, 785.
47. PURVES, H. D., and GRIESBACH, W. E., Changes in the gonadotrophs of the rat pituitary after gonadectomy, *Endocrinology*, 1955, **56**, 374.
48. FARQUHAR, M. G., and RINEHART, J. F., Electron microscopic studies of the anterior pituitary gland of castrate rats, *Endocrinology*, 1954, **54**, 516.
49. FARQUHAR, M. G., and RINEHART, J. F., Cytologic alterations in the anterior pituitary gland following thyroidectomy: an electron microscope study. *Endocrinology*, 1954, **55**, 857.
50. SIPERSTEIN, E., NICHOLS, C. W., JR., GRIESBACH, W. E., and CHAIKOFF, I. L., Cytological changes in the rat anterior pituitary from birth to maturity, *Anat. Rec.*, 1954, **118**, 593.
51. PURVES, H. D., and GRIESBACH, W. E., Functional differentiation in the acidophil cells and the gonadotrophic basophil cells of the rat pituitary, *Proc. Univ. Otago Med. School*, 1952, **30**, 27.
52. HEDINGER, C. E., and FARQUHAR, M. G., Elektronenmikroskopische Untersuchungen von zwei Typen acidophiler Hypophysenvorderlappenzellen bei der Ratte, *Schweiz. Zschr. allgem. Path. u. Bakt.*, 1957, **20**, 766.
53. SEVERINGHAUS, A. E., The cytology of the pituitary gland, *in* The pituitary gland, *Proc. Assn. Research Nerv. and Ment. Dis.*, 1938, **17**, 69.
54. RINEHART, J. F., and FARQUHAR, M. G., Electron microscopic studies of the anterior pituitary gland, *J. Histochem. and Cytochem.*, 1953, **1**, 93.
55. FARQUHAR, M. G., "Corticotrophs" of the rat adenohypophysis as revealed by electron microscopy, *Anat. Rec.*, 1957, **127**, 291.
56. PEARSE, A. G. E., The cytochemical demonstration of gonadotropic hormone in the human anterior hypophysis, *J. Path. and Bact.*, 1949, **61**, 195.
57. KONEFF, A. A., HOLMES, R. O., and REESE, J. D., Prevention of adrenalectomy changes in the anterior pituitary of the rat by sodium chloride administration, *Anat. Rec.*, 1941, **79**, 275.
58. GROLLMAN, A., and FIROR, W. M., The rôle of the hypophysis in experimental chronic adrenal insufficiency, *Am. J. Physiol.*, 1935, **112**, 310.
59. BRACHET, J., Nucleic acids in the cell and the embryo, *Symp. Soc. Exp. Biol.*, 1947, **1**, 207.
60. CASPERSSON, T., The relations between nucleic acid and protein synthesis, *Symp. Soc. Exp. Biol.*, 1947, **1**, 127.

61. FICQ, A., and BRACHET, J., Distribution de l'acide ribonucléique et incorporation de la phénylalanine-2-^{14}C dans les protéines, *Exp. Cell Research*, 1956, **11**, 135.
62. KELLER, E. B., ZAMECNIK, P. C., and LOFTFIELD, R. B., The role of microsomes in the incorporation of amino acids into proteins, *J. Histochem. and Cytochem.*, 1954, **2**, 378.
63. DESCLIN, L., Détection de substances pentosenucléiques dans les cellules du lobe antérieur de l'hypophyse du rat et du cobaye, *Compt. rend. Soc. biol.*, 1940, **133**, 457.
64. HERLANT, H., Recherches sur la localisation histologique des hormones gonadotropes femelles au niveau de l'hypophyse antérieure, *Arch. biol.*, 1943, **54**, 225.
65. TUCHMANN-DUPLESSIS, H., and LABAT, J., Répartition des acides nucléiques au niveau du lobe antérieur de l'hypophyse de la souris blanche, *Compt. rend. Soc. biol.*, 1946, **140**, 240, avril.
66. WOLFE, J. M., Cytochemical studies of the anterior hypophyses of rats receiving estrogen, *Am. J. Anat.*, 1949, **85**, 309.
67. DESCLIN, L., Contribution à l'étude de la structure et du fonctionnement de l'hypophyse pendant le post partum, *Arch. biol.*, 1945, **56**, 261.
68. SEVERINGHAUS, A. E., A cytological study of the anterior pituitary of the rat, with special reference to the Golgi apparatus and to cell relationship, *Anat. Rec.*, 1933, **57**, 149.
69. VAN DYKE, D. C., SIMPSON, M. E., LI, C. H., and EVANS, H. M., Survival in the circulation of the growth and adrenocorticotrophic hormones as evidenced by parabiosis, *Am. J. Physiol.*, 1950, **163**, 297.
70. CONTOPOULOS, A. N., Production and removal rate of pituitary follicle stimulating and thyrotrophic hormones in the rat, *Acta Endocrinol. Suppl.* **51**, 1960, 295.
71. MARTIN, S. J., The effect of complete suprarenalectomy on the oestral cycle of the white rat with reference to suprarenal-pituitary relationship, *Am. J. Physiol.*, 1932, **100**, 180.