Progress in CLINICAL PATHOLOGY

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VOLUME IV

Editor

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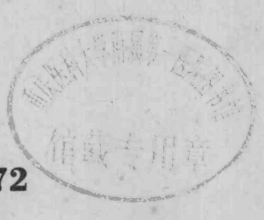
Danville, Illinois



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Preface

THIS VOLUME, like the earlier volumes in the series, is directed primarily to the clinical pathologist. It is published during the "year of clinical pathology" when the advancements in this comparatively new field are subject to review and when the future role of the clinical pathologist is subject to question. Also, from time to time, both personal statements and official publications express the reluctance of the "pure" pathologist to recognize the apparent inevitability of his growing role in the field of clinical pathology. It is interesting to ask why, in most instances, the function of the clinical pathologist has fallen on the institutional pathologist, creating the problem of identification which continues to plague the "pure" pathologist and the clinical pathologist alike.

In the first volume of this series, James N. Patterson chronicled the development of clinical pathology from the viewpoint of a man who grew up with the field and is one of those mainly responsible for the evolution of clinical pathology into a full-fledged branch of pathology. In the United States it would appear that clinical pathology fell to the pathologist by default, although it is difficult to determine at what point the pathologist first departed from the pathologic tradition of Morgagni and Bichat to involve himself in this new field. In Europe, the clinician and the semeiologist (a subspecialist in the field of internal medicine and surgery) have traditionally been responsible for the application of principles and techniques of chemistry and bacteriology to the diagnosis of the patient. This approach, which started with Richard Bright and continued with van den Berg and Morawitz, could be expected in continental Europe where most specialists are hospital based. In this country the tradition of care is centered in the community hospital where, logically, laboratory semeiology is the province of the pathologist, whose interest in the detection and interpretation of disease is direct and continuing. Thus, the older pathologist was drawn into a field somewhat alien to his training and experience; and the transition caused remarkable changes in the teaching of pathology.

It may be asked whether special bacteriological, chemical, immunological, and radionuclide procedures should properly be called pathology and form a part of it. (There seems to be less question about the competence of the pathologist to handle the hematologic aspects of disease.) Today's pathologist, initially by default and now by training, is expected to assist his clinical confrères in using the new "instrument semeiology" to diagnose disease and manage patients. So long as financial considerations within community hospitals prevent the departmentalization sometimes necessary for optimum results in clinical pathology, this arrangement will prevail. Of course, the pathologist is taking upon himself a task which will become increasingly difficult with the rapidly expanding progress in the field. This remains true even when the pathologist is surrounded by a cohort of technical specialists. Charges of superficial competence or of outright incompetence may be expected, mostly from those who think that the basic scientist is best qualified to handle the new "laboratory semeiology." This is a moot point.

On the one hand, the high level of specialization of the basic scientist makes it financially difficult for the community institution to assure sufficient coverage in each field of clinical pathology. On the other, the pathologist may be less qualified than the basic scientist to handle the technical aspects of the complex arsenal which has become the core of modern clinical and, especially, of modern chemical pathology. He may be less able than the basic scientist to see himself safely through the highly technical requirements of mod-

ern bacteriologic, chemical, immunologic, and radionuclide procedures. He is likely to be less versed than the chemist in such problems as quality control and instrumentation.

In the pathologist's favor, however, is the versatility which gives him, in most instances, an adequate working knowledge of the theoretical and practical aspects of technical procedures. Furthermore, his medical training and experience allow him to correlate the laboratory findings with the clinical status of the patient. Obsolescence will not befall the pathologist who communicates intelligently with his clinical colleagues, since they expect him to elucidate the meaning of test results and to suggest certain tests which may be available for the solution of special clinical problems. Intelligent communication and interpretation remain the best qualifications of the pathologist, since the clinician seeks not the general interpretation of a test but rather the interpretation of the result as it applies to his patient.

The preceding should not be construed as an attempt to minimize the contribution of the basic scientist to the field of clinical pathology. The future of clinical pathology depends on the harmonious cooperation of all scientists interested in the improvement of laboratory semeiology as an aid to diagnosis. Ideally, the specialized basic scientist will provide highly reliable techniques and results that the clinical pathologist and the clinician will utilize in patient diagnosis, a trend already evident in larger centers.

The clinical pathologist has certain problems all his own; for him the major problem seems to be the need to keep abreast of the ever-mounting mass of clinical, technical, and theoretical material with which he must be familiar for best performance on the job. Knowledge of basic biochemical and physiologic advances, of new radiologic techniques, of new therapeutic and even surgical procedures is needed for the new breed of pathologists. This series has tried to aid the new pathologist and will continue in this endeavor. Like the preceding volumes, the fourth volume tries to bring to the clinical pathologist basic facts and developments necessary for his practice, as well as familiarity with new concepts and technical tools which are likely to affect the field of clinical pathology in the near future.

A provocative article on reagent kits discusses systems on which every pathologist depends, at times perhaps with less than full understanding of the problems connected with their use. Another chapter offers an in-depth review of technical aspects and interpretation of endocrine profiles in clinical medicine from the viewpoint of a writer who is a clinical pathologist and clinician as well as an investigator. This chapter is complemented by another whose subject is the relatively new technique of radioimmunoassays. Control of hospital infection, infectious mononucleosis, Australia antigen – all the focus of considerable interest – are reviewed, whereas another chapter discusses the potential contributions of the laboratory to the diagnosis of atherosclerotic and coronary artery disease. An article of considerable interest describes a new understanding of acid/base, gas, and blood electrolyte balances; in the editor's experience, the topics presented are of immediate impact in the daily care of patients. The final chapter reviews advances in veterinary clinical pathology; some of our readers may wonder why such a chapter was included. It was felt that it would emphasize the many points of contact between human and veterinary clinical pathology. Also, where veterinary laboratories are not available, the clinical laboratory may be asked to help in veterinary situations, a fairly frequent experience in the editor's own practice. Finally, the existence of models of human diseases in animals is of great help to the experimental clinical pathologist.

With this volume, the series has had for the first time the benefit of a formally ap-

pointed editorial board. This innovation seemed necessary for a better selection of topics and a more comprehensive review of the material presented for publication. The members of the editorial board have been active in both respects, and the editor would like to express his appreciation to them. The editor is also grateful to Mr. Duncan Mackintosh, former vice president, Grune & Stratton, Inc., for his helpful support and to Mrs. Glen Gould for her highly competent editorial assistance in the preparation of this volume.

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CHAPTER 1

Endocrine Profiles in the Clinical Laboratory

By Habeeb Bacchus, Ph.D., M.D., F.A.C.P.

THE CLINICAL PATHOLOGIST is constantly called upon to aid the clinician in establishing diagnoses in several areas of medical practice. This is especially true in the field of endocrinology and metabolic diseases. Either the pathologist is asked to recommend the tests necessary for a diagnostic investigation of a special problem, or his department is expected to perform the tests ordered. Several of the special endocrine tests (e.g., those for GH, FSH, and LH levels) are performed in special endocrine and reference laboratories. Yet since there are several useful tests which can be performed in the clinical laboratory without requirement of very specialized equipment, such tests will be discussed in this section. Satisfactory performance of these tests of endocrine functions necessitates knowledge both of the metabolic basis of the disease processes and of the chemical principles involved in the performance of the tests.

In this section the functions and disorders of the following endocrine glands will be discussed: hypothalamic-neurohypophysial system, anterior pituitary, adrenal medulla and chromaffin system, parathyroid glands, adrenal cortex, ovaries, and testes. The thyroid is not included here as it was covered in detail in a previous volume of *Progress in Clinical Pathology*.* Discussion of the glands listed includes a summary of hormone synthesis and actions and a brief consideration of the clinical entities associated with abnormalities in their functions, and a detailed description of the various clinical investigative procedures and chemical tests, with their rationales and methodology, as well as the application of these procedures to clinical problems. When feasible, diagnostic schemata are presented in the hope that the pathologist will find these a useful guide for tests to be recommended to the practicing clinician for a work-up of a given problem.

Table 1 presents normal values for various clinical laboratory endocrine tests.

HYPOTHALAMIC-NEUROHYPOPHYSIAL SYSTEM

The term neurohypophysial system in this discussion refers to the neurosecretory system,

Dedicated to my friend and colleague Kenneth L. McCoy, M.D., Director of Laboratories, Providence Hospital, Washington, D.C., and to my trainees, past, present, and future.

*See Volume III, Chapter 9.

Table 1. – *Normal Values of Some Endocrine Parameters in the Clinical Laboratory*

Test	Normal Values
Urine:	
VMA	1.8-7.0 mg/24 hr
Metanephrines	<1.3 mg/24 hr
Catecholamines	20-100 mg/24 hr
17-Ketosteroids	8-18 (men) and 7-15 (women) mg/24 hr
17-OHCS	2.5-8.4 mg/24 hr
17-KGS	8-18 (men) and 7-16 (women) mg/24 hr
"11-Oxy"-17-OHCS	2.5-8.0 mg/24 hr
11-Deoxy-17-OHCS	<0.2 mg/24 hr
Polar 17-OHCS	1.2-4.0 mg/24 hr (usually ½ of THF, THE)
Pregnanetriols	1.5-2.6 mg/24 hr
Pregnanediol, folliculoid phase	0.9-1.8 mg/24 hr
Pregnanediol, luteal phase	2.0-4.8 mg/24 hr
Total Estrogens:	
Follicular phase	20-40 μg/24 hr
Luteal phase	40-80 μg/24 hr
Estriol, third trimester of pregnancy	5-60 mg/24 hr
Free cortisol	60-150 μg/24 hr
Urine calcium, on 150-mg-Ca-diet	<200 mg/24 hr
Tubular reabsorption of phosphate	>90%
Plasma:	
Free cortisol, 8:00 a.m.	5-25 μg %
Free cortisol, 5:00 p.m.	2.5-12.5 μg %
Free corticosterone, 8:00 a.m.	1-8 μg %
Free corticosterone, 5:00 p.m.	1-8 μg %
Serum:	
Calcium, mg/100 ml	8.8-10.6 mg/100 ml

including the hypothalamic, paraventricular, and supraoptic nuclei, the neurohypophysial axons to these nuclei, the median eminence, the pituitary stalk, and the posterior pituitary gland. The endocrinologic functions of this system are (a) the formation and release of vasopressin – the antidiuretic hormone (ADH); (b) the formation and release of the oxytocic hormone, oxytocin; and (c) the formation and release of hormonal substances which regulate certain secretory functions of the anterior pituitary.

The antidiuretic activity of posterior pituitary extract was established clinically in 1913 [10, 26] when the extract was found to decrease urine volume in patients with diabetes insipidus. In 1937, working on whale tissue, Geiling et al. [11] demonstrated that the antidiuretic principle was confined to the posterior pituitary in that species. Later neurophysiological and anatomical studies clearly showed the association of the hypothalamus and of the posterior pituitary as the neurohypophysial system. It is now established that the hormones vasopressin and oxytocin are synthesized in the hypothalamic nuclei in the form of secretory vesicles enclosed by membranes. These secretory vesicles are transported down the neurohypophysial tract to the posterior pituitary [20].

CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE NEUROHYPOPHYSIAL HORMONES

Vasopressin

The hormone is made up of eight amino acid residues, five amino acids linked by a S—S bond and a tail composed of three amino acids. The structure of human vasopressin was discovered by Du Vigneaud et al. [9], who also showed that species differences between vasopressins resided in the replacement of arginine in the "tail" (human vasopressin contains arginine) by lysine, which is unique to pig vasopressin. These molecules are small enough to be dialyzable; the active hormone is bound electrostatically to a carrier protein of approximate molecular weight 30,000 [24]. Phylogenetic differences between the pituitary hormones have been reviewed [21].

Hypothalamic Neurosecretions Regulating Anterior Pituitary Functions

At least six activities regulating the anterior pituitary have been identified. Some biological properties of these substances have been described, but complete structures are not yet identified. The known data on these factors follow:

Corticotropin Releasing Factor (CRF). Two extracts isolated from the neural lobe possess corticotropin releasing activity [12, 13]. One extract, termed α-CRF, may be similar to α-MSH (melanocyte stimulating hormone) in the chemical structure, with the addition of threonine, alanine, and leucine. The β-CRF extract is described as resembling vasopressin in molecular size.

Thyrotropin Releasing Factor (TRF). Minute amounts of a substance which is capable of discharging TSH from the pituitary have been found in hypothalamic tissue. On the basis of studies on "relatively pure" TRF fractions, Guillemin has suggested that the active principle is probably a small nonpolypeptide molecule [13].

Luteinizing Hormone Releasing Factor (LRF). Animal studies have provided convincing evidence for the existence of a factor which releases LH from the anterior pituitary. The factor is suggested to be a small basic polypeptide of estimated molecular weight between 1200 and 2000; this molecule is unlike vasopressin and oxytocin in lacking the disulfide bridge. The following amino acids are found in the structure: aspartic acid, glutamic acid, glycine, alanine, lysine, histidine, arginine, threonine, serine, proline, and leucine [13].

Follicle Stimulating Hormone Releasing Factor (FRF). Animal studies suggest the existence of this factor, which releases FSH from the anterior pituitary. It is probably an amine, although its chemical structure is not yet fully known.

Prolactin Inhibiting Factor (PIF). The existence of a factor from the hypothalamus which regulates the release of prolactin by the anterior pituitary has been demonstrated in animal and clinical studies. This factor is implicated in the syndromes of inappropriate lactation in human patients. Although its chemical structure is not definitely known, this factor is presumed to be a polypeptide.

Melanocyte Stimulating Hormone Inhibiting Factor (MIF). Animal studies suggest the presence of a factor which inhibits the release of MSH. The chemical structure is presumably that of a polypeptide.

Despite the significant amount of information on the physiological roles of several of the factors produced by the neurohypophysial system, the clinical implications are not completely understood. Since well-defined clinical problems are associated with vasopressin secretion, the remaining part of this chapter will be largely confined to this hormone. The regulation of its secretion and its action will be briefly reviewed prior to consideration of clinical tests used to assess this function of the neurohypophysis.

PHYSIOLOGIC ROLE OF VASOPRESSIN OR ANTIDIURETIC HORMONE (ADH)

The major physiologic role of this hormone is in the regulation of water balance by the renal tubule. It was clearly established by the classic studies of Verney [25] that the increase of the osmotic pressure of plasma in the district of the internal carotid is followed by release of antidiuretic hormone and the resultant formation of a concentrated urine. It was shown that the receptors responding to the osmotic challenge

are located in the anterior hypothalamus. Conversely, dilution of body fluid suppresses the release of ADH with the resultant excretion of urine which is hypotonic in relation to the body fluids. These findings indicate that a feedback between the renal tubule and the anterior hypothalamus operates to maintain the normal tonicity in body fluids. Later studies [1] showed that there is also a close anatomic and physiologic interrelation between the anterior hypothalamic ADH mechanism and a thirst center. This interrelationship also serves to maintain normal fluid balance in the body. There are other stimuli of nonosmotic nature which may influence the release of ADH; these include pain, emotional states, stimulation of certain nerves, as well as pharmacologic agents such as alcohol (which inhibits the release), nicotine [5], morphine [7], acetylcholine [17], barbiturates [8], bradykinin [19], ferritin [2] and cinchoninic acid [6].

Various studies have revealed that second to the osmotic factors in the regulation of ADH activity is a reduction in effective plasma volume. The mechanism involves the participation of right atrial stretch (volume) receptors which sense decreased plasma volumes and stimulate a release of ADH in an attempt to conserve fluid volume. This physiological pathway seems operative even in the presence of hypotonicity of plasma, suggesting that tonicity as a stimulus may well be of secondary importance under certain conditions [23].

The antidiuretic hormone exerts its action on water balance by increasing the permeability of the distal tubular luminal cells to water, so that hypotonic luminal fluid becomes equilibrated with interstitial fluid. This action is independent of sodium or of reabsorption of other solutes. There are some data which suggest that, because of its ability to induce water retention, ADH may secondarily induce sodium excretion via a suppressed aldosterone secretion due to increased glomerular filtration rate [16].

Under certain conditions ADH may exhibit a significant vasopressor activity (hence the name vasopressin). Antidiuretic levels of ADH cause widespread vasoconstriction affecting peripheral and visceral vessels. There may occur a decrease in cardiac output. This action, coupled with coronary artery vasoconstriction, may be dangerous in patients with decreased coronary reserve.

Vasopressin is distributed in the bloodstream linked to a protein; however, it is not certain what proportion of the hormone is actually protein bound. The hormone is metabolized by the liver and the kidneys, but measurable amounts are still recoverable in the urine. By comparison of extraction ratios, Lauson has concluded that the metabolism of ADH by the kidney exceeds that of the liver by a factor of 2 [15].

CLINICAL DISORDERS ASSOCIATED WITH CHANGES OF VASOPRESSIN SECRETION

Deficiency of Vasopressin (Diabetes Insipidus)

The most significant result of a deficiency of vasopressin is the *secretion of dilute urine with resultant increase in serum osmolality*. In the absence of sufficient fluid intake, the rise in serum osmolality will lead to serious clinical symptoms eventually culminating in death. When the thirst center is active, and provided the patient is not unconscious because of trauma, coma, or anesthesia, such dire consequences are rare, since secondary polydipsia is induced.

Causes of Vasopressin Deficiency [18]. Primary diabetes insipidus may be *idiopathic* (in approximately 45% of all cases) or *familial* (in about 1% of all cases). The familial type often starts in infancy and the idiopathic type in childhood or later life. Secondary diabetes insipidus is observed in head trauma, accidents, head surgery, primary neoplasms, metastatic neoplasms (especially breast carcinoma), sarcoidosis, birth injuries, eosinophilic granuloma, and certain metabolic disorders.

The major clinical symptoms of diabetes insipidus are secondary to the loss of fluid volume (patients excreting 5 to 10 liters of urine daily, with comparable polydipsia). The specific gravity of urine ranges from 1.001 to 1.005, with corresponding low urine osmolality. The specific gravity of urine may increase somewhat with fluid restriction. There are no other major clinical manifestations ascribable to the diabetes insipidus per se, but death may ensue if there is an associated defect in the thirst center or if the patient is unable to consume the required fluids.

Differential Diagnosis. Diabetes insipidus, a deficiency of antidiuretic hormone, should be distinguished from two other conditions which are associated with polyuria and hyposthenuria. There are (a) inability of the kidney to elaborate a concentrated urine despite adequate vasopressin secretion, and (b) persistent excessive water intake such as in psychogenic polydipsia, which may itself result in a relative unresponsiveness of the renal tubule to antidiuretic hormone.

Any interference of the normal renal concentrating mechanism to render it unresponsive to the action of vasopressin may result in a syndrome clinically undistinguishable from diabetes insipidus. *Hypercalcemia,* such as in primary hyperparathyroidism, and *hypokalemia,* due to various causes, may impair the maximum concentrating ability of the renal tubules. In most such patients, the urine specific gravity is lower than normal, but only in a few is it sufficiently decreased and the osmolality sufficiently low to suggest diabetes insipidus. Renal concentrating ability may also be significantly decreased in *chronic renal disease* of varying etiology; such defects are usually accompanied by other evidence of the basic renal disorder. The natural history of *acute tubular necrosis,* due to infection, trauma, renal allotransplantation, etc., will include a polyuric phase subsequent to the oliguric phase.

Increased Secretion of ADH

A syndrome of hyponatremia and hypoazotemia without cardiovascular failure has been described in patients with bronchogenic carcinoma [22]. The presence of hypernatruria distinguishes this syndrome from the hyponatremia found in congestive heart failure or in cirrhosis of the liver. As the clinical syndrome evolves, there is an apparently paradoxical natriuresis ascribed to an escape from the maximal renal effect of ADH due to intra- and extrarenal response to the progressive expansion of total body water. The essential clinical laboratory features of this syndrome include: (a) hyponatremia; (b) decreased serum osmolality; (c) decreased blood urea nitrogen; (d) natriuresis inappropriate to the hyponatremia; (e) an increased urine osmolality; (f) normal adrenal function; and (g) normal renal function [27]. The findings in the syndrome essentially duplicate those observed in normal individuals who are given injections of a long-acting vasopressin and allowed access to a normal fluid intake [16]. The common feature is secretion of ADH inappropriate to the existing water balance status. In addition to bronchogenic carcinoma, pancreatic carcinoma, lymphosarcoma, adenocarcinoma of the duodenum, subdural hematoma, brain tumors, subarachnoid hemorrhage, cerebrovascular thrombosis, skull fractures, cerebral atrophy, seizures, Guillain-Barré disease, tuberculous meningitis, acute intermittent porphyria, and myxedema may be found in the background of this syndrome [4]. Similar finding have been noted after administration of morphine or barbiturates and after anesthesia. Measurable amounts of ADH have been found in the tissues of some of the tumors associated with the "inappropriate ADH syndrome" [27].

PROCEDURES FOR ESTABLISHING A DIAGNOSIS OF DIABETES INSIPIDUS

Water Restriction

With this screening procedure, changes in volume, specific gravity, and osmolality of urine are measured after a timed period of fluid restriction. Normal subjects on fluid restriction may reduce their urine flow to 0.5 ml/min or less, increase the urine specific gravity to 1.020 or more, and increase the urine osmolality to 300 mosmols/kg or greater. The test should be supervised carefully, especially in patients in whom diabetes insipidus is suspected. Water or other fluid intake should be restricted completely for up to 24 hr, depending on the tolerance of the patient. The body weight should be determined at the inception of the water restriction and should be checked frequently. A body weight loss of less than 5% is safe, but any excessive weight loss may lead to circulatory collapse. Patients with diabetes insipidus, or with lack of response to ADH, will excrete urine with specific gravity of 1.001 to 1.005 persistently, unless severe dehydration supervenes. The urine volume will not decrease to the 0.5 ml/min or less as occurs in normal subjects, nor will the urine osmolality increase significantly. It is possible, however, if dehydration

supervenes, that the urine osmolality may rise to 300-400 mosmols/kg; occurrence of dehydration will be avoided if the test is discontinued before the patient has lost 5% of body weight.

The patient who fails to significantly increase specific gravity and osmolality of urine, or fails to reduce the urine volume in a like manner, should be studied further. This screening test does not provide conclusive differentiation between the alternative possibilities of inadequate vasopressin secretion versus nonresponsiveness of the tubule.

Intravenous Infusion of Hypertonic Saline (Hickey-Hare Test)

This procedure is an alternative method of increasing serum osmolality in order to cause release of antidiuretic hormone. The principle of the method is that the increase in serum osmolality induces release of ADH, which, in turn, acts on the renal tubules, decreasing urinary output and increasing concentration of urine.

As recommended by Hickey and Hare [14], the patient is hydrated initially with a volume of glucose in water (8-10 ml/min) sufficient to induce a diuresis of 5 ml/min. The glucose-water infusion is then replaced with a 2.5% sodium chloride solution, infused at the rate of 0.25 ml/min/kg of body weight, which is continued for 45 min. Voided urine flow is determined every 15 min. Normal subjects should exhibit a sharp decrease in urine volume while receiving the hypertonic saline infusion.* Failure to respond in this test with decreased urinary volume still does not distinguish between inadequate release of ADH and lack of responsiveness of kidney tubules.

Nicotine Infusion Test

A third method of inducing ADH release is pharmacologic in nature. Nicotine stimulates release of ADH by the neurohypophysial system. Accordingly, healthy individuals exhibit a sharp decrease in urine flow after the injection of nicotine.

Water diuresis is induced first, as is done in the Hickey-Hare test. Nicotine is then given intravenously (0.5-1 mg to nonsmokers and as much as 3 mg to habitual smokers). The administration of nicotine may be hazardous in some patients and, in adequate dosage, will cause salivation or nausea and may induce vomiting, dizziness, and syncope. Especially with syncope, vascular changes may cause alterations in renal blood flow sufficient to void accuracy of the test. Normal individuals exhibit a significant decrease in urinary volume and increase in specific gravity and osmolality after the injection of nicotine. Failure to respond suggests either inadequate ADH release, or inadequate renal response to ADH, or both.

Response to Exogenous Vasopressin

Volume, specific gravity, and osmolality of urine are monitored after the administration of an adequate amount of exogenous vasopressin. Provided the renal tubules are responsive, normal subjects and patients with diabetes insipidus should exhibit maximal concentration of urine (i.e., specific gravity of >1.025 and osmolality or around 700 mosmols/liter). The patient empties his bladder at the start of the study. Vasopressin in normal saline (5 milliunits per minute) is infused over a 1-hr period (total, 0.3 units). Measurements are made of the specific gravity and osmolality of the voided urine collected every 15 min.

An alternative procedure is to use pitressin tannate in oil. This more convenient procedure is performed as follows: The patient receives an intramuscular injection of 5 units of pitressin tannate in oil at 7 p.m. and empties his bladder at bedtime. On arising, and at 3-hr intervals thereafter, voided urine specimens are collected while fluid intake is restricted. Significant concentrating ability indicates normal tubular responsiveness. The above procedures readily distinguish between diabetes insipidus and nephrogenic diabetes insipidus, the latter failing to respond to the vasopressin challenge. Failure to concentrate urine after exogenous vasopressin is a reflection of tubular nonresponsiveness to the hormone. It is now clear that this could also result from tubular insensitivity associated with long-standing compulsive water drinking — psychogenic polydipsia. Because continued polydipsia lowers the maximal uri-

*This test is hazardous in patients with decompensated cardiovascular disease.

nary concentration after solute changes (induced tubular defect), it is occasionally difficult to distinguish between diabetes insipidus and psychogenic polydipsia on the basis of the above tests. It has been suggested [3] that if, after water restriction, the urine-concentrating ability exceeds that found after vasopressin, then the ability to secrete ADH is normal, regardless of how low the urinary concentration may be. If vasopressin infusion is followed by higher urine concentration than after water restriction, secretion of ADH is abnormal, regardless of how high the urine concentration may be.

THE ANTERIOR PITUITARY

CHEMICAL AND PHYSIOLOGIC CHARACTERISTICS OF THE HORMONES OF THE ANTERIOR PITUITARY GLAND

The anterior pituitary gland influences a variety of biological processes through the production of polypeptide and glycoprotein hormones. Body growth is regulated through the synthesis and release of growth hormone (GH, somatotropic hormone, STH), and the structure and activity of several other endocrine glands are controlled by the anterior pituitary through the secretion of various tropic hormones.

The hormones secreted by the anterior pituitary gland are not readily measured in the clinical laboratory. Only recently has reliable methodology (radioimmunoassay methods) been introduced to quantitate the circulating levels of these substances. These methods are available in a small number of specialized laboratories only. Some of the older biological methods for quantitating anterior pituitary hormones are also useful but also are best performed in specialized laboratories. These remain simpler clinical laboratory procedures which are useful in the work-up of problems involving the anterior pituitary. In addition, the participation of the clinical laboratory is required in many of the procedures preliminary to radioimmunoassays. For example, in quantitating response of GH to hypoglycemia, the clinical laboratory performs the glucose determinations, whereas sample aliquots to be assayed for GH are submitted for radioimmunoassay. Many laboratory procedures designed to measure the effects of the various pituitary hormones on their target organs may also be conducted in the clinical laboratory. For example, assessment of pituitary adrenocorticotropic (ACTH) activity often involves measurement of urinary 17-hydroxycorticosteroids (17-OHCS) after metyrapone challenge. Thus, a good understanding of the activities of the various pituitary hormones is essential to the clinical laboratory personnel..

The anterior pituitary gland (adenohypophysis) is derived from ectodermal cells (Rathke's pouch) in the roof of the primitive oral cavity. After migration of these cells upward and separation from the oral cavity by mesoderm, the cells eventually assume a position anterior to the neurohypophysis. The pituitary gland (anterior hypophysis and neurohypophysis) weighs approximately 500 mg in the normal adult male and as much as 1 g in the pregnant female. The adenohypophysis accounts for approximately 75% of the entire pituitary size. The gland is supplied by branches from the internal carotid arteries. The anterior pituitary itself is supplied by a system of portal veins from a capillary network around the median eminence and the neural stalk.

The anterior pituitary gland is composed of several types of cells, which have been characterized by staining and immunofluorescent techniques. Different cell types presumably secrete specific hormones. Thus, acidophilic cells secrete growth hormone and prolactin; the basophilic cells secrete thyroid stimulating hormone (TSH), follicle stimulating hormone FSH), luteinizing hormone (LH), and adrenocorticotropic hormone (ACTH). Chromophobe cells may also participate in the secretion of the pituitary hormones.

ACTH, melanocyte stimulating hormones (α-MSH, β-MSH), GH, and prolactin exhibit protein or polypeptide structures, whereas TSH, LH, and FSH are glycoproteins. TSH, ACTH, LH, and FSH maintain the structure and biological activities of specific target endocrine glands. GH affects several organ systems and is involved in several biochemical reactions. Recent data indicate that ACTH, TSH, and LH increase the activity of adenyl cyclase in the cell membranes of the adrenal cortex, thyroid, and corpus luteum, respectively. The activation of adenyl cyclase accelerates the formation of

cyclic adenosine-3′,5′-monophosphate (cyclic AMP) from ATP. Cyclic AMP serves as the intracellular mediator of the action of these hormones.

Growth Hormone (GH)

This is a single-chain polypeptide composed of 188 amino acids, with a molecular weight of 21,500. An intrachain disulfide bond forms a ring structure [8]. There are species differences in immunologic as well as in biological activity; only GH from primates has significant biological activity in man. The mechanism of action of GH is not known, but the hormone appears to influence several biochemical mechanisms [1, 7] including stimulation of protein synthesis, intracellular transport of amino acids, increased ribosomal protein synthesis, and intracellular lipolysis. The activity of GH also leads to increased plasma free fatty acids and to increased fatty acid oxidation and ketogenesis [4]; it decreases the responsiveness to insulin and the conversion of glucose to fat in adipose tissue; GH stimulates collagen synthesis, increases intestinal absorption of calcium, and induces hypercalcemia; under certain conditions, GH also induces retention of sodium and phosphate as well as increase in serum alkaline phosphatase. Finally, the hormone is also diabetogenic in certain species.

It is estimated that one pituitary gland contains 5-10 mg of GH. In normal individuals, in the fasting and resting state, the plasma concentration of GH is less than 5 ng/ml. The half-life of the circulating hormone is 20 min. GH level in the plasma is increased by exercise, by hypoglycemia, and by infusion or ingestion of some amino acids, e.g., arginine. The hormone level decreases in the presence of hyperglycemia.

Prolactin

A polypeptide hormone, prolactin has a molecular weight of 23,000; the molecule contains an intrachain disulfide bond. The hormone plays an important role in growth and development of breasts and in lactation [10], these functions being dependent on the availability of corticosteroids, estrogens, and progesterone. The mammotropic and lactogenic actions of prolactin have been utilized in a biological assay. Prolactin stimulates the crop sac of the pigeon, which is the base of a bioassay procedure.

Follicle Stimulating Hormone (FSH)

This hormone is a glycoprotein with estimated molecular weight of 29,000. It stimulates maturation of the ovarian follicles in the female and increases the growth of seminiferous tubules and the process of spermatogenesis in the male. Administration of FSH in women is followed by increased levels of estrogens and pregnanediol in urine [3]. FSH extracted from human postmenopausal urine has been employed to induce ovulation and pregnancy in patients with ovulatory failure; in such patients a high incidence of multiple births has been reported.

FSH is quantitated in urine by a biological assay procedure which utilizes the uterine weight of immature mice as the end point of FSH action. This procedure does not clearly separate FSH from LH activity, however. More recently, levels of FSH have been measured by radioimmunoassay methods, which seem promising [12].

The secretion of FSH by the pituitary gland is inhibited by estrogens; conversely, in estrogen deficiency (e.g., hypogonadism), there are increased levels of FSH in the urine. In the male, testosterone is relatively ineffective in inhibiting FSH release.

Luteinizing Hormone (LH)

This is a glycoprotein with an approximate molecular weight of 30,000. The hormone stimulates the secretion of progesterone by the corpus luteum; the released cyclic AMP functions as the intracellular mediator of the steroidogenic action of LH [11]. In the male, LH (called interstitial cell stimulating hormone, ICSH) stimulates the production of testosterone by the interstitial cells of the testes. Its ability to increase the weight of the ventral prostate in the rate is the basis of a biological assay method for LH.

Radioimmunoassay has been employed in the measurement of LH in plasma [17]. In prepubertal children the level of LH is estimated to be 0.5 mμg/ml. At puberty, in males, the levels reach 0.7 mμg/ml, increasing up to 1.7 mμg/ml in the fourth decade of life. In females,