SPECIAL REPORT

Adrenal Cortex, Aldosterone, and Hypertension

Report of an International Workshop

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THE OPENING SESSIONS of the workshop were about methodology. Coghlan reviewed the recently developed, double isotope-dilution methods for determining aldosterone in peripheral plasma and pointed out that all were time-consuming, expensive, and required use of excellent isotope technic, but do not present extreme technical difficulties. Coghlan emphasized that methodologic errors can be controlled and that equal attention ought to be directed toward standardization of the physiologic status of the patient.

The results reported by Coghlan, Luetscher, and Tait on measurement of aldosterone in peripheral blood reemphasized that postural changes are a powerful stimulus to secretion of aldosterone. Even placing the subject 30° from the horizontal will markedly raise concentrations in the blood. It is, however, necessary to place the subject in a fully upright position before the metabolic clearance rate is markedly changed. Care is needed to avoid corticotropin release in such experiments and the sitting position may provide a suitably quiet but effective stimulus.

The presentations and discussions did not make clear whether the double isotope assay is so laborious and has enough potential sources of error that a simpler method is needed. Nevertheless, there is now reasonable agreement between groups concerning the values in peripheral blood. One notable exception is the assays in patients with essential hypertension in whom Genest's group, but not Coghlan and Peterson, reported raised blood values.

Methods of measuring the renin-angiotensin system were discussed by several workers. Skinner reviewed the renin assay methods and made a plea for uniform terminology. He stated that plasma renin activity may be influenced greatly by plasma substrate concentration and in man the renin substrate concentration is well below the level necessary for the maximum rate of angiotensin formation. Sheep renin substrate has a high affinity for human renin, and in his method of assay Skinner adds sheep substrate to achieve zero order kinetics. Measures of plasma renin activity and plasma renin concentration provide different information about the level of renin in plasma. A rise in endogenous renin substrate observed in pregnancy is associated with increased renin activity but decreased renin concentration. Little is known about the control of substrate production.

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In addition to the concentration of renin and substrate in plasma, other factors influence the rate of reaction. Bumpus reported on a renin inhibitor that interferes with the reaction of renin with the substrate in vitro and in vivo. This substance, a phospholipid, is now called "renin pre-inhibitor" because it is converted into an active inhibitor (a lysophospholipid) by a phospholipase. When the active material is injected intramuscularly into the normotensive, acute or chronic Goldblatt hypertensive, DOCA hypertensive, or spontaneously hypertensive animal, it significantly reduces plasma renin activity. Blood pressure is reduced in all groups save the normotensive and spontaneously hypertensive rat. Bumpus suggested that renin is involved in the maintenance of DOCA hypertension even though the renin levels in the circulating blood are low. He felt that plasma renin levels should be considered with other parameters since a low plasma renin level in one condition may have the same biologic action as an elevated level under other physiologic states. The question was raised whether the natriuretic action of the inhibitor might not be responsible for the decrease in blood pressure, especially in the DOCA hypertensive rats. Bumpus felt that the failure to lower the blood pressure in spontaneously hypertensive rats was against this possibility. It was argued, however, that a natriuretic action is more apt to lower the blood pressure of an animal made hypertensive by sodium than in one in which the hypertension is not sodium dependent.

Problems of radio-immunoassay of angiotensin received much attention. Most laboratories have now been able to generate antibodies to angiotensin II and have published technics. The labelling of the antigen is generally done by the methods of Hunter and Greenwood, and most participants used dextran-treated charcoal for separation of the complex and the free angiotensin. Opinions differed as to whether angiotensin should be extracted from plasma or whether it could be measured directly in it. Peart, Coghlan, and Page each indicated that extraction is the preferable method since slightly lower angiotensin values for normal subjects are obtained. This raised the possibility that small amounts of nonspecific material were being measured in the immunoassay applied directly to plasma, but the issue has not been finally settled. Peart indicated that the normal venous blood level for angiotensin was 8 picograms/ml, in a subject on a normal sodium diet after being recumbent for at least 1 hour. A major problem with immunoassay for angiotensin II is that plasma levels are so low that one works in the lower range of sensitivity of the method. Because of this, fairly large volumes of blood (10 to 15 ml) may be necessary to demonstrate the angiotensin.

Radio-immunoassay of angiotensin I is now being done in a number of laboratories. Both Page and Peart reported on their experiences. The major value of the method is that it is a more specific and a less time-consuming method of measuring renin activity than the conventional bioassay. The group also felt that the angiotensin I immunoassay was better than the immunoassay of angiotensin II for measurement of renin since there is no convincing information that angiotensinases could be inhibited without inhibition of the converting enzyme. Details of the immunoassay for angiotensin I are essentially the same as those for angiotensin II except for the difference in antibodies and the need for incubation of renin with substrate. In general, this incubation is usually carried out for 3 or 4 hours, and angiotensin I levels between 50 and 500 picograms/ml/hour have been obtained from venous blood from normal individuals recumbent for 1 or more hours. There was agreement that extraction of angiotensin I was not absolutely necessary and that satisfactory results could be obtained by measurements made directly in plasma since large amounts of angiotensin I are generated in the system and there is little or no difference between results obtained with and without extraction.

A need was expressed for a central source for angiotensin antibodies since one animal is able to generate a large amount of a potent

antibody and might be able to serve the majority of investigators. This would also help to standardize methods.

Control of Renin

The control of renin secretion was the subject of an evening's discussion. Thurau presented further data supporting his hypothesis that the juxtaglomerular cells and the macula densa constitute a mechanism by which each cortical nephron can regulate its own filtration rate. According to this hypothesis, an increase in the concentration of sodium in the tubular fluid bathing the macula densa causes the release of renin from the juxtaglomerular cells in the afferent arteriole supplying that nephron. The renin reacts with a substrate locally to form angiotensin I, and converting enzyme in the vicinity converts angiotensin I to II. The angiotensin II produces constriction of the afferent arteriole with consequent reduction of the filtration pressure in the nephron. Thurau and colleagues dissected out an individual juxtaglomerulus apparatus, incubated it with purified renin substrate, and found that angiotensin II was produced. This observation indicated the presence of converting enzyme.

The importance of the sympathetic nervous system and circulating catecholamines was emphasized by Ganong. He and his associates have shown that hypoglycemia produces a marked rise in circulating renin without any change in blood pressure but is associated with increased epinephrine secretion. Denervation of the adrenal medulla, but not renal denervation, abolished the renin response. Stimulation of the pressor region of the medulla oblongata produces a rise in renin that is blocked by renal denervation. He concluded that both circulating epinephrine and renal sympathetic activity can stimulate renin secretion.

The importance of hypovolemia as a stimulus to renin release was questioned by Meyers and his colleagues. Reduction of intravascular volume by hemorrhage has much smaller effect on plasma renin activity than administration of furosemide, yet the increase in plasma renin activity following furosemide occurs even when hypovolemia is prevented by a uretero-venous fistula.

Regulation of Secretion and Biosynthesis of Aldosterone

The importance of the renin-angiotensin system in regulation of aldosterone was questioned. Denton presented the anti-renin data on sheep accumulated by his group. Infusions of angiotensin cannot maintain an elevated aldosterone secretion for a prolonged time. The aldosterone secretion rate and renin or angiotensin levels are dissociated during the correction of sodium deficiency, and angiotensin cannot duplicate the effect of sodium depletion on stimulating the conversion of corticosterone to aldosterone. In the discussion, it was pointed out that angiotensin infusions have not maintained an elevated aldosterone secretion in the rat and dog. Unresponsiveness of aldosterone secretion to prolonged infusions of angiotensin has not been observed in man, but there are few studies of this problem. Murlow questioned the importance of the renin system in regulating the response of aldosterone to sodium depletion in the rat. Nephrectomy does not lower the elevated levels of aldosterone in sodium depleted rats and aldosterone secretion can be stimulated by sodium depletion in nephrectomized rats. The pituitary gland, in a permissive way, is of critical importance in the rat's response to sodium depletion. There appears to be some factor in the pituitary gland in addition to ACTH that is important. This factor may be growth hormone or possibly another factor. Dr. Muller stated that no known substance acting in vitro in the rat could stimulate the conversion of corticosterone to aldosterone the way that sodium depletion does. Muller reported that serotonin stimulated the production of aldosterone in rat adrenal quarters in vitro. The physiologic significance is unknown as circulating concentration of serotonin is low in vivo.

Abnormalities in Steroid Biosynthesis and Hypertension

Abnormalities in steroid biosynthesis associated with hypertension were reviewed. Ap-

proximately 35 patients have been reported with 11-hydroxylase defect. Eberlein stated that the hypertension is the result of excessive secretion of desoxycorticosterone (DOC) but the absence of hypokalemia and alkalosis is unexplained. New described the high rates of secretion of DOC and 11-desoxycortisol (compound F) S and low rates of secretion of corticosterone (B), cortisol (compound F), B, F, and aldosterone in this syndrome. Some patients with 21-hydroxylase deficiency have overproduction of aldosterone but neither hypertension nor hypokalemic alkalosis develop. Bartter suggested that the hyperaldosteronism is secondary to progesterone-induced sodium loss. Biglieri described the 17-hydroxylase deficiency syndrome characterized by hypertension, hypokalemia, amenorrhea, and suppressed renin. The enzymatic defect is present in both the ovary and adrenal glands. The excess secretion of B and DOC produces the hypertension and hypokalemia, and the low aldosterone secretion is thought to be due not to another biosynthetic defect but to the suppressed renin and possibly to excessive ACTH. Dexamethasone can readily reverse the hypertension and hypokalemia, but these patients are unusually sensitive to dexamethasone. It was pointed out that prolonged ACTH stimulation results in an immediate rise in aldosterone secretion with a return to pre-ACTH levels and then to a marked fall following cessation of ACTH administration. The mechanism of the profound post-ACTH suppression is not known.

Another form of dexamethasone-suppressible hypertension was reported by Laidlaw and New. Hyperaldosteronism, hypokalemia and suppressed plasma renin are present, but no obvious enzymatic block has been found. Although patients with primary aldosteronism may have a 30 to 50% decrease in aldosterone secretion when treated with dexamethasone, blood pressure and serum potassium values do not change.

Contraceptive Drugs

Sims reported that the menstrual cycle may have a profound influence on aldosterone secretion. Aldosterone secretion is stimulated in the luteal phase. Skinner commented that renin activity also is increased two to threefold in the luteal phase of the menstrual cycle. The effect of oral contraceptive drugs on the renin-angiotensin-aldosterone system was reviewed by Luetscher. The data appear convincing that the pill may cause hypertension in some women. Renin substrate concentration is uniformly increased, but effects on renin concentration, renin activity, and aldosterone secretion are irregular. The mechanism of the hypertension is not clear, and indeed, little is known about the effects of the oral contraceptive drugs on water and electrolyte balance.

Steroid Hypertension

The mechanism by which steroids produce hypertension was discussed. Bartter mentioned that mineralocorticoid treatment of normal subjects or patients with primary aldosteronism following surgery produces little change in blood pressure and suggested that prolonged administration is necessary to induce some bodily change necessary for hypertension.

Tobian argued that the lipid-filled interstitial cells of the renal medulla are influenced by hypertension. The number of granules in these cells as well as the sodium content of the medulla diminish markedly in hypertensive rats. In fact, there is a strong correlation between the numbers of granules and the sodium and urea concentrations in the renal papilla. It was brought out in the discussion period that water loading also diminishes the number of granules in the interstitial cells. The function of these cells is still unknown.

Brownie reported his group's studies on adrenal regeneration and androgen-induced hypertension in the rat. In both conditions, morphologic changes in the adrenal mitochondria are associated with impairment of 11-beta hydroxylation of DOC. Brownie suggested that both of these conditions are a form of mineralocorticoid hypertension due to excess secretion of DOC. In the discussion, Rapp supported this hypothesis by showing elevated DOC blood levels in adrenal regeneration hypertension.

Symington described his histologic findings in the adrenal gland on using light and electron microscopy in 45 cases of primary aldosteronism. Electron microscopy was helpful in distinguishing nodules containing zona glomerulosa cells from those containing zona fasciculata nodules. The histologic picture varies from simple tumor with pleomorphism of cells and with both fasciculata and glomerulosa cells to simple hyperplasia of the zona glomerulosa. In 73% of the cases the aldosteronism was due to adenoma which may be associated with small nodules. Hyperplasia of the zona glomerulosa is also associated with small nodules. The weight of the adrenal gland in primary aldosteronism is unusually low.

Russell reviewed the case histories and adrenal histology of autopsy cases at Johns Hopkins Hospital from the late 1800's to present. The incidence of hypertension in patients with nodules 1.5 cm in size or greater has not increased. In Negro males with accelerated hypertension, however, the incidence of multiple small nodules has increased; Russell thought that these were secondary to the hypertension.

Genest reviewed his long and extensive studies of the adrenal secretion in patients with hypertension. He has found increased aldosterone excretion in about 50% of patients with essential hypertension, and more recently an increase in plasma aldosterone concentration in 50% of essential hypertensive patients. In the discussion, Peterson and Coghlan stated that they had found normal plasma aldosterone levels in patients with essential hypertension.

Kaplan reviewed the evidence in the published reports of Jerome Conn that suggests a significant incidence of aldosterone-producing adenoma in patients with essential hypertension, and took issue with each point of evidence. In his own series, Kaplan found a low incidence of primary aldosteronism. In the discussion, Genest quoted that Conn found 17 cases of primary aldosteronism in 225 consecutive hypertensive patients, an incidence of 7.5%. Melby claimed an incidence of 5% among the patients he studied. Liddle measured aldosterone secretion rates in 90 consecutive patients with hypertension and found a low incidence of normokalemic primary aldosteronism (about 2%). He studied a group of patients with low plasma renin and normal aldosterone secretion rates. These patients had normal serum electrolytes and normal DOCA secretion rates. The explanation for the normal aldosterone secretion rate in face of low plasma renin activity is not clear. Liddle treated these patients with the adrenal inhibitor, aminoglutethimide, and found a fall in blood pressure while none occurred in those hypertensive patients with normal renin activity. These studies suggested that an abnormal steroid secreted by the adrenal gland or unusual sensitivity to a normal secretion rate is playing a role in the pathogenesis of hypertension.

Brown emphasized the importance of repeated measurements of plasma potassium in the diagnosis of primary aldosteronism. Of 82 patients with the disease, 72 had hypokalemia, but in 50% the hypokalemia was intermittent. He emphasized that plasma and not serum potassium should be measured and that the arm should not be exercised before bloodletting. Not all patients who are operated on get better, especially those without a solitary adenoma. Brown stated that the response to a protracted course of spironolactone (Aldactone) in large doses, 300 to 400 mg a day for more than 6 weeks, is a good prognostic test. If the blood pressure responds, surgical intervention will be successful; if the blood pressure does not fall, surgery will fail.

Melby reported similar findings and also noted that the blood pressure of patients with secondary aldosteronism and hypertension did not respond to spironolactone. Most patients who responded did so within 3 weeks.

A series of papers reported a high incidence, about 20%, of suppressed plasma renin in hypertensive patients. Despite the low renin activity, aldosterone production was normal. Several reports indicated that patients with primary aldosteronism with either nodular hyperplasia or zona glomerulosa hyperplasia

respond poorly to adrenal surgery. Liddle recommended subtotal adrenalectomy in these patients with primary aldosteronism since drug therapy can correct the hypokalemia and hypertension.

Melby reviewed his experience with bilateral adrenal vein catheterization for localizing the adenoma by measuring adrenal vein aldosterone concentration and venography. Twenty-five per cent of the tumors may be seen on venography.

Ben-Raad reported an interesting observation. Potassium loading of patients on a low sodium diet for several days results in increased aldosterone secretion and decreased plasma renin concentration. Administration of aldosterone cannot reproduce this effect.

Several investigators reported a wide variety of pathologic changes in the adrenal gland in patients with primary aldosterone syndrome. Although single adenoma comprised the largest group, there were many cases of multiple adenoma, zona glomerulosa-cell hyperplasia, and even normal adrenal glands. Biglieri reported that no test he has used will distinguish preoperatively primary aldosteronism with tumor from primary aldosteronism with hyperplasia. There was general agreement with this statement.

Dangers of Isolation
Samuel Johnson (1773)

The change of religion in Scotland, eager and vehement as it was, raised an epidemical enthusiasm, compounded of sullen scrupulousness and warlike ferocity, which, in a people whom idleness resigned to their own thoughts, and who, conversing only with each other, suffered no dilution of their zeal from the gradual influx of new opinions, was long transmitted in its full strength from the old to the young, but, by trade and intercourse with England, is now visibly abating, and giving way too fast to the laxity of practice and indifference of opinion, in which men, not sufficiently instructed to find the middle point, too easily shelter themselves from rigour and constraint.—From ERIC S. ROBERTSON: *Old St. Andrews.* London, E. P. Dutton & Co., 1923, p. 187.