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REORIENTATIONS IN HYPERTENSIVE DISORDERS*

BY

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In a decade or more of therapeutic endeavour with blood-pressure-reducing agents some rewards have been achieved in gains of life and health, particularly in severe and malignant hypertension (Harington *et al.*, 1959). The application of these therapeutic tools has given opportunities for more extensive and detailed studies on the natural history and pathology of these disorders and the underlying factors in their genesis. I welcome this opportunity to comment on some ideas developing out of the growing experience of myself and my immediate colleagues. I appreciate that we are only one small group among many teams in various parts of the world contributing continuously to the vast and bewildering volume of literature on this subject. Two eminent British schools of thought have presented their experience to this College recently through Peart (1959) and Rosenheim (1954) in their distinguished Goulstonian and Oliver-Sharpey Lectures respectively. No doubt the comments I now present will omit many important facets of the subject, and perhaps some of the "facts" on which my conclusions are based may prove to be no more than shifting sands. My only claim is that they represent a stage in the evolution of ideas based on clinical investigative experience backed by such experimental data as seem relevant.

As the major therapeutic attack on hypertension has fallen on the autonomic system I shall begin by a brief review of the place of the sympathetic system in the control of the arterial pressure and deal subsequently with the problems of renal hypertension, endocrine and electrolyte factors, retinitis and its relationship to severe hypertension, atherosclerosis and hypertension, with a subsequent summing up.

Regulation and Measurement of Arterial Pressure

The arterial pressure is the resultant of two forces: cardiac output and peripheral resistance to blood flow. Sympathetic control of the arterioles has been recognized since the days of Claude Bernard, and our thinking was long dominated by the acceptance of arteriolar tone as the principal variable in pressure control. However, Grimson *et al.* (1937) found that in extensively sympathectomized dogs the fall in blood-pressure induced was closely correlated with depression of the cardiac output; Homer Smith *et al.* (1939) noted that after interference with sympathetic outflow by spinal anaesthesia in man there was little evidence of decrease in peripheral arteriolar resistance and that the measurements suggested

a depression of cardiac output due to peripheral pooling. Pugh and Wyndham (1950) repeated these observations and found that spinal anaesthesia as high as the second thoracic segment interfering with sympathetic tone to the point of dilating skin vessels and making the toes pink and warm produced surprisingly little evidence of vasodilatation elsewhere. In the recumbent position cardiac output fell proportionately to a modest reduction of arterial pressure, but on tilting the feet down the arterial pressure might fall profoundly along with a further drop of cardiac output, and indeed the patient might faint. Relaxation of venous tone together with interference with reflex baroreceptor reaction to a falling cardiac output accounts for most of these phenomena.

The part played by sympathetic regulation of the venous tone has only gained acceptance relatively recently, following the earlier pioneer work of Gollwitzer-Meier and Bohn (1930), Fleisch (1931), and others: the subject was reviewed again by Sharpey-Schafer in his recent Oliver-Sharpey Lectures. In the past few years we have also seen the brilliant series of studies by Sarnoff and his school (1960) showing the role of cardiac sympathetic stimulation in improving the output response of the heart to any given filling

FIG. 1.—Neural regulation of arterial pressure.

pressure. Thus sympathetic nervous activity may exert a pressor effect at a variety of points of action (Fig. 1). It may contract the arterioles; it may constrict the spongework of venules, squeezing more blood towards the central pump and thus promoting an increase in cardiac output; or it may enhance the contractility of the heart itself and increase its output without accompanying change in venous filling pressure; and, of course, it can also increase the heart rate, with further improvement of cardiac performance.

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It has become abundantly clear that pressure reduction by ganglion-blocking or sympathicolytic drugs may result not only from arteriolar dilatation but also from depression of the cardiac output resulting from simultaneous relaxation of venous and arterial tone in the periphery with consequent pooling. All these effects are enhanced by the standing position, and it is easily understandable that some depression of myocardial contractility from sympathetic paralysis could be involved as well (Beck, 1958). Such effects would of course be demonstrable only in the innervated heart, and claims that the isolated heart is unaffected by these drugs may not be relevant to the situation in the intact mammalian organism.

Although our main therapeutic weapons against hypertension act through the autonomic system there has been a general disinclination to ascribe to this system any primary role in the genesis of hypertension. The early experiments of Pickering (1936) showed that in essential hypertension release of sympathetic tone to the vessels of the hand by warming the body was not followed by any undue increase in local blood flow to the hand which might have been expected from the high perfusion pressure if the sympathetic system had been primarily involved in the development of locally increased vascular tone. Duff (1956) also found no evidence of sympathetic vasoconstriction in the hands in the early milder stages of hypertension, describing the vasoconstriction found in the more severe stages as the result of reduced vascular calibre as the disorder progresses. Measurements of regional blood flow in the splanchnic area and brain in hypertensive subjects have usually been within normal limits, indicating a general and evenly spread elevation of vascular resistance. Brod (1960), however, claims that resistance to flow in skeletal muscle vessels in hypertensive subjects is actually low, and this is accompanied by a correspondingly elevated autonomic visceral vasoconstrictive reaction in renal and splanchnic arterioles: the part played by imbalanced autonomic cardiovascular regulation cannot be neglected.

Neurogenic Factors

Neurogenic factors are of enormous importance in the actual setting of any particular pressure level and they are certainly responsible for the considerable fluctuations encountered when frequent blood-pressure readings are taken in normotensive and hypertensive states. It has long been realized that single casual measurements of blood-pressure are liable to substantial fluctuations, and Smirk (1957) has tried to take into account the fluctuations between basal and random readings. Pressures measured under basal conditions, he finds, are a better guide to prognosis, and the closer one approaches to this basal figure the less the rise of pressure appears to be with age; the corollary is that much of the supplemental pressure responsible for casual high pressures is neurogenic.

My colleague Dr. Shillingford has recently designed a pressure recorder which stays on the base of the finger quite comfortably, signalling the systolic pressure in this situation at intervals of five or of one and a half minutes according to choice. A normal resting reading of 120 systolic may fall to 70 during deep sleep and rise to 160 during the mental strain of difficult arithmetic or the visit of a very important person. It was, I think, Ayman who pointed out that an observer might alter

the blood-pressure by the expression on his face as he manipulated the cuff. In Smirk's clinic the medical staff do not use sphygmomanometers in their out-patient rooms, all pressures being taken under relaxed conditions by pleasant girl technicians who are considered to generate less emotion than a familiar and comforting doctor. The well-known "placebo" effect of the patient's confidence in the mere interest, care, examination procedures, and comforting words of his medical adviser can itself abate the pressure and make judgment of the effects of certain milder hypotensive agents exceedingly difficult. Ayman (1930) recognized the value of "enthusiastic treatment of the worried patient," which in certain instances may be just as important as the drugs used as adjuvants. Emotion is one of the most potent of all the influences increasing the cardiac output. My colleague Dr. John Tuckman has shown that amylobarbitone sedation in a hypertensive subject reduces the pressure almost solely by depressing cardiac output, the peripheral resistance remaining unchanged.

Conversely, some of the pressor tests applied to hypertensive and normal subjects may have more cardiac than peripheral vascular effects. It used to be thought that the immersion of a limb in ice-cold water caused a more intense reflex vasoconstriction in hypertensive than in normal subjects. Boyer *et al.* (1960) question this and point out that the rise in pressure is just as often determined by increased cardiac output as by vasoconstriction. The pressor reaction is thus an integrated response of cardiac output and arteriolar contraction, either of which may dominate. In hypertensive subjects, however, it is possibly a little more often vasoconstrictive, and in contrast to normals the reaction certainly lasts longer (Brod, 1960).

In past years when surgical sympathectomy was often used in an effort to reduce hypertension we realized that the procedure failed frequently, perhaps because of the impossibility of eliminating all the ganglia, particularly those in the spinal roots (Boyd and Monro, 1949). Compensation for extensive but inevitably incomplete surgical sympathectomy is no doubt achieved by greater activity of the remaining fibres, and such sympathectomized subjects are well recognized as more sensitive to ganglioplegic drugs. We sometimes fail to remember that these drugs do not *paralyse* the ordinary flow of tonic impulses through the ganglia but rather they *damp* sustained reflex stimulation approximately in proportion to its intensity (Paton, 1952-3). In this manner we account for the postural fall of pressure when no effect is noted in recumbency: reflex postural vasoconstriction fails and hypotension parallels the postural fall in cardiac output. Section of the afferent buffer nerves from the carotid sinus in dogs unleashes excessive activity of the vasomotor centres, with substantial elevation of pressure, and such animals are also unusually sensitive to ganglioplegics (Page and McCubbin, 1951).

Enhancement of Pressure Reduction

We can apply this principle of damping of active vasoconstrictor reflexes to the interpretation of a variety of influences which may enhance pressure reduction in patients under treatment with sympathicolytic drugs. Reduction of blood volume is normally countered by increased sympathetic activity: potent diuretics such as chlorothiazide reduce the blood volume, and sensitivity to autonomic blockade is thus enhanced: sensitivity can be abolished by dextran infusions (Dollery *et al.*,

1959a). Hot baths, hot weather, and febrile illnesses all induce vasodilatation in the skin, which is normally balanced by increased sympathetic tone elsewhere, and once again under all these circumstances sensitivity to ganglion-blocking agents is increased. Exercise dilates the blood-vessels in working muscles, but this is compensated by vasoconstriction in the splanchnic area and elsewhere with a consequent increase in sensitivity.

Patients under the influence of adrenergic blocking agents like bretylium (Taylor and Donald, 1960) or guanethidine (Dollery *et al.*, 1960) may show a profound fall of arterial pressure during and after exercise. This fall of pressure is not due to failure to increase the cardiac output, but according to Taylor and Donald (1960) it is related to vasodilatation or at least lack of vasoconstriction in areas outside the working muscles. Taylor and Donald have demonstrated that vasoconstriction in the splanchnic area and non-working muscle is a normal reaction to the vasodilatation in exercising muscles, and this vasoconstriction is reduced under the influence of bretylium, especially in the non-working muscle mass, where it may fail altogether. Further study of this selective variable sensitivity of particular vascular areas will be of great interest and importance.

The autonomic system thus plays a most important part in setting the level of pressure in any individual patient according to circumstances. We can even define certain hypertensions as being completely determined by neurogenic causes. Poliomyelitis and brain-stem neuro-encephalitis are unusual but well-established primary causes of hypertension. Ostfeld (1961) suggests that the poliomyelitic type may be related to the extent of muscle damage. In human patients with paraplegia from high spinal injuries the spinal cord deprived of central inhibition seems to release potent reflexes causing gross elevation of the blood-pressure under such sensory stimulation as bladder distension (Cunningham *et al.*, 1949). The stress of battle (Graham, 1945) or of violent disasters such as the Texas City explosion (Ruskin *et al.*, 1948) may induce hypertension for a limited period, but its neurogenic origin is not so clear-cut, since adrenal hormones may also circulate in excess under these circumstances. A variety of other neurological conditions associated with hypertension have been collected by Mandell (1959). The data on the relation of hypertension to impaired perfusion of the hind brain (Dickinson and Thomson, 1960) have been received very critically (Lowe, 1961); nevertheless this problem requires exploration by more refined techniques.

We should remember that Conway (1953) has emphasized the very high susceptibility of elderly hypertensives to ganglion-blocking drugs, suggesting a strong neurogenic component in this age range. Grimson (1949) reported the production of hypertension in 180 dogs by section of the vagus and carotid sinus afferents. Over four years of observation, with arterial pressures raised to 200–280 mm. Hg systolic, none of the animals developed a malignant phase or renal damage. Thomas (1944) thought that cardiac output was often augmented in such experiments and noted that the blood-pressure often fell with rest. While experimentalists prefer to produce renal hypertension, which has more dramatic consequences, these experimental neurogenic hypertensions may well bear an important relationship to benign and almost harmless hypertension, so common especially in the elderly.

Non-neurogenic Agents

There are, however, abundant grounds for the incrimination of agents other than neurogenic in the majority of cases of hypertension. Folkow *et al.* (1958), in Sweden, have induced the maximal possible physiological vasodilatation in the forearm by immersion in hot water to the point of skin-redness, and added vasodilating influences of muscular work and reactive hyperaemia. Under these circumstances there still remained in hypertensives a high vascular resistance as calculated by blood flow and local arterial pressure measurements. Flow resistance in their units of measurement were as follows:

Normal	...	...	...	1.81
Essential hypertension	...	...	...	2.48
Renal hypertension	...	...	...	2.09

Somewhat similar results in the vessels of the great toe were obtained by Caliva *et al.* (1959) except that the residual high resistance after sympathetic blockade of the vessels was of a high order only in severe hypertensive patients with marked eye-ground changes. He found little measurable abnormality in mild cases. These findings agreed with those of Duff (1956), who found a reduced hand blood-flow only in severe hypertensives with diastolic pressures above 130 mm. Hg. While residual high resistance is clearly related to the non-neurogenic component of hypertension it is not necessary to ascribe it to an actual anatomical narrowing of the lumen. This would lead back to the old Gull and Sutton theory of arterio-capillary fibrosis as an anatomical cause of hypertension. It is generally accepted nowadays that the arteriolar obstruction in hypertension is of a functional rather than an anatomical character.

Increased responsiveness of hypertensive hand vessels to adrenaline has been shown by Ginsburg and Duff (1958), but this may of course depend on pre-existing narrowing of vessels in which contraction of circumferential smooth muscle cells, by a given unit of length, will cause a greater increase of resistance. These authors claim that similar increased sensitivity of digital vessels to adrenaline may be induced in normal subjects by premedication with hydrocortisone. This does not mean that essential hypertension is a *forme fruste* of Cushing's disease but that humoral rather than anatomical factors may be responsible for the early development of the hypertensive abnormality. It has been noted (Murray *et al.*, 1958) that in the stage of partial relief of renal hypertension by kidney-grafting the arterial pressure is highly sensitive to pressor stimuli, and these reactions are abolished only by removal of the diseased kidneys of the recipient.

Renal Hypertension

Though more than a century has now elapsed since Richard Bright noted the relationship between nephritis and hardening of the pulse, the exact mechanism of this relationship still intrigues us. Substantial steps forward have been Tigerstedt and Bergmann's recognition of renin, Goldblatt's clipped renal artery experiment, and the subsequent efforts of Wilson and Byrom and of Pickering and their schools in this country, as well as the work of groups led by Page, Grollman, and the late Braun-Menendez abroad. The whole subject has been magnificently reviewed by Peart (1959) applying his elegant methods for the recognition of angiotensin, and

creating not a little consternation by his failure to demonstrate this substance in the renal vein in patients with hypertension known to be due to unilateral renal disease. It was with some relief that I heard the recent communication by Peart and Lever (1961) that renin has now been demonstrated in the kidney lymphatics of dogs with narrowed renal arteries, even though the amounts are small.

Clinically a substantial advance was made when Platt (1948) demonstrated that in some 75% of hypertensive subjects under the age of 40 the hypertension was secondary to renal or some other definable disorder. Overt or, more frequently, latent pyelonephritis became increasingly recognized as a cause of hypertension, largely as a result of the studies of Longcope (1937). The possible vascular character of the disturbance in renal damage from pyelonephritis was demonstrated by Kincaid-Smith (1955), who showed that occlusive changes, often with recanalization, took place in the arcuate arteries within the kidney and that areas of the kidney in the immediate neighbourhood had undergone an anatomical shrinkage defined as partial infarction. It is of course possible that scarring in the kidney could also have an effect comparable to the pressor influence of a rigid casing round the kidney induced by the cellophane-wrapping technique of Page (1960). Hawthorne *et al.* (1953) have also shown that experimental constriction of the renal blood-supply can induce hypertension even although there may be no reduction of the mean pressure beyond the constriction. Diminished pulsation, suggested by Kohlstaedt and Page (1940), may thus be an important element in the mechanism of renal hypertension.

In our own studies of the natural history and pathology of almost 200 patients with malignant hypertension it became clear from necropsies that we had not infrequently overlooked cases resulting from partial occlusion of a renal artery and that our clinical methods of detection of latent pyelonephritis were inadequate. About the same time Hodson (1957) was demonstrating that careful measurement of the size of the renal shadows often revealed inequalities in which shrinkage of one kidney had resulted from vascular damage or pyelonephritis. Influenced by his techniques of retrograde catheterization of the aorta from the femoral artery, we have now been able to demonstrate unilateral renal arterial disease in a considerable number of patients who would have been overlooked by our older methods. As an indication of the practical results of this type of investigation, we had in Hammersmith Hospital between 1940 and 1957 seven patients with severe hypertension in whom unilateral nephrectomy was practicable. Since 1958 we have recognized 14 such treatable patients out of twice that number with definable renal vascular insufficiency. This represents perhaps a twentyfold increase in positive diagnosis, often combined with therapeutic success. Even with these improvements, however, we may still overlook potentially treatable cases (see Case 1, Lecture 2).

Routine aortography should always be carried out:

- (1) In hypertensive patients under the age of 40 in whom no other primary cause of hypertension is apparent.
- (2) When severe hypertension has suddenly developed in a subject previously known to be normal.
- (3) In all patients in whom the renal outlines on simple radiographic or pyelographic examination show one or other kidney to be significantly smaller than its neighbour.

(4) When an audible vascular bruit is heard on auscultation over the renal area.

In middle age the cause of partial occlusion of the renal artery is usually a mural thrombus on an atheromatous plaque; this aetiology may also be demonstrable in many of the younger patients. In other instances, however, particularly in younger subjects, the pathology of the vascular narrowing is more obscure and other abnormalities such as congenital aneurysms with distal narrowing may be found to involve a renal artery. An interesting consequence of narrowing of the renal artery is the decrease in volume and sodium content of the urine on the ischaemic side. This gives another method of recognition of an ischaemic kidney (Howard *et al.*, 1954), but collection of urine from two kidneys is a difficult technical procedure and from a practical standpoint aortography is probably more decisive. More work is required with isotope-labelled substances specifically excreted by the kidney for the recognition of inequalities of perfusion.

Towards the end of our studies on the natural history of malignant hypertension (Kincaid-Smith *et al.*, 1958) we became aware that some of these patients developed a severe potassium-losing state with accompanying muscular weakness. There was no evidence at necropsy of a primary aldosterone-secreting tumour of the adrenal. Dollery *et al.* (1959b) observed a potassium-losing state in patients with unilateral renal artery obstruction in whom removal of the offending kidney cured both hypertension and hypokalaemia. It is now considered that in such patients there is in fact an excessive secretion of aldosterone (Laragh, 1961).

The work of Gross (1960) and his associates has done much to clarify the relationship of renin to disturbances of sodium and potassium metabolism (Fig. 2). They

FIG. 2 (after Gross).—The interrelationships of renin, aldosterone, and electrolyte exchange.

showed that the administration of excess sodium chloride or deoxycortone (D.C.A.) caused renin to disappear from the kidneys. Conversely, renin is responsible for a secondary secretion of aldosterone, which in turn induces a potassium-losing state. It is of interest to note that over 20 years ago Pickering and Prinzmetal (1940) noted that renin could induce a marked sodium diuresis in rabbits. Whether this sodium loss is the stimulus to aldosterone secretion, however, is uncertain. While the biochemical consequences of excess aldosterone secretion and hypertension may be corrected by removal of an ischaemic kidney in man, it is also important to remember that a similar potassium-losing state may also occur in malignant essential hypertension unrelated to *unilateral* renal ischaemia.

These electrolyte disturbances constitute strong indirect evidence of renin participation in malignant hypertension, although, in a gross form, they may be relatively infrequent.

On the other hand, it would be unwise to deny that mechanisms other than a direct effect of angiotensin on the arterioles probably play some part in renal hypertension, and it is certainly doubtful whether the amount of renin or angiotensin formed is adequate to maintain hypertension by its vascular action alone. Kolff (1957) suggests that small amounts of angiotensin, inadequate to raise blood-pressure directly, may interfere with a pressure-reducing function of normal kidney tissue. The successful transplantation of a normal kidney into an identical twin with renal hypertension led to a temporary fall of blood-pressure, but the pressure soon rose again, and removal of the diseased kidneys was necessary to achieve a sustained reduction of pressure: this seems to be the usual pattern (Murray *et al.*, 1958). In a similar transplantation case, however, Hume (1958) achieved no pressure-reduction until the diseased kidneys were removed. In both dog and rabbit renal ischaemia does not produce hypertension unless the opposite healthy kidney is removed. Braun-Menendez (1958) suggested that reduction of functional renal mass was an important factor stimulating the formation of renotrophin, a growth hormone of (?) pituitary origin, and he claimed that hypophysectomy reduced or prevented Goldblatt hypertension.

The recent elegant experiments of Cook and Pickering (1959) showing the location of renin in the area of the juxtaglomerular apparatus and the macula densa has focused attention on the changes in this situation. The close proximity of these tissues to the convoluted tubules has led Gross (1960) to suggest that renin may have an important local function in relation to electrolyte exchange. In experimental "Goldblatt" animals the juxtaglomerular cells undergo increased granulation on the ischaemic side (Tobian *et al.*, 1959).

My colleague Dr. Everson Pearse has shown in rats that the Goldblatt experiment leads to a marked increase in glucose-6-phosphate dehydrogenase (G.P.D.) granulation in the macula densa on the ischaemic side with depletion on the untouched side, the significance of the change in concentration of this enzyme being as yet uncertain. Interestingly enough, the same change of decreased G.P.D. granulation also occurs in the salivary duct cells in Goldblatt rats (Gross, 1960). The whole physiological relationship of these important cells to hypertensive disease remains to be more fully elaborated. In cases of human Goldblatt kidney with renal artery stenosis, and even where nephrectomy has led to cure, this granulation phenomenon has not yet been demonstrable. Acute and chronic experiments often differ. The importance of species difference, too, is brought out by the frequent therapeutic success of removal of ischaemic human kidneys after many years of hypertensive disease as contrasted with some animal experiments which have suggested that after a few weeks of renal ischaemia the hypertension induced may become irreversible.

There are many other facets of renal hypertension which remain perplexing. Peart (1959) points out a strong association of hypertension with glomerular renal lesions and its absence in anuric tubular necrosis. Until the final uraemic stages, however, glomerular nephritis, acute or chronic, is often unaccompanied by any very

dramatic degree of hypertension—for example, 160/100—and, conversely, patients with anuric tubular necrosis not infrequently get moderate rises of arterial pressure of the same order. "Renoprival" hypertension, as it was called by Ledingham (1954), is a well-established clinical and experimental entity. In anuric patients at least the problem of maintaining an accurate fluid and electrolyte balance is such that water and salt retention could not be denied as contributory in producing some of these hypertensive reactions. It is also worth remembering that the kidney in acute tubular necrosis is far more severely ischaemic than the kidney with a moderate degree of obstruction to the main renal artery.

Goldblatt (1958) claims that in the early acute phase of clipped kidney hypertension the blood-pressure is singularly resistant to ganglioplegic agents, a point which also emerges from the work of Lavery and Smith (1961). Acute nephritic hypertension (Rosenheim, 1951) is very resistant to ganglion-blocking drugs, an observation which I can personally confirm, although very high dosage of hexamethonium will often succeed in reducing pressure. This observation, however, is in contrast with some earlier work of Pickering (1936) and of Arnott and Matthew (1939), who showed that relaxation of the skin blood-vessels by warming the body was accompanied by a substantially increased hand blood-flow in patients with acute nephritis, suggesting a significant neurogenic component in this disorder. Salt and water retention in acute nephritis may introduce factors in the genesis of the hypertension quite different from those involved in any renin-angiotensin vasoconstrictor effect.

Endocrine and Electrolyte Factors

The realization that the treatment of Addison's disease with D.C.A. and salt could overstep the physiological limit and induce hypertension was documented by Loeb (1940). Continuous excessive administration of salt has also been shown to induce hypertension (Meneely, 1954), while Ross (1956) found an increase in total exchangeable body sodium which was most conspicuous in severely hypertensive patients. Verney and Vogt (1938) found that the administration of salt aggravated experimental Goldblatt hypertension in dogs. Conversely, sodium restriction, such as a therapeutic rice diet, could exert a beneficial influence on the course of severe hypertension. While it is also true that adrenalectomy can reduce a pathologically elevated blood-pressure it does so only by inducing manifestations of Addison's disease. The adrenals are thus not involved in the production of hypertension, although their presence is necessary for hypertension to develop. Cottier *et al.* (1958) have shown that although there is an increased excretion of sodium by the kidneys in hypertension this is in fact dependent on the elevated blood-pressure, as it disappears with hypotensive treatment. While the level of exchangeable body sodium may be increased in certain instances, the abnormality of sodium excretion is probably secondary to elevated vascular pressure in the kidney, and it has nothing to do with the genesis of hypertension.

The administration of D.C.A. and salt to normal subjects if continued long enough can induce oedema, water retention, elevated venous pressure, and a moderate rise in arterial pressure, a condition closely simulating the circulatory phenomena of acute nephritis. The moderate elevation of arterial pressure in acute

nephritis may in fact be more closely related to water and salt retention than to renin secretion.

The relationship of aldosterone to sodium balance in response to the formation of renin or angiotensin has been discussed. The administration of renin causes an increase in the width of the zona glomerulosa layer of the adrenal gland, and this is one of the bits of evidence in favour of this site of formation of aldosterone. Conn's syndrome of primary aldosteronism is usually associated with moderate hypertension, which is reduced by removal of the tumour: the associated potassium loss can also induce tubular damage and pyelonephritis (Milne *et al.*, 1957) with other consequences on the blood-pressure. The obviously treat-

able hypertension of pheochromocytoma we can regard as a partially solved problem. The careful work of von Euler (1956) has shown quite clearly that in the vast majority of essential hypertensives there is no excess excretion of noradrenaline. The distribution of vasoconstriction under the influence of noradrenaline is also entirely different from that seen in essential hypertension or induced by angiotensin. While cortisone by itself may not induce hypertension, in Cushing's syndrome some other unidentified intermediary steroid may be responsible (Grob, 1960): pressure often subsides slowly over a few months after adrenalectomy.

[The second lecture, together with a list of references, will appear in our next issue.]

VACCINATION AGAINST MEASLES

PART I. PREPARATION AND TESTING OF VACCINES CONSISTING OF LIVING ATTENUATED VIRUS

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In 1958 Katz, Medearis, and Enders showed that the Edmonston strain of measles virus adapted to chick-embryo-tissue cultures was considerably attenuated for both monkeys and man. Live virus vaccines for trials in the United States were prepared by them (Enders *et al.*, 1960) and by other workers from the same strain (Stokes *et al.*, 1960; Schwarz *et al.*, 1960).

The studies reported here were undertaken to determine the degree of attenuation shown by four batches of measles vaccine derived from different sublines of the Edmonston strain. Two of these batches were prepared at these laboratories and the other two in the United States. The preparation and testing of the vaccines is described in this paper; Parts II and III describe the clinical and antibody responses of children in clinical trials in England and Nigeria.

Materials and Methods

Strains of Virus.—The passage history of the four vaccine strains is summarized in Table I. The early passages of all four were done by Enders *et al.* (1960). The 3C subline was started from the fourteenth-chick-embryo-tissue-culture passage received from Dr. S. L. Katz. The fifteenth to thirty-first chick-tissue-culture passages were made in these laboratories. From the eighteenth passage onwards the incubation temperature was lowered to 32° C. Passages 27 and 28 were made at limit dilution. The 4A subline corresponds closely to vaccine B of Enders *et al.*; the final three chick-tissue-culture passages were made in these laboratories, starting from material received from Dr. S. L. Katz. The subline for vaccine 8 had been passaged 39 times in

the Parke, Davis Laboratories after receipt from Dr. S. L. Katz. Vaccine L was prepared for Dr. S. L. Katz in the Merck Sharp and Dohme Laboratories, five passages beyond the vaccine A of Enders *et al.*

Titration of Virus.—Serial dilutions in 0.5 log steps were prepared and 0.5-ml. volumes were inoculated into HEP-2 cell cultures, using five cultures per dilution. Cultures were incubated in a roller apparatus at 37° C. and were examined microscopically for the cytopathic effect of measles virus at intervals up to 14 days after inoculation. Titres were calculated by the method of Kärber and were expressed as TCID₅₀ per 0.5 ml.

Titration of Neutralizing Antibody.—The sera from experimental animals and from vaccinated children were examined for neutralizing antibody. Pre- and post-vaccination samples were examined as a pair in the same test. The sera were inactivated at 56° C. for 30 minutes and diluted, using twofold dilution steps. Equal volumes of serum dilution and virus at a final dilution of 100 TCID₅₀ per 0.5 ml. were incubated at 20° C. for one hour. Two or four HEP-2 cell cultures were each inoculated with 0.5 ml. of the mixture. Cultures were observed for 14 days for cytopathic effect. The neutralization end-point was calculated by the method of Kärber. Titres are expressed as the reciprocal of the final dilution in the serum-virus mixture.

Preparation of Vaccines

Vaccines 3C and 4A.—Virus was grown in chick-embryo-tissue cultures. Primary and secondary seed lots were produced under the same conditions as vaccine lots. Trypsinized cells from decapitated chick embryos of 7–11 days' incubation were grown at 37° C. in Roux flasks, using Earle's balanced salt solution plus 0.5% lactalbumin hydrolysate, 5% of heated normal horse serum, and neomycin and polymyxin as antibiotics. Confluent cultures were washed twice with Earle's balanced salt solution, the seed virus inoculum was introduced and left in contact with the cell sheet for one hour at room temperature. At the end of this period the cell sheet was washed twice with Earle's balanced salt solution and re-fed with synthetic medium 199. Inoculated cultures were incubated at 32° C. for

TABLE I.—*Passage History of Vaccine Strains*

Early Passages	Vaccine	Additional Passages
Edmonston strain: 24 in human kidney	3C	17 in chick-embryo-tissue cultures at 37° C. 14 " " " " " " " " 32° C.
+		
28 in human amnion	4A	13 in chick-embryo-tissue cultures 6 in embryonated eggs 1 in chick-embryo-tissue cultures at 37° C. 3 " " " " " " " " 32° C.
+		
6 in embryonated eggs	8	53 in chick-embryo-tissue cultures
	L	19 in chick-embryo-tissue cultures