# RECENT PROGRESS IN HORMONE RESEARCH

*Proceedings of the 1963 Laurentian Hormone Conference*

Edited by

GREGORY PINCUS

# RECENT PROGRESS IN HORMONE RESEARCH

*Proceedings of the*
*1963 Laurentian Hormone Conference*

Edited by
**GREGORY PINCUS**

VOLUME XX

COMMITTEE ON ARRANGEMENTS

E. B. Astwood
R. W. Bates
G. A. Grant
A. D. Odell
E. C. Reifenstein, Jr.
J. F. Tait
A. White
G. Pincus

1964

ACADEMIC PRESS, New York and London

ACADEMIC PRESS INC.
111 Fifth Avenue, New York, New York 10003

*United Kingdom Edition published by*
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: MED. 47-38

PRINTED IN THE UNITED STATES OF AMERICA

# PREFACE

The 1963 Laurentian Hormone Conference met at Mont Tremblant Lodge in the province of Quebec, Canada, during the week September 1 to 6. During that period the papers contained in this volume were delivered to the Conference, and they underwent the discussion recorded here also. The remarkable range of biomedical interests pertinent to endocrinology is epitomized (but far from defined) by the topics presented and discussed. Hormones as antigens, as metabolic regulators, as neuroeffective agents, as promoters of protein synthesis, of fertility, and of infertility are described here. Their genesis, the control of their secretion, the natural and synthetic inhibitors of their activities are discussed in detail. And from these presentations of hormone action and interaction, of negative and positive feedback mechanisms, of developmental sequences in hormonal systems, there continues to emerge the impression of an enormously busy hierarchy of organs and tissues, each cell of which feels and responds to the impact of a remarkable complex of chemical regulators.

The meeting and this record of its proceedings are made possible by a loyal group of contributors to the expenses of the meeting. The sponsors of the 1963 meeting were: Abbott Laboratories; Armour Pharmaceutical Company; Ayerst Laboratories; Ayerst, McKenna & Harrison, Ltd.; Baxter Laboratories, Inc.; Ciba Company Limited; Ciba Pharmaceutical Products; Cutter Laboratories; Charles E. Frosst & Co.; General Mills; Hoffmann-LaRoche Inc.; Lederle Laboratories Division, American Cyanamid Co.; The Lilly Research Laboratories; Mattox & Moore, Inc.; Mead Johnson Research Center; Merck Sharp & Dohme Research Laboratories; The Wm. S. Merrell Company; Nordic Biochemicals, Ltd.; Organics, Inc.; Organon Inc.; Ortho Research Foundation; Parke, Davis & Company; Chas. Pfizer & Co., Inc.; Riker Laboratories, Inc.; Schering, A. G.; Schering Corporation; Searle Chemicals, Inc.; G. D. Searle & Co.; Smith Kline & French Laboratories; Smith, Miller & Patch, Inc.; E. R. Squibb & Sons of Canada; The Squibb Institute for Medical Research; Sterling-Winthrop Research Institute; Syntex Laboratories, Inc.; The Upjohn Company; Warner-Lambert Research Institute; and Wyeth Laboratories, Inc. Their continuing interest and support is gratefully acknowledged. Their contributions made it possible for us to have as special guests from abroad Dr. Roger Guillemin of the College de France and Dr. Roger V. Short of the University of Cambridge. The Committee on Arrangements is responsible for the invitation of speakers, the mechanics of program proceedings, the recording of the discussion and related details. The discharge of these duties is fortunately

facilitated by the help of a number of individuals. Among these are the men who acted as chairmen for the sessions of the Conference; the informed and expert cooperation of Drs. P. H. Henneman, F. C. Bartter, A. E. Wilhelmi, L. L. Engel, S. Lieberman, R. K. Meyer, and W. J. Haines is sincerely appreciated. The efficient conduct of the meeting arrangements would not have been possible without the kind offices of Mrs. Jacqueline C. Foss. She and Mrs. Mina Rano performed with superior competence as secretaries to the meeting. Mrs. Louise P. Romanoff has again earned our gratitude for her preparation of the subject index to this volume. To the staff at Academic Press we are indebted for the care and competence that they continue to exercise in the publishing of these proceedings.

Our stay at Mont Tremblant was again made pleasant by the kindness of our hostess at the Lodge, Mrs. Mary R. J. Robinson, and the courtesy of her staff. For local arrangements and assistance in various recreational activities we are indebted to Drs. C. H. Sullivan, G. A. Grant, J. C. Beck, and E. C. Reifenstein, Jr.

The Laurentian Hormone Conference every year invites applications from qualified investigators in the hormone field to attend its meeting. Every attempt is made to have informed research workers at the meeting who are interested in contributing to critical discussion of data and concepts presented by our speakers. We continue to solicit the active participation of experts in the hormone field.

GREGORY PINCUS

*Shrewsbury, Massachusetts*
*May 1964*

CONTENTS

## IV. Steroid Hormones



## V. Testis Hormone Physiology

# I. POLYPEPTIDE HORMONES

## Some Studies with Antisera to Growth Hormone, ACTH, and TSH[1]

E. E. McGarry, J. C. Beck, L. Ambe, and R. Nayak

*McGill University Clinic, Royal Victoria Hospital, Montreal, Canada*

### I. Introduction

In 1948 Leathem (46) spoke before this group on the subject of antihormones, the existence of which had been postulated by Collip (15) to explain the resistance that may develop on repeated injections of a hormone. The concept of antibodies to hormones was not generally accepted for several reasons. In only relatively rare instances could antibodies to hormones be demonstrated; impurity of hormone preparations and lack of adequate techniques at that time made it difficult to determine whether the antibody was directed against the hormone itself, and finally, the thinking of immunologists was paralyzed by Ehrlich's dictum of *horror autotoxicus*, which states, in essence, that the body cannot, in fact it dare not, develop antibodies to normal endogenous constituents.

Over the past ten years immunochemical techniques have become more sensitive and more versatile, hormones have become purer, and the concept of autoimmunity has become respectable. In 1958 the reports of Hayashida and Li (39) and of Read and Stone (67) on the production of specific antisera to purified growth hormone preparations were among the first of an increasing number of papers reporting studies utilizing antisera to hormones. The present paper is not intended as a review. Many interesting papers have appeared to which no reference will be made. No comprehensive review has appeared, but a good deal of information is contained in a recently published colloquium (14).

For the past six years we have produced antisera to a number of hormones, HGH[2] (25), bovine TSH (5, 25), porcine and human ACTH (25, 58), human

[1] These investigations have been supported by grants from the Medical Research Council of Canada (MA-724) and the United States Public Health Service (HD-00511-04).

[2] Abbreviations used in this chapter: ACTH, adrenocorticotropic hormone; FFA, free fatty acids; FSH, follicle-stimulating hormone; GH, growth hormone; ICSH, interstitial cell-stimulating hormone; MSH, melanocyte-stimulating hormone; TSH, thyroid-stimulating hormone. H, B, and P preceding abbreviations for hormones indicate, respectively, human, bovine, and porcine preparations.

FSH (59), bovine GH (25), porcine GH (25), sheep prolactin and growth hormone, human chorionic gonadotropin and insulin. Since this report will be concerned mainly with studies utilizing antisera to growth hormone preparations, to ACTH and to TSH, some of the immunochemical characteristics of the antisera to these hormones will be briefly outlined, others will be dealt with in subsequent sections of this paper.

TABLE I
*Cross Reaction of Various ACTH Preparations*[a]

| Preparation | Inhibition factor |
|---|---|
| Porcine ACTH, 21 units/mg. (i.v.) (original antigen) | 1 |
| Human ACTH | 1 |
| ACTH fractions | |
| $\alpha_1$ $\alpha_2$ | 100 |
| $\beta$ | 4 |
| $\gamma_1$ $\gamma_2$ | 100 |
| $\Delta_1$ | 100 |
| Human MSH | 313 |
| Porcine ACTH, 40 units/mg. (original antigen) | 1 |
| Li "pure" ACTH | 1 |

[a] The smallest amount of porcine ACTH required to inhibit the reaction between porcine ACTH-sensitized red cells and antiporcine ACTH serum is used as the standard of comparison in each experiment; it is given a value of 1. The inhibition factor represents the relative amount, by weight, of other preparations required to produce inhibition of the reaction. Each factor represents at least three experiments done in duplicate.

The antisera to GH are hormone specific and species specific (25). We have never been able to demonstrate a cross reaction with human albumin reported by others (35, 39) by hemagglutination inhibition and only rarely by double gel diffusion techniques (17, 64).

The antisera to bovine ACTH cross reacts with human ACTH and with various other ACTH preparations (25) (Table I). Early antisera to ACTH showed a slight cross reaction with TSH, but more recently the ACTH preparation used as antigen has a potency of 121 I.U. (intravenous administration, i.v.) per milligram, and a cross reaction with TSH cannot be consistently demonstrated by hemagglutination inhibition.

Three bovine TSH preparations have been used as antigens. There is no cross reaction of either ACTH or TSH with primate gonadotropin preparations nor with human growth hormone, although slight cross reactions with gonadotropins of other species occur (5).

The work to be reported here will be discussed under three main headings: the use of antisera to clarify and elucidate biological activity; the use of antisera to identify hormones in tissues; and finally, the use of antisera to measure hormones.

## II. The Use of Antisera in Biological Systems

It had been shown that the release of free fatty acid by the rat epididymal fat pad *in vitro* was markedly increased in the presence of epinephrine and of ACTH. Our first interest in adipose tissue stemmed from the hope that since human growth hormone induced the release of free fatty acids *in vivo* (66), growth hormone preparations might do so also *in vitro*, thus providing a convenient bioassay method. Rat adipose tissue was used initially as an exercise in techniques to be applied to the study of human adipose tissue. Since the rat is responsive to various growth hormone preparations *in vivo*, a bovine growth hormone preparation was added to the rat epididymal fat pad *in vitro* and was found to stimulate the release of free fatty acids (5). This preparation of bovine growth hormone consistently causes a release of free fatty acid, as little as 1 μg. per milliliter of medium giving a release twice that of the control value. Small amounts of TSH were found to cause a release also of free fatty acids (13). The fat pad studies were initiated at about the same time that the studies on antisera to hormones began, and it was only logical to utilize whatever systems we had at hand to gain evidence for the specificity of the various antisera to their respective hormones. Antiserum produced in the rabbit was added to the medium in a concentration of 1:5 and was shown to inhibit the release of free fatty acids induced by this bovine growth hormone preparation. The release of free fatty acids was also equally inhibited to a similar degree by normal rabbit serum. These and all sera used in inhibition experiments in the fat pad were dialyzed prior to use to eliminate the possible inhibitory effect of glucose.

In the hope that this nonspecific inhibition resided in some portion of the plasma other than the γ-globulin, rabbit serum was separated and the effect of γ-globulin on free fatty acid release induced by bovine growth hormone was then studied. It was found that normal rabbit γ-globulin itself stimulated the release of free fatty acid but inhibited the release stimulated by bovine growth hormone, by ACTH, and by TSH and also the release induced by epinephrine (5) (Fig. 1). Thus it appeared that there was in normal rabbit serum and in normal rabbit γ-globulin a non specific inhibitor of hormone-stimulated free fatty acid release.

Nonspecific inhibitors in serum and plasma have been reported for many

antigen-antibody reactions, including tests for rheumatoid factor (11) and for passive cutaneous anaphylaxis (10) and in immunoassay of hormones using hemagglutination inhibition (57, 58, 45). In the latter instance it became apparent that in that system nonspecific inhibition was never seen with serum dilutions beyond 1:128. It seemed worthwhile, therefore, to see whether the nonspecific inhibitor of free fatty acid release could also be diluted out. It was then found that normal rabbit serum did not significantly inhibit the release of free fatty acids stimulated by any hormone if the dilution of the serum exceeded 1:200 (5, 60). The experiments with the various preparations that had been used to stimulate free fatty acid release

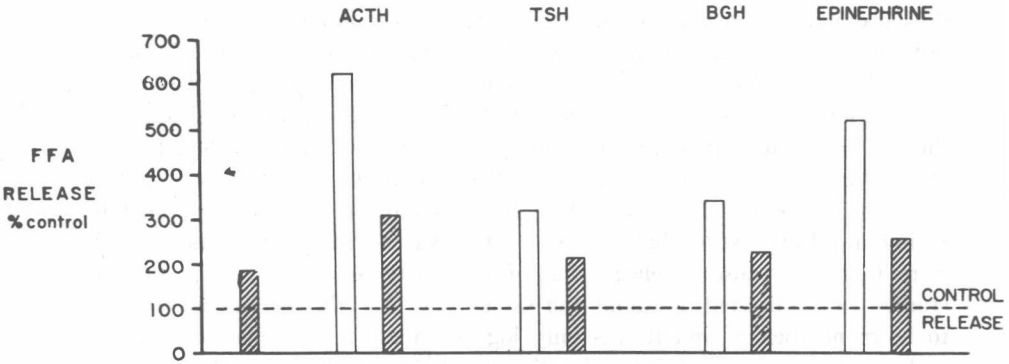


FIG. 1. Effect of rabbit γ-globulin on hormone-stimulated release of free fatty acids. Open bars: No γ-globulin. Hatched bars: 1% γ-globulin.

were then repeated using antisera dilutions of 1:400 to 1:800, and specific inhibition was demonstrable. It was found that the release of free fatty acids induced by ACTH was not inhibited by antisera to TSH but was inhibited by the specific antiserum to ACTH (58) (Fig. 2). The TSH-stimulated release was completely inhibited by antisera to TSH but was not significantly decreased by antisera to ACTH (5) (Fig. 3). In addition, it was found that the free fatty acid release induced by ACTH was inhibited by plasmin but that induced by TSH was not. Other evidence that TSH-stimulated release is not due to ACTH contamination has been reported by Frienkel (27). In addition to providing evidence of the specificity of the antisera, the results of these experiments suggested three things. First, that the antisera to TSH was specific for that hormone as was the antiserum to ACTH. Second, that the release of free fatty acids induced by TSH could not be attributed to contamination with ACTH. Third, that it was unlikely that the free fatty acid release induced by TSH and ACTH was

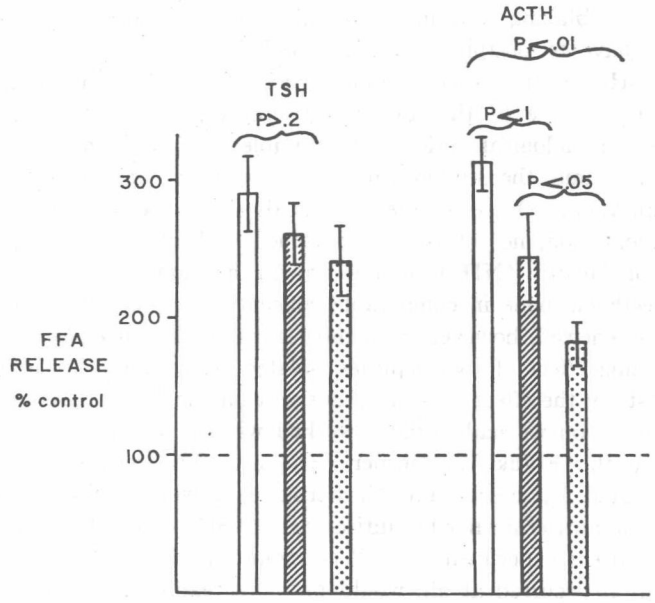


FIG. 2. Effect of diluted normal rabbit serum and anti-ACTH rabbit serum on FFA release stimulated by TSH and ACTH. Hatched bars: Normal rabbit serum, 1:800. Dotted bars: Anti-ACTH rabbit serum, 1:800.

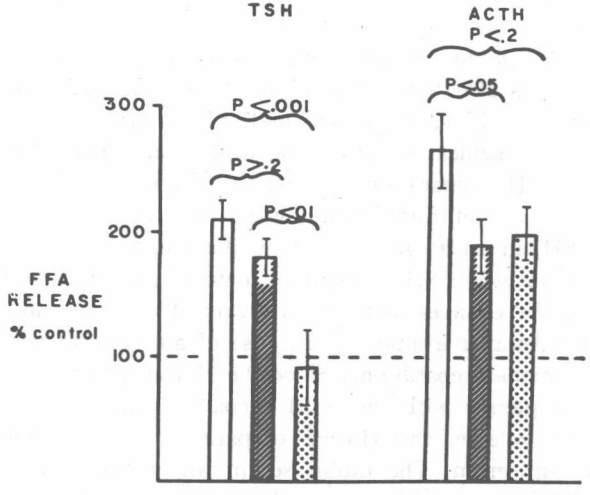


FIG. 3. Effect of diluted normal rabbit serum (hatched bars) and anti-TSH rabbit serum (dotted bars) on FFA release stimulated by TSH and ACTH (open bars).

due to a fat-mobilizing substance contaminating both hormone preparations. One would have to postulate two fat-mobilizing hormones, one a contaminant of TSH and the other a contaminant of ACTH. These experiments then are an example of the use of antisera to elucidate the question of specificity of a biological activity attributable to a hormone.

In the meantime, the studies with bovine growth hormone had not been followed up. While release of free fatty acids was always obtained with the original preparation, no release was obtained with several lots of National Institutes of Health (NIH) bovine growth hormone or with porcine or human growth hormone in comparable concentrations, i.e., 1–5 μg./ml. Our interest was renewed, however, with the report of Friesen *et al.* (28) of the lipid-mobilizing effect of two peptides isolated from porcine pituitaries. We decided to study the effect of various antisera on the FFA release stimulated by the preparation we had initially worked with, a bovine growth hormone prepared by the Frank W. Horner Company. The release of free fatty acids stimulated by bovine growth hormone, however, was not inhibited by the specific antiserum nor by antiserum to TSH or ACTH. In the ACTH and TSH antisera experiments, smaller amounts of HGH were used so that inhibition, if it occurred at all, would be more readily apparent. Failure to inhibit BGH-stimulated release suggested that either there was a fat-mobilizing substance in the Horner preparation to which no antibodies had been formed or that the antigenic determinants were different and probably spatially removed from the portion of the molecule responsible for free fatty acid release.

For many years immunologists have utilized antisera to purify antigen mixtures and it has also been utilized in the field of immunochemistry as applied to hormones. Li used antisera to ICSH to remove traces of ICSH, not detectable by chemical or physiochemical means, from FSH, thus obtaining a pure FSH preparation that was found to have little or no biological action (50), confirming earlier work of Greep *et al.* (34). Li also found that ICSH, in an amount which by itself had no biological activity, restored to the purified FSH the full biological activity usually associated with FSH, i.e., the changes seen in the ovary, the uterus, and in vaginal cytology (50). (Another example of the use of antisera to define biological activity of a hormone preparation.) Since the Horner preparation apparently cross-reacted completely with the NIH preparation and the antiserum did not inhibit FFA release, the Horner preparation was absorbed with the specific rabbit antiserum. The rabbit serum was removed with goat anti-rabbit serum. The supernatant was found to have no lipid-mobilizing ability. The supernatant obtained when BGH was added to normal rabbit serum

followed by absorption with goat antirabbit serum was found to have retained lipid-mobilizing activity. This attempt to use antisera to isolate a lipid-mobilizing factor failed. There are several possible explanations, but one interpretation of these results is that the lipid-mobilizing effect may be associated with one of the antigenic moieties but so situated on the molecule to be uninhibited by antibody combination. There are, of course, many other examples of failure to inhibit biological activity with antisera, the best known of which is insulin. In the great majority of instances in man, antisera to insulin do not interfere with biological activity.

To summarize the results thus far presented: Antigen-antibody reactions may be utilized not only to demonstrate the specificity of the antibody, but also to clarify specificity of a biological reaction.

## III. Localization of Hormones in Tissues

These studies have been carried out using the immunofluorescent technique of Coons (16). Coons showed that the γ-globulin fraction of an antiserum conjugated with fluorescein isocyanate remained firmly bound to antigen in tissues and could be visualized in a microscope using a fluorescent light source. Modifications to this technique have been introduced, such as the use of fluorescein isothiocyanate (68) instead of the isocyanate, the use of other fluors, e.g., rhodamine (72), and the use of Sephadex instead of dialysis to remove excess fluor from the conjugates (26).

In 1951 Marshall (54) reported the localization of antisera to ACTH in basophiles. However, there was also localization of the antisera to other tissue elements not only in the pituitary, but also in the kidney. Cruickshank and Currie (18) reported on the production of antisera to several hormones of pituitary origin and, while they clearly showed the antisera were not hormone specific, they showed localization only in the pituitary —though not to any single cell type. With the availability of purer hormone preparations it seemed worthwhile to repeat the experiments of earlier workers.

Leznoff *et al.* (48) showed that fluorescein-conjugated anti-HGH γ-globulin was localized specifically in eosinophilic cells of the human pituitary gland and in the cells of two eosinophilic adenomata. Grumbach (35), using the fluorescent labeled antibody technique, also reported localization of growth hormone in acidophiles. It was later shown that fluorescein-labeled rabbit antibodies to ACTH were localized in basophilic cells of human pituitaries. This was confirmed by Pearse (63), who found the antiserum localized in the R type of mucoid cells according to the classification of Adams and Pearse. While we have not looked specifically at the staining of cells showing Crookes' change, Pearse has done so and has found the results

difficult to evaluate. He found weak fluorescence in the perinuclear region and often a ring of fluorescence around the periphery of the cells in material obtained at biopsy.

The eosinophile has long been considered the site of production of growth hormone so that localization of growth hormone antisera to this cell type was considered confirmatory. The cell type responsible for the production of ACTH, however, has been a subject of some controversy. ACTH has been "localized" to the eosinophile, and to the various varieties of basophiles or mucoid cells depending on the tinctorial qualities of the various chemical stains used. There has been a tendency to assign a single hormone to a single granular cell type, an exercise fraught with the difficulty that with most of the stains used there are not enough cell types to go around. The exception is the method of Herlant (40) and modifications of it, which have appeared in the European literature but have been largely ignored by the English-speaking workers.

Hymer and McShan (44), using chromatographic separation of pituitary particulates, were able to obtain homogeneous populations of granules of various sizes. ACTH was found to be associated not with any particular granule fraction but with the microsomal fraction, an observation that makes it difficult to localize it to any one cell population. Barnes (3) studied the cytology of the mouse pituitary by both electron and light microscopy and concluded that groups of cells homogeneous with respect to staining affinity may not be homogeneous with respect to function.

Localization of a hormone antiserum demonstrates at best only the presence of the hormone in the cell. It gives no proof that the hormone is synthesized by that particular type of cell. There has been only one report in the literature of studies attempting to correlate the site of synthesis of a hormone with the localization of the fluorescein-conjugated antiserum.

Midgley and Pierce (55), using fluorescent-tagged antisera, found that HCG was localized in the syncytium of immature human placenta. It could not be identified in mature human placenta. Midgley and co-workers (56) concluded from studies with tritiated thymidine and electron microscopic studies of monkey placenta that the syncytium synthesizes HCG and that the cytotrophoblast consists of indifferent rapidly dividing cells from which the syncytium is derived. It is of interest that Grumbach and Kaplan (36) found anti-HGH localized in the syncytium of human placenta.

In the pituitary, however, the assumption has been made that where a hormone is localized it is also synthesized. Recent work suggests very strongly that ACTH is synthesized by chromophobe cells. Siperstein (70) studied pituitaries of adrenalectomized rats which had received tritiated thymidine

at intervals up to 12 hours before death. She found in autoradiographs increased grain counts in a particular type of chromophobe cell. These cells could also be identified in the pituitaries of normal rats but were fewer in number. She puts forward the hypothesis that ACTH is synthesized by chromophobe cells. Chromophobe adenomas are no rarity in Cushing's syndrome. We have found brilliant fluorescence with labeled anti-ACTH serum in the cells of a chromophobe adenoma removed from a patient with Cushing's syndrome (49). It is of interest that from 1944 to 1954 there were 30 patients at the Royal Victoria Hospital and the Montreal Neurological Institute in whom the diagnosis of acromegaly was made. Of the ones in whom there is good evidence that active acromegaly was present and who came to craniotomy, 6 of 9 had chromophobe adenomas. Thus in well-documented active disease in which there is apparently active synthesis and secretion of a pituitary hormone, chromophobe adenomata are more frequently seen than chromophile adenomata. We have not compiled statistics for the past nine years but the pituitaries of 4 patients who had active acromegaly with removal of a chromophobe adenoma in the past four years have been stained with anti-HGH. In three, no fluorescence was seen using either the direct or indirect method. In the fourth there was a faint dusting of fluorescence only suggestive of specific staining. Grumbach (35) has reported patches of fluorescence in a chromophobe adenoma stained with fluorescein-conjugated anti-HGH. While findings based on studies in tumor tissue should perhaps be applied to cells of normal pituitaries with caution, it would seem worthwhile to consider the possibility that at least some chromophobe cells may be actively synthesizing and secreting hormone.

We have also attempted to stain pituitaries with the antisera to TSH. There is in the literature some disagreement as to species specificity of TSH as determined by immunochemical techniques. It had been shown that our antisera would inhibit the TSH activity in serum of patients with primary myxedema (61). We interpreted this finding as evidence of cross reaction between human and bovine TSH. Werner *et al.* (76) were also able to inhibit TSH-like activity of human sera, as were Levy and his co-workers (47). Arquilla and associates (1) using TSH prepared from human pituitaries by the method of Condliffe and Bates (4) and an Armour preparation of TSH could show no evidence of cross reactivity. One of the disturbing things about these results is that the antiserum to human TSH could be almost completely absorbed with Raben human growth hormone, which itself had no TSH activity. One possible explanation of the failure to demonstrate a cross reaction between human and bovine TSH in this study is that the hormones were prepared by different methods, since it would appear that

tertiary structure plays an important role in antigenicity. For example, while sperm whale insulin and pork insulin are reported to have identical amino acid sequences, Berson (7) has reported that immunochemically they behave very differently. A hundred times as much sperm whale insulin may be required to inhibit binding of $I^{131}$ pork insulin. Pork and human insulin differ only in the C-terminal amino acid of the B chain. Lockwood *et al.* (52), removing this amino acid from pork insulin, found antigenicity unaltered. Studies with the antisera to this preparation led these authors to conclude that tertiary structure is important in antigenicity. Arquilla and co-workers indeed have invoked the importance of tertiary structure in discussing the very marked cross reaction obtained between their human TSH and human growth hormone (1). This criticism cannot, however, be invoked against Utiger *et al.* (73), who tested antiserum to human TSH against several bovine preparations. Finally, Oki (62) has reported cross reaction between porcine and human TSH. Many of these studies have not been quantitated. It may be that if enough antiserum to bovine TSH is used, a cross reaction can be demonstrated. Indeed, Levy (47) found that much more antibovine TSH was needed to inhibit human TSH than was required to inhibit bovine TSH. In spite of these conflicting reports we decided to try and stain human pituitaries with antibovine TSH.

Because we planned to apply labeled antisera to two different hormones to one section of pituitary tissue, anti-TSH γ-globulin was conjugated with rhodamine (72). Fluorescein-conjugated anti-ACTH γ-globublin was used. In sections stained only with the rhodamine conjugate, the pinkish-orange fluorescence was localized to the cytoplasm of certain basophiles. In sections in which the rhodamine conjugate was followed by the anti-ACTH fluorescein conjugate, on first glance no rhodamine fluorescence could be seen. On closer inspection, there was a hint of faint rhodamine fluorescence buried under the brilliant green fluorescence, suggesting that the same cells were stained with both conjugates.

Using normal human pituitary, one mirror image was stained with fluorescein-conjugated anti-TSH γ-globulin and the other mirror image was stained with fluorescein-conjugated anti-ACTH γ-globulin. Both conjugates were found to localize in the same cells (Fig. 4). Prior application of unlabeled anti-TSH serum did not diminish the fluorescence obtained with anti-ACTH but it did inhibit subsequent localization of labeled anti-TSH. Application of unlabeled ACTH to the tissue resulted in marked diminution of staining when labeled anti-ACTH was then applied whereas fluorescein-labeled TSH antiserum still gave brilliant fluorescence.

Mack and co-workers (53) have reported selective localization of anti-