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THE RENIN-ANGIOTENSIN SYSTEM

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In the four years since the major review of angiotensin by Page and Bumpus (222), the renin-angiotensin system has been the subject of the most intense endeavour. This undoubtedly stems from the discovery of the relation between the kidney, the suprarenals and aldosterone secretion, brought out by the work of Gross and his colleagues (131) and Davis and his colleagues (88, 89), and further by the direct demonstration of the stimulation of aldosterone secretion by angiotensin (123, 184). The very names used for the circulating substance—hypertensin, angiotonin, angiotensin—retarded the study of this field. Once it was clear that this substance had actions other than vaso-constrictor or pressor

actions, work developed rapidly to re-define the possible roles of renin and angiotensin in normal and pathological physiology.

I. COMPONENTS OF THE SYSTEM

A. Purification of renin

In most descriptions of the use of renin experimentally, this material has been only partially purified, and it may well be that the impurities yielded misleading or inaccurate results. The major work on purification of renin as an enzyme has been that of Haas, Goldblatt, and their various associates (138, 139, 140; see especially 138). They followed the well established techniques of alcohol or acetone treatment, aqueous extraction, acidification and ammonium sulphate precipitation and achieved a purification of 56,000-fold from the initial extracts (140). The activity is difficult to assess precisely but about 1 μg of protein is equivalent to 1 Goldblatt unit of renin, which raises the blood pressure of the dog about 30 mm Hg. Methods of protein purification have advanced considerably during the last few years, particularly those involving chromatography, molecular sieving on gels, electrophoresis, especially in media capable of molecular sieving, and ultra-centrifugation. These physico-chemical means of separating proteins may be made even more effective by the use of immunological criteria of purity. None of these techniques in itself is sufficient to establish the homogeneity and complete purity of protein, but taken together they give greater confidence than hitherto that a high standard of purity has been achieved. The application of modern techniques started with the use of diethyl-amino-ethyl (DEAE) cellulose chromatography (170, 223, 227). This has been used to measure the concentration of renin in the plasma of various species (60, 61, 192). Gel filtration on columns of Sephadex was first described by Lever and Peart (190) in studies on renin occurring in lymph. Carboxy-methyl and sulpho-ethyl cellulose Sephadex have been used as DEAE cellulose was used (273). Starch gel electrophoresis in many ways is one of the most exacting tests of homogeneity of protein, since it depends not only on the electrical charge but also on molecular sieving; it has been used by Nairn *et al.* (217) and Peart *et al.* (228). Immuno-electrophoresis has recently been applied in both starch gel and agar gel (228) as a test of homogeneity. Antisera to pig renin raised in the rabbit were used to identify various immunological components at various stages of purification. A preparation was obtained to which only one precipitin line appeared on immuno-starch gel electrophoresis; yet this cannot be taken as a proof of complete purity. The preparation of pig renin obtained by use of most of the techniques described has a molecular weight of about 40,000 to 50,000 as judged by its behaviour on Sephadex gel filtration. On immuno-starch gel electrophoresis, it runs in the pre-albumin position relative to serum and to it antirenin serum gives only one precipitin line. Its potency is such that 5 ng injected into the rat anaesthetised with pentobarbitone and treated with pentolinium (224) usually raises the blood pressure about 20 mm Hg (228).

1. Properties of renin. As was apparent from the early extraction procedures of Haas and Goldblatt (138), renin is a very stable enzyme which withstands quite

wide extremes of temperature and pH. It withstands a pH as low as 2 to 2.5 without marked deterioration, and in a crude state will even withstand a pH of 10 for some time. It is not destroyed by raising the temperature to 60° for half an hour but is destroyed at 80° within twenty minutes. As the preparations become purer, stability does become a problem, and in dilute solution it is essential to store it at 0°, if one is to prevent its deterioration. Repeated freezing and thawing in dilute solution lead to considerable loss, and freeze-drying also is harmful. As in the case of many other enzymes, frothing in solution leads to loss of activity (272).

Enzymatic properties. When the renin is incubated with substrate from plasma from which it makes angiotensin, the initial velocity of reaction is of zero order (192), and the amount of angiotensin produced depends only on the amount of substrate available for conversion. The reaction is a very quick one, and while earlier it was thought that this proceeded best at pH 7.3, subsequent study has shown that the optimum pH lies at about 5.7 (192). This optimum varies for renins from different species using a common substrate derived from the ox (60, 61, 192). Whether this variation also occurs in reactions where the species of renin and substrate is the same, is not known. The pH optimum is not very sharp and extends roughly over ±0.5 pH unit. The bond specificity of the enzyme requires further study with the purer renin. The only relevant work is that of Skeggs *et al.* (254, 260) using both a synthetic and a naturally prepared tetradecapeptide, from which renin split off the C-terminal tetrapeptide, leaving the angiotensin decapeptide. The split occurred at the leucyl-leucyl bond. It is not yet clear whether this is the only site of attack or the most susceptible bond to be attacked by renin. Deodhar *et al.* (95) suggested that renin is an SH enzyme since *p*-chloromercuribenzoate destroys activity, but the latter has not been confirmed with pig renin (223a).

2. Criteria of renin-like activity. In discussing renin-like material in any tissue, it is necessary to define what is meant by the term renin. Certain criteria suggested before (226) can now be amplified. The unknown should be identical with standard renin in 1) biological actions, 2) physico-chemical, chromatographic and electrophoretic characteristics, 3) immunological characteristics, and 4) the initial enzyme-substrate velocity curves. In most claims of the demonstration of renin, nearly all these criteria are found wanting. For the assay of the very small quantities of renin present in many situations, enzyme kinetics makes the best index.

3. Location of renin. In an earlier review (227) the author concluded that renin is located close to the vascular pole of the renal glomerulus, in or near the juxtaglomerular apparatus (82, 83). Since the original observations of Goormaghtigh (128), who suggested on histological grounds that the juxtaglomerular apparatus might well be the site of production of renin, there has been a great deal of work done on the morphology and less, but perhaps more directly important, work on the extraction of different parts of the juxtaglomerular macula densa complex. The way in which the size and degree of granulation in the juxtaglomerular and macula densa cells vary in relation to electrolyte balance, to the administration of

adrenal cortical hormones, and to interference with the blood supply to the kidney has been studied by numerous workers (143, 144, 145, 276, 277, 278, 285). It is clear that in the absence of the adrenals the granules in the complex increase. In the rat, granules increase not only in the afferent arteriole, but also in the efferent arteriole, while dense cytoplasmic bodies appear in the macula densa (7). The most prominent workers in this field are the Hartrofts (144), who showed that the degree of granulation is diminished by the administration of deoxycorticosterone (DOCA). Like Goormaghtigh (128), they found that granulation increases when the renal artery is constricted. Sodium restriction increases the granulation; excess sodium decreases it. Hypertrophy of the zona glomerulosa usually parallels the degree of granulation, but hypokalaemia leads to hypertrophy of the zona glomerulosa without change in juxtaglomerular granulation. ACTH increases granulation even in the absence of the adrenals (201). Marx and Deane (202) showed that in rats on a low sodium diet, the glucose-6-phosphate dehydrogenase activity in the macula densa, measured by histochemical means, increases. The administration of angiotensin to the rat in relatively large doses is associated with degranulation of the juxtaglomerular cells (169, 181, 203, 204). It has been very widely assumed that the degree of granulation always runs parallel to renin secretion, and the loose assumption has been made that the granules themselves are the site of renin production. This may be so, but the evidence for it is not yet adequate. There is no doubt that one can extract more renin from kidneys that have increased granulation, and that, after treatment with DOCA, as the degree of granulation diminishes, so does the amount of extractable renin (134, 233, 234, 285). Similarly in the rat with renal-clip hypertension, the increased renin beyond the clip is associated with increased granulation, and the decrease to zero in the unclamped kidney parallels the disappearance of granules (23, 233, 234). Nevertheless the enzymic changes in the macula densa on the clipped side (234) (and, interestingly enough, in the parotid gland duct epithelium) show that other factors must be considered.

The localisation of renin has been attempted by two different methods. The first has depended upon very fine dissection techniques. Cook and Pickering (83) separated out glomeruli and showed that renin was present only in the vascular pole, and Cook (81), by sucrose gradient centrifugation, showed that renin was associated with one particular sedimenting layer that contained granules. Bing and his collaborators (25, 26, 27, 28, 29, 32) have similarly managed to separate out fragments of the juxtaglomerular apparatus and have concluded that renin is probably found mainly in the macula densa. The other approach has been by use of immuno-fluorescent techniques. The Hartrofts (106, 144) claimed that using antirenin tagged with fluorescein, only the granules in the afferent arteriolar wall are stained and not the macula densa. The answer to this problem is a matter of considerable moment, since in considering the appropriate stimulus for release of renin, the site of its production and storage assumes great importance. While the approach of Bing and his colleagues (25) is obviously extremely careful, it cannot be regarded as completely conclusive. The biggest criticism of the fluorescein antibody technique is that the antibody used is impure, since the renin anti-

gen is impure. The solution to the problem will come only from either more minute dissection or a more specific antibody. A new observation on the distribution of renin, that bears on the stimulus for production, is that of Brown *et al.* (54). Originally Cook *et al.* (82) found that renin content was correlated with the number of glomeruli in the outer third of the renal cortex, but in the middle and deeper layers renin was absent while the glomeruli were still plentiful. This led to the conclusion that renin is associated either with a particular type of glomerulus or with some other structure present only in the outer cortex. Brown and his colleagues (57a) showed that when a clip was put on the renal artery, renin then developed close to the deeper glomeruli. On dissecting out all glomeruli arising from a branch of the interlobular artery, this difference was demonstrated for individual glomeruli. The juxtaglomerular apparatus became more prominent in these deeper glomeruli. Bing and Kazimierczak (30) observed that in the newborn pig renin could be found in the sub-capsular zone, which is free of afferent and efferent vessels and contains only tubular tissue; they offered this finding as evidence in favour of their view that the macula densa is the site of origin of renin. An alternative possibility is that arterial precursor cells were present and contained renin.

Claims of the presence of renin or renin-like material in organs other than the kidney have been numerous. The organ extract most likely to contain true renin is the enzyme extracted from the submandibular gland of the white mouse (279, 290, 291, 292, 293). The product of this enzyme from substrate seemed identical with octa- and decapeptide angiotensin. Attempts to extract a similar enzyme from the salivary glands of other species have failed. In the past Dengler (93) claimed that there was a renin-like material in extracts from the pig arterial wall, which, on incubation with plasma, produced a vaso-constrictor that also contracted the intestine and uterus. The nature of this substance still remains open to doubt but Gould *et al.* (129), also using pig artery, claimed that renin-like material was present. Nearly 75% of the renin seemed to be present in the adventitia of the artery. They suggested this was due to the presence of the vasa perforans, but since these penetrate into the media, this interpretation is unlikely. Extracts of liver, placenta and pregnant uterus exhibit similar activity. Using different and probably less sensitive techniques, Pickering and Prinzmetal (231) could not find renin in many other tissues, including the liver. The amounts relative to those in the kidney must be very small and the identity of the enzyme with renin is dubious, *e.g.*, in the demonstration of renin-like material in the placenta of the cat (266), and more recently the uterine wall of the rabbit, whether pregnant or non-pregnant (136). These renin-like materials could be made in these various sites or they could be adsorbed from the blood stream and stored. Extracts from similar organs in nephrectomised animals would be of interest. The other major sites outside the kidney in which a renin-like enzyme has been claimed to be present are the blood, lymph, urine, and amniotic fluid (47, 52, 53, 150, 190, 192). The older references, of course, started with the original demonstration by Braun-Menendez *et al.* (49) of the appearance of a pressor substance in blood of the renal vein. Measurement of renin in blood and

elsewhere depends clearly upon the adequacy of assays for very small quantities of renin and this will be discussed later (Section IIA).

B. Substrate

The presence of the substrate of renin in the *alpha*-2-globulin range on electrophoresis had been established by Green and Bumpus (130). Walaszek and Huggins (287) fractionated plasma by a modified Cohn procedure and obtained a fraction which, when incubated with amylase, produced a pressor and smooth-muscle-stimulating substance. By applying newer methods of protein fractionation, Skeggs *et al.* (259) have brought a further advance. They suggested that there are five different substrates which, on incubation with renin, produced angiotensin-like material. Three of these substrates were obtained in a pure enough form to compare amino acid composition. They were very nearly identical, and the investigators suggested that their different electrophoretic mobilities might be due to variation in sialic acid content. The rate of reaction of renin with these three substrates seemed to be the same and the angiotensin produced in each had aspartic acid as the N-terminal amino acid as in the angiotensin produced from whole plasma. This work may be compared with previous investigations of these authors into substrate (254, 260), in which they used enzymatic digestion of plasma proteins with subsequent chromatographic separation of the peptides to obtain a tetradecapeptide which, on incubation with renin, was split into decapeptide and tetrapeptide at the leucyl-leucyl bond. As a further outstanding contribution they were able to synthesise this tetradecapeptide and show that it behaved exactly like the natural one. The source of renin substrate has not been established. While the liver may be the origin, the evidence for this is not strong. The specificity of renin substrate has been studied by Fasciolo (109). After nephrectomy in dogs the renin substrate levels rose whereas bradykinin substrate did not. Renin has been previously shown to exhaust its substrate without modifying substrate for bradykinin (108). It seems likely that kallidin and angiotensin may be derived from the same or very similar substrates.

Converting enzyme. All that is known about the enzyme which converts the decapeptide first produced by the action of renin on substrate to octapeptide, is due to Skeggs *et al.* (258), who showed that it was dependent on the presence of sodium chloride.

C. Angiotensin

Studies on the physico-chemical properties of angiotensin have been very few but the isoleucyl[5] angiotensin octapeptide is most likely to have a helical structure (265). This view was supported by its behaviour on dialysis (86). Efforts to prepare radio-active angiotensin have met with varying success. The original description (294, 295) of I^{131}-angiotensin is almost certainly misleading, since Barbour and Bartter (8) subsequently showed that iodination led to considerable loss of activity. In their hands also tritiation led to loss of activity, but Khairallah *et al.* (176) have managed to prepare a randomly tritiated angiotensin, and this has been used in metabolic studies.

D. Angiotensinase

It is clear that there must be more than one form of angiotensinase. Of the better defined enzymes in the body, trypsin, chymotrypsin and leucine amino peptidase destroyed the activity of angiotensin very quickly (107a). Studies on angiotensinase activity of various organs and body fluids have not hitherto been very precise. However, Khairallah *et al.* (173) studied peptidases in normal human plasma and red cells and showed that preparations from both had the same pH optima and were calcium dependent. Perhaps because of this, the enzymes were inactivated by disodium EDTA (3×10^{-3} M). The substrate requirements were for *alpha*-aspartic or asparagine as the N-terminal amino acid. When the link at the N-terminal end of the angiotensin is through the *beta* carbon of aspartic, the molecule is resistant to digestion by blood angiotensinases. This resistance is perhaps responsible for the prolonged pressor effect of *beta*-aspartic angiotensin on intravenous injection (64, 159). Klaus and Biron (178) used electrophoresis to separate serum angiotensinases. The activity ran mainly in the *alpha*-1-globulin fraction in normal man, although in hepatitis the angiotensinase activity spread into the *alpha*-2 region in company with other amino peptidases. They believed it likely that there were several amino peptidases comprising angiotensinase activity in the plasma. Undoubtedly more exacting separation techniques are needed to draw conclusions about this aspect (177, 179).

II. ASSAY OF THE COMPONENTS

It is wise before discussing the rate of production of a substance to consider the methods of measurement. I have stated before (226) that one of the biggest handicaps was the lack of adherence to reasonable criteria. The same statement can still be made to-day about assay procedures for renin and angiotensin. At the present time of rapid expansion, it is easy to predict that there will be a large mass of confusing and inaccurate data produced by methods which are not quantitative. It seems timely to re-state the requirements of an acceptable assay for the various substances involved in the renin and angiotensin system. It should be specific, quantitative (with predictable recoveries at various levels), reproducible, and sensitive at physiological levels.

A. Renin

In the measurement of very small quantities of renin, criteria of specificity are the most difficult to apply but the most sensitive quantitative technique at present is that of enzyme kinetics. These aspects have been fully discussed in various papers (60, 61, 192). In this method (192) renin activity was extracted from the plasma or other body fluid; the initial velocity of its reaction with a standard substrate was proportional only to the renin content. The other main methods in current use (47, 150) do not give data which are fully satisfying from the quantitative aspect. They may indicate whether there is a lot or a little renin present, but even there they could be misleading and, on the basis discussed here, could not safely be regarded as quantitative assays. In all instances, the final product of the reaction of renin with substrate is measured pharmacologically as

angiotensin, and fortunately the techniques for this are very sensitive (224, 255). The final product may be measured as decapeptide or octapeptide; in most assay systems with blood pressure measurement the result will be the same. If an assay system with different sensitivities to octa- and decapeptide were used, then control of the amount of converting enzyme would be essential.

B. Angiotensin

Page and Bumpus (222) described most of the current methods for recovery and assay of angiotensin. The first involves the extraction of angiotensin into organic solvents, followed by adsorption, *e.g.*, on alumina (257) or on ion exchange resins (212, 214, 246), and final biological assay after elution. The other method depends on a biological assay in which an attempt is made by the use of different blocking agents to achieve specificity. The rat colon has been used in this way (236). With some of the methods, difficulties of reproducing results in different laboratories have been extreme, but the method of Scornik and Paladini (246) seems to permit reproducible recovery of small amounts of angiotensin. Promising results have been reported (67) with an approach which may supercede these; it depends on isotope dilution techniques using tritiated angiotensin. The concentration of angiotensin in the blood is usually so low that techniques for assay will probably always remain difficult and subject to unpredictable variations. The biological assays always used in any of the current procedures have the merit of extreme sensitivity; they will show a few ng of angiotensin per ml of fluid (147, 224, 236, 255). An assay for renin based on enzyme kinetics *in vitro* will always be more sensitive. In theory, as long as the reaction proceeds to completion, the amount of angiotensin which can be produced is limited only by substrate concentration. The use of a biological assay without a preliminary physico-chemical separation of renin or angiotensin must always be somewhat suspect and a parallel pharmacological assay would then add great weight.

C. Substrate

The same arguments as were applied to the assay of renin and angiotensin apply to the measurement of substrate. So far there are no fully reliable quantitative data about variation in amounts of plasma substrate because the conversion of substrate into angiotensin has not been properly quantified. Naturally the presence of angiotensinase in plasma complicates the problem. A suitable method requires the measurement of the amount of angiotensin produced by a standard renin free of both substrate and angiotensinase when the substrate is free of renin and angiotensinase activity.

D. Angiotensinase

The assay of angiotensinase is a relatively simpler problem than the measurement of other components of the serum. The effect of organ homogenates in destroying angiotensin has been studied by simple incubation of synthetic angiotensin with the tissue (22, 235); the destruction is probably due to multiple enzymes. Khairallah *et al.* (173) showed that EDTA blocks the action of one

angiotensinase requiring *alpha*-aspartic or *alpha*-asparagine as the N-terminal amino acid for its action. The pH optimum for this enzyme is between 7.5 and 8. Anticoagulants have properties apart from anticoagulant activity and it may well be that angiotensinase activity in plasma *in vitro* may depend upon these properties. The simplest method for assay is to add synthetic angiotensin to the plasma and follow its decay curve under the given conditions (151, 183). An adequate assay requires that 1) the action of renin on substrate in the serum must be completely prevented, 2) the anticoagulant itself must be without effect on the angiotensinase activity, and 3) when a decay curve is being followed, the substrate (angiotensin) should be present in excess.

III. ACTIONS OF RENIN AND ANGIOTENSIN

Since most of the data about the actions of renin strongly suggest that it exerts its action through the production of angiotensin, various effects will be discussed jointly.

A. Haemodynamic effects

Most of the data available concern angiotensin, mainly in the form of the synthetic val^5 octapeptide. Little work has been done with the purer preparations of renin and much that has been reported is therefore doubtful.

1. Heart. The effects of synthetic angiotensin are not very different from those of cruder preparations reported earlier (210). Some of the discrepancies result from the fact that the analysis of the precise actions on the function of the heart have differed according to the technique used. In the intact animal the intravenous infusion of angiotensin leads to a slowing of the heart rate and a decrease in cardiac output secondarily (42, 91). In the intact non-anaesthetised animal, however, the influence of the peripheral circulation and the nervous system as well as hormonal effects, such as the release of adrenaline from the suprarenal (111), have to be taken into account. It is important to turn to studies on the isolated heart. An earlier study was that of Bianchi *et al.* (19) on the isolated heart of guinea-pigs and rabbits; very large doses of angiotensin (100–1,000 μg) produced a decrease of contractile force and coronary flow, followed by an increase. Berry *et al.* (18), using an isolated heart with an oxygenator, found slowing of sinus rate when the angiotensin reached the heart, followed by rise in rate and force of contraction. They suggested that the direct effect on either the myocardium or coronary vessels was responsible for the initial depressor effect. The *beta* blocker pronethalol (nethalide) in a dose of 2.5 mg per kg prevented the change in rate and contractile force; this suggests an action *via* the sympathetics. Fowler and Holmes (116) showed in the dog that there was a decrease of cardiac output and ventricular force with rise of atrial pressure. In the cat, when left ventricular function was studied relatively independently, continuous infusion of angiotensin led to myocardial depression and lowered coronary blood flow. In contrast, noradrenaline caused a large increase in ventricular contractility and an increase in coronary flow (103). The effect of angiotensin on isolated ventricular and atrial muscle shows varying results. Koch-Weser (180) used isolated kitten

papillary muscle and claimed a consistent positive inotropic action. It was not as potent as noradrenaline (wt/wt). Fowler and Holmes (116) confirmed this in the cat. On the isolated atrium, the resting and stretching tension development was unaffected. No ectopic impulses arose and there was no direct effect on the sino-atrial node. Unlike sympathomimetic amines, angiotensin caused no increase in duration of systole. The increased force of contraction appeared with concentrations of 10^{-9} M in the bath. Appreciably higher concentrations were obtained in the blood during therapeutic or experimental administration of angiotensin at rates of 1.6 to 5.2 μg per minute (180). Previously Yu *et al.* (297) suggested that infusion of 1 μg per kg per minute increased myocardial contractility, but this was in the intact subject. Beaulnes (16), using cat papillary muscle and the atria of cat and rabbit, found increased rate and strength of contraction at concentrations of angiotensin less than 0.1 μg per ml; above this, depression occurred. The concentration of 0.1 μg per ml represents an enormous dose in the whole animal. The action on isolated cardiac muscle was blocked by dichloroisoproterenol (DCI) as well as by noradrenaline; this result suggests that part of the action was due to liberation of catecholamines. However, the dose-response curve on the atria of the rabbit was not altered by reserpine, which causes depletion of catecholamines. In discussing cardiac function, apart from a direct action on cardiac muscle, the effect of angiotensin on the coronary arteries is important (209). Fowler and Holmes (116) found that direct injection of 1 μg into the coronary artery of the dog caused constriction, as did infusion intravenously, with blood levels calculated at 0.42 μg per litre. In the cat heart Downing and Sonnenblick (103) found slight reduction of coronary blood flow, whereas noradrenaline produced an increase. Bianchi *et al.* (19), using massive doses of angiotensin also found a decrease. Further studies with much lower doses of angiotensin injected into the coronary artery would seem to be desirable to draw a final conclusion. Despite difficulties of interpretation of the effect of angiotensin in the whole animal, there is general unanimity about cardiac actions. In man the bradycardia induced as the pressure goes up decreases the cardiac output when the infusion rate is between 0.02 and 0.1 μg per kg per minute (91). The bradycardia is almost certainly mediated reflexly through the vagus, for it can be blocked by atropine (45). As the systemic pressure rises, cardiac work increases, and, probably for this reason, the venous pressure rises in every species studied (3, 42, 91, 112). When the rise in arterial pressure was buffered by a mechanical compensator in dogs, there was little change in heart rate. Denervation of the sino-aortic area led to increase of blood pressure and heart rate with intravenous injection of 10 μg angiotensin (220). Interpretation of direct cardiac action in the intact animal at the smaller dose range is difficult to analyse. There is little evidence of a marked direct stimulating or depressive action on the heart in the dose range which produces marked peripheral haemodynamic effects.

2. Regional circulation. There has been dispute as to whether angiotensin constricts *pulmonary* arteries. In normal man, when angiotensin is infused intravenously, pulmonary artery pressure rises before any rise in the systemic pressure; this points to pulmonary vaso-constriction (91). Chimoskey *et al.* (77),

using a dose of 2 μg per kg in the dog, found a rise in pulmonary artery pressure. Other workers, using different animals with different anaesthetic regimes, have suggested that angiotensin has little effect on the pulmonary vessels (19, 208, 240). The effect of the anaesthetic used may be important. Yu *et al.* (297) in man and dog found increased pulmonary resistance. The most likely conclusion is that there is an increase of pulmonary artery resistance and pulmonary artery pressure. This is not as marked as the effect on the systemic vessels and it is overshadowed by the systemic effects.

The general effect of intravenous infusion of angiotensin is to increase the *total peripheral resistance* (44, 91, 112, 160). This is brought about by increased resistance in all territories studied, *e.g.*, skin (44, 91), muscle (44), splanchnic area and liver (11, 76, 91, 248), and the kidney (12, 40, 43, 91, 112, 299). Barer's study directly on renal blood flow in the cat (12) confirms the conclusions of studies of renal and splanchnic blood flow by indirect means in man. Bradykinin could antagonise the reduction in renal flow due to angiotensin. Other effects on the kidney will be discussed later.

There have been few measurements of *cerebral flow* but Ikeda *et al.* (158), using the unanaesthetised rabbit, showed that angiotensin constricts the vascular bed of both the internal carotid and external carotid, in contrast to noradrenaline, which had a definite effect only on external carotid flow. The dose comparison is interesting since 0.1 μg of angiotensin octapeptide reduced internal carotid flow, whereas 5 μg noradrenaline did not. Doses up to 5 μg per minute of angiotensin had no obvious effect on cerebral performance in man (91). In the human retina (102) the arteriolar calibre was more markedly reduced with angiotensin than with noradrenaline and, somewhat surprisingly, the retinal veins were reduced more by angiotensin than by noradrenaline. This effect on the veins will be discussed later since noradrenaline is a more potent venoconstrictor than angiotensin (wt/wt). Measurement of pressure drops down the arterial tree in the upper limb suggested that the major site of action of angiotensin was on the small arterioles (172). The distribution of the major changes of peripheral resistance on intravenous infusion of angiotensin (0.05 to 0.5 μg per kg per minute) has been studied using the altered distribution of rubidium-86 in the rat (200). The investigators claimed that the cerebral vascular resistance was reduced or unchanged. The gut and skin resistance increased, and renal vascular resistance increased most. Other studies suggested that the renal vascular bed was perhaps the most sensitive in man (91). The doses considered hitherto (0.02 μg per kg per minute or greater) are probably above levels at which angiotensin is ever circulating in man or animal. When angiotensin was infused at the lower rates of 0.001 to 0.005 μg per kg per minute, there was no increase in blood pressure in man, nor was there an increased rise in arterial pressure on exercise; cardiac output was slightly to moderately diminished (241).

3. Effects on small vessels. It has already been suggested that the major effect of angiotensin is on the arterioles and that the rise in venous pressure is mainly associated with increased cardiac work (91). Haddy *et al.* (141), using the dog forelimb and mesentery and the rabbit ear, showed that the effect was largely on

the arterioles, especially in the mesentery. There was little effect on venous pressure in small veins. With a predominantly arteriolar effect, one of the consequences of intra-arterial administration would be a sharp fall in post-capillary venous pressure. Watson (289) studied this problem by measuring the vascular distensibility of the veins in the hand. Angiotensin reduced the venous distensibility as would be expected. Folkow and his colleagues (115) measured inflow pressure and limb volume simultaneously in the cat forelimb and showed that while angiotensin raises the inflow arterial pressure more markedly than noradrenaline, it has little effect on limb volume, in contrast to the large reduction produced by noradrenaline. This means that there is little constriction of veins by angiotensin and much by noradrenaline. In the forearm veins of man, de Pasquale and Burch (98) could not show an effect of angiotensin on venous tone, in contrast to noradrenaline. There were similar findings on pre- and post-capillary vessels in the finger (97). The reduction of the calibre of the retinal veins observed by Dollery *et al.* (102) is presumably due to reduction in blood flow through the arterioles.

B. Renal effects

In general most of the renal effects of renin, like other effects in the body, seem to be mediated by the formation of angiotensin. The only possible exception is the proteinuria obtained on intravenous injection (232). Brandt and Gruhn (48) suggested that diminished tubular re-absorption of protein was the cause. Addis *et al.* (2) attributed proteinuria to increased glomerular permeability and considered that this was dependent on the pressor action of the extract. Sellers *et al.* (250, 251) concluded that this was not true since renin given intramuscularly produced massive proteinuria without elevation of blood pressure. Some of these experiments may be misleading in that pig renin was used in the rat and proteinuria is known to occur with other foreign proteins (195). This does not apply, however, to the original observations of Pickering and Prinzmetal (232) in the rabbit. Deodhar *et al.* (95) showed that the presence of intact enzymatically active renin was necessary for proteinuria and confirmed the observation of Sellers *et al.* (250) that adrenalectomy prevented the proteinuria even when pressor amounts of renin were used. They believed it was angiotensin which caused the proteinuria since it had the same effect. However, the degree of proteinuria was very much less with angiotensin and much less consistent. Renin may have an effect of its own in addition to angiotensin. Of course, the renin used is not pure and the effect of impurities has to be considered. It seems conclusive that changes in glomerular permeability are the cause of the heavy proteinuria. Masson *et al.* (205, 206) produced severe vascular and parenchymal renal damage by injections of crude kidney extracts as well as purer renin. Renin-containing extracts plus DOCA, or aldosterone and renin, produced even worse renal damage (207), and DOCA and salt alone produced similar damage. It may be that the pressor effects of renin are responsible for the additive action. Byrom (69), using massive doses of angiotensin, found gross vascular damage and necroses probably due to prolonged vaso-constriction in the intra-lobular arteries. In

nephrectomised rats, injections of crude kidney extracts (124, 218) cause widespread vascular damage and increased vascular permeability with oedema, serous effusions, and serum protein in vessel walls. Angiotensin as well as purified pig renin has the same effect (124). These observations are like those of Asscher and Anson (4), who reported a decrease of plasma volume due to increased vascular permeability. It seems likely that this factor affecting vascular permeability will turn out to be renin. The role of the intact kidney in prevention of this effect, whether it be by removing renin or modifying its action, is not yet understood.

Apart from the proteinuria, as far as is known most of renin's *effects on renal function* are exerted through the effects of angiotensin. Pickering and Prinzmetal (232) were the first to observe the diuresis and natriuresis caused by injections of renin in the normal rabbit. They further showed that angiotensin preparations had a similar effect. The effect of angiotensin in general on renal function in different animals is variable. In normal man, intravenous infusion at all dose levels produced an antidiuresis and reduction in excretion of electrolytes (43, 91). In the rabbit or rat, diuresis and increased excretion of electrolytes are the rule (156, 205, 230, 232, 250). The effect on renal function is almost immediate and, as Barer (12) has shown in the cat, there is always a reduction in renal blood flow. In man, reduction in urine flow and sodium output is accompanied by reduction in the clearance of inulin, creatinine and *para*-aminohippurate. It seems likely that, within the dose range 0.04 to 0.4 μg per kg per minute intravenously, a reduced glomerular filtration rate due to a vascular effect might cause the decreased sodium output. It has been strongly suggested that angiotensin must have a direct action on tubular re-absorption of sodium to explain the diuresis and natriuresis seen in the rat, rabbit and dog. This was supported by Leyssac *et al.* (194) who claimed that angiotensin prevented the uptake of radio-active sodium into tubular cells *in vitro*. Vander (283), using a stop-flow technique with and without injection of angiotensin into the renal artery, has provided evidence that in addition to the vascular component, there must be some direct tubular action. In man with hypertension, infusion of doses of the order of 0.04 to 0.4 μg per kg per minute causes diuresis with natriuresis (62). The return of the blood pressure to normal achieved by either surgical correction of unilateral obstruction of the renal artery or unilateral nephrectomy, or by the use of hypotensive drugs, leads to a return of the normal antidiuresis. The effect of angiotensin on inulin and PAH clearance is negligible in the hypertensive subject and marked in the normal. This fact suggests that the renal resistance vessels show little response to angiotensin at the high pressures, and when the blood pressure is brought down, the response to angiotensin is restored to normal. This interpretation implies that it is the effect of the blood pressure on the vessels which prevents the action of angiotensin. A direct tubular action of angiotensin, preventing sodium reabsorption, might be revealed because the renal vessels in the hypertensive subject have become insensitive. The differences between normal man and the rat and rabbit may be due to a greater effect on the vessels in man so that the direct tubular action could never be revealed. Another approach, however, is that provided by Thurau and Deetjen (274). They suggested that one of the mecha-

nisms involved in urinary concentration and dilution is alteration in medullary blood flow. If angiotensin had little effect on the medullary vessels and, by raising the blood pressure increased medullary flow, then the medullary concentrating gradient could be affected. Since it is possible to bring about both antidiuresis in normal man and diuresis in hypertensive man without altering the perfusion pressure (62), there must be a local renal effect. Changes in glomerular filtration rate too small to be measured with current techniques, produced by variations in afferent and efferent arteriolar pressure, would probably be enough to allow for some of the changes in water and sodium output. In patients with cirrhosis and ascites there was a diuretic response like that shown in subjects with hypertension (185, 186). As these subjects usually have high rates of aldosterone secretion with sodium retention and low urinary sodium, the relation of angiotensin to aldosterone has to be considered. Since angiotensin stimulates the production of aldosterone (123, 184) in doses which are subpressor or marginally pressor in man (33, 184) or dog (280, 281), then the possible modifying role of aldosterone on the renal response to angiotensin needs evaluation. As pointed out earlier, the effect of angiotensin on renal performance is immediate and leads to a decrease in excretion of sodium and potassium. The effect of aldosterone injected into the renal artery is delayed for half an hour and then causes sodium retention and increased potassium excretion. Even though some of the renal action of angiotensin must be exerted through aldosterone, a large part of it cannot. The action of large amounts of aldosterone in cirrhosis may lead to the altered renal response, and some patients with Conn's tumour and primary aldosteronism have been reported in whom angiotensin produced a diuresis (92). This may be an effect of hypertension, however. Some normal subjects deprived of sodium and presumed to have a high secretion of aldosterone, may show a diuresis and natriuresis with angiotensin (185). One might speculate that in these circumstances angiotensin is acting directly as an aldosterone antagonist comparable to spironolactone. The adrenals are not essential to the antidiuretic action of angiotensin (267), nor is the posterior pituitary, as in patients with diabetes insipidus (91). Since angiotensin has such marked actions on renal function, it is important to consider the possible effects of local intra-renal release, shown by the presence of renin and angiotensin in renal lymph (190). Alteration in blood flow even with cortical necrosis, or direct effect on tubular re-absorption, could occur.

C. Modification of the pressor actions of renin and angiotensin

The original discoverers of renin (275) described the increased and prolonged pressor response to renin in the nephrectomised animal. Bing (24) has discussed fully the mechanism of the potentiation. Cross circulation of nephrectomised and normal rats and injection of plasma from nephrectomised rats into normal rats potentiated the response to renin (31, 39). Plasma from nephrectomised animals gave increased yields of angiotensin. It is most likely that the increased sensitivity is due to increased amounts of substrate, which is about ten times normal in the rat 24 hours after nephrectomy. Is the increased concentration due to accumulation caused by an absence of renin? Another implication would be that

with diminished physiological or pathological renin production there might be a rise in substrate. The increased sensitivity of DOCA-treated animals to renin (131, 206) may therefore be due to a decreased renin production and consequently a decreased utilisation of substrate. Satisfactory evidence for this would require that the initial velocity of reaction of purified renin with the substrate from nephrectomised animals and normal animals should be exactly the same. This has not yet been shown. Blaquier *et al.* (39) have suggested that the addition of purified substrate to normal plasma did not increase the amount of angiotensin formed in the same way as addition of plasma from nephrectomised animals. This is probably incorrect. One of the puzzles about thiss ituation is that the blood pressure of nephrectomised animals given renin remains high for many hours longer than in normal animals, yet Gross *et al.* (135, 239, 243) found by cross circulation that there seemed to be no excess renin present in the blood at this time. Maybe renin is adsorbed on the surface of vessels and remains enzymatically active.

1. Tachyphylaxis. Tigerstedt and Bergman (275) were the first to point out that diminished responses could be obtained if successive big doses of renin were given intravenously. Conceivably substrate can be exhausted by this means, but no direct measurement of substrate has been made under these conditions.

Decreasing response to repeated injections of angiotensin has been described by Gross and Bock (41, 132). Tétreault (270) found a gradual decrease in the pressor response with repeated injections of 0.01 to 1 μg per kg in the rat. The decrease is not very great unless very large doses are used. There were identical findings on the perfused isolated hind limb and kidneys of the rat (271) and they regained sensitivity after 90 minutes. The isolated rabbit heart behaved similarly (16). The development of insensitivity might be due to altered electrolyte flux across the vessel wall or to the exhaustion of a substance liberated by angiotensin. The possibility of release of noradrenaline from the arterial wall stores is suggested by the observation that angiotensin releases adrenaline from the adrenal medulla (111). Dogs treated with reserpine (0.2 mg per kg), which depletes noradrenaline stores, were studied by Baum (15); angiotensin by intra-arterial injection (0.1 to 0.2 μg) had less effect than in the normal dog. On the whole animal there was no block of the pressor effect of angiotensin by Hydergine, Dibenamine or tolazoline (137, 269). In the dog hind limb the constrictor response to angiotensin was reduced after acute sympathectomy, whereas the response to noradrenaline and tyramine remained the same (298). Similar results were obtained after hexamethonium or cervical cord section. These findings suggest that angiotensin does not cause local release of noradrenaline from nerve endings or from stores in the vessel wall. Chlorothiazide decreased the renal vaso-constriction produced by angiotensin, adrenaline, or noradrenaline (125). The effect rapidly disappeared after stopping the chlorothiazide. The concentration of chlorothiazide used in the infusion was 0.02 mg per ml and the relation between this and therapeutic levels is not known. Bendroflumethiazide had no effect on the contraction of rabbit aortic strips to angiotensin, but reduction in sodium concentration in the bath increased the response to angiotensin and increase had the

reverse effect (219). This is unlike the results of altering the sodium environment in the whole animal (see below). Changes in the acid base balance produced in cats by various means altered responses to angiotensin and noradrenaline. During metabolic acidosis the vaso-constrictor effect of angiotensin was decreased (68). Huidobro and Paladini (157) claimed that the action of angiotensin on smooth muscle or on the blood pressure was increased by using the solutions at pH 10, and believed this was due to altered molecular configuration. These results might be partly explained by adsorption of angiotensin on the glass container (225, 261). In the intact dog, atropine enhanced the pressor effect of intravenous angiotensin, particularly increasing the diastolic pressure (45). The effect of changes in electrolyte concentrations and balance in the whole animal on the actions of angiotensin has been closely studied, since there are situations in which there has been an apparent lack of correlation between the effects expected from the measured levels of renin or angiotensin and the effects of infusions of these substances into the normal subject. In the sheep, intravenous infusion of angiotensin octapeptide had a greatly reduced pressor effect when sodium deficiency was induced (38). There was a similar reduction in the effect of phenylephrine, metaraminol, or adrenaline. It seems likely that there is a reduction of intra-vascular volume as well as cardiac output in sodium deficiency in the sheep as in man. This failure of pressor response might be mediated either through failure of cardiac output to rise or of peripheral resistance to increase. Doyle and Fraser (104) reported that the effects of intra-arterial infusion of angiotensin were the same in sodium-depleted or normal man. Discussion of electrolyte change and vascular sensitivity leads naturally to the effects of aldosterone. Johnston and Jose (161), in studies on patients with secondary aldosteronism due to cirrhosis of the liver or the nephrotic syndrome, found reduced pressor responses to angiotensin. The effects were very large in certain patients. Laragh *et al.* (186) found that the decreased pressor response to infusions of angiotensin in patients with cirrhosis and ascites did not apply to noradrenaline, and this was confirmed (161). It may be important that most of the patients also were receiving chlorothiazide. Aldosterone administered to rabbits in big doses reduced the pressor effects of intravenous angiotensin (168). Kaplan and Silah (167) reported that patients with various diseases were not as sensitive to angiotensin as normal. Those with a Conn's tumour or malignant hypertension fell into this group. Sodium deprivation in both normal and hypertensive subjects, or chlorothiazide given for seven days, also reduced the pressor effect of angiotensin. Noradrenaline behaved like angiotensin except in patients with cirrhosis and ascites (186). The importance of a reduced vascular response is that large amounts of angiotensin could circulate without the expected haemodynamic effects, and if stimulation of aldosterone production occurred, then there could be a normal blood pressure with high production of aldosterone. The nearest clinical analogue to this is perhaps Bartter's syndrome in children, in which marked potassium deficiency with grossly increased production of aldosterone was associated with hypertrophy of the juxtaglomerular apparatus and decreased sensitivity to infused angiotensin was claimed (14). Another physiological state in which the pressor effect of angioten-

sin is reduced is pregnancy (1, 73, 74, 75). While dose-response curves were not performed in any of the studies, the difference from the normal is large. This is perhaps in line with the decreased effect of angiotensin on urine volume and sodium excretion in pregnant women at all levels of pressor response (73, 74, 75). The work of Brown *et al.* (50) on infusions of angiotensin into the rabbit brings out another aspect relevant to tachyphylaxis. They found that at a dose of 1 μg per kg per minute, the blood pressure rose initially and then fell to normal and did not rise while the infusion continued. At the dose range around 0.1 μg per kg per minute a sustained pressor response could be maintained for months. When the infusion was stopped at either of these dose levels, the blood pressure fell below the starting value and stayed there for a varying period of time. The tachyphylaxis to bigger doses of renin may be due to this depressor effect in response to angiotensin.

IV. MODE OF ACTION

The relation of angiotensin to the nervous system, and also release of catecholamines from the adrenal medulla, has also received attention. The first observation was in the cat where, after cocaine, angiotensin caused contraction of the nictitating membrane and phenoxybenzamine blocked this effect (237). This was possibly the first indirect demonstration of the release of adrenaline and noradrenaline from the adrenal medulla by angiotensin. Subsequently, direct intra-arterial injection of angiotensin was shown to release adrenaline, as indicated by contraction of the nictitating membrane (111). In the rat, suprarenalectomy reduced the action of angiotensin, and direct perfusion of the suprarenals with angiotensin led to increased output of catecholamines (72). It has been shown in the dog that infusion of DMPP (1,1-dimethyl-4-phenylpiperazinium iodide), a ganglion stimulating agent, caused increased pressor response to angiotensin (163). It was believed this was a form of sensitisation, but obviously adrenal discharge of adrenaline and noradrenaline occurred, since phentolamine or adrenalectomy removed the response. Stimulation of the superior cervical ganglion of the cat by angiotensin (0.1 to 0.3 μg), causing contraction of the nictitating membrane, has been reported (193). Ganglionectomy or cutting the post-ganglionic fibres abolished the response. Whether these effects are important in the haemodynamic actions of angiotensin is further raised by the work of Buckley *et al.* (20, 66) who showed that angiotensin (0.2 to 0.4 μg per kg) had a central action in the internal carotid artery territory. The central pressor responses could be blocked by benzodioxane while the effects of intravenous injection of angiotensin were unchanged. Denervation of the carotid sinus area had no effect. There were similar observations on the rat brain (188). McCubbin and Page (197, 198) claimed that infusion of angiotensin or renin in small amount increased the response to various drugs or reflexes initiated in the sympathetic nervous system. Since response to injected noradrenaline was little affected, this action depended upon some other factor in the sympathetic nervous system. They suggested that this mechanism, which appears very quickly after renal artery constriction in the dog, may indicate that one aspect of renal clip hypertension

might be an increased responsiveness of the vascular system to some substances like tyramine. Direct application of angiotensin to the carotid sinus had little effect on its activity compared with that of noradrenaline (199). Another relation of angiotensin to the nervous system is brought out by the contraction of the isolated intestine. Robertson and Rubin (238) found that a natural angiotensin was blocked by an anticholinesterase. Botulinum toxin denervated the ileum by interference with the cholinergic supply, and, while the response to acetylcholine was unaltered, that to angiotensin was abolished. Khairallah and Page (174, 175) had previously found that atropine would antagonise angiotensin on the guinea-pig ileum. The direct action of angiotensin on ileal smooth muscle was shown to be slight. Both the oxytocic action and gut contraction were decreased by adrenaline and noradrenaline. Adrenergic blockers, such as phenoxybenzamine and phentolamine, also blocked the action of angiotensin on these two tissues. This is an odd occurrence and is perhaps to be attributed to a non-specific action of the adrenergic blockers. Walaszek *et al.* (288) confirmed the blocking effect of atropine on the ileum and found that the action of substance P was similarly blocked. Morphine shared with phenoxybenzamine the property of blocking the effect of angiotensin, although that of bradykinin was not affected. In another study (17), contraction of the vas deferens of the guinea-pig, stimulated *via* the hypogastric nerve, was strongly potentiated by angiotensin, which did not itself cause contraction. Splenic contraction in the cat behaved similarly. Further, in anaesthetised and spinal cats in which sympathetic post-ganglionic transmission was blocked by nicotine or tetramethylammonium, the pressor responses were reduced. In keeping with the other evidence, this supports the idea that angiotensin may increase the local release or effect of noradrenaline on stimulation of the nerve but is incapable of releasing noradrenaline from stores in smooth muscle tissue despite its ability to release adrenaline from the suprarenal. The pressor response that slowly develops over a few days when small concentrations of angiotensin (0.006 to 0.02 μg per kg per minute) are given intravenously to the rabbit, may be related to an effect either on ganglia or on the brain (101). The oxytocic action of angiotensin on the rat uterus seemed to depend upon the presence of calcium for its action (100). The use of various smooth muscles to distinguish between angiotensin, bradykinin, oxytocin, vasopressin and substance P has been described (35). It was shown that the rat uterus and guinea-pig ileum seemed to be the most sensitive structures for angiotensin, followed by the rat colon. Recently the rat colon has been described as a discriminating and sensitive test for angiotensin (236). In approaching the precise mode of action of angiotensin on the arterial smooth muscle, contractions due to groups of substances such as potassium, caesium, barium chloride and histamine were differentiated from the type of contraction produced by adrenaline and angiotensin (182). It is possible that there are two different contractile systems for vascular muscle, the one for transient regulation of tension, the other for the maintenance of tonic contraction. The tension of the latter seems to depend on the concentration of potassium in muscular cells (187). The evidence at present for this seems a little indirect but the idea is of considerable interest. In view of the effects of angiotensin on renal function, its lack of effect on sodium transport in the abdominal

skin of the toad, in contrast to vasopressin, is striking (9, 10). An isolated observation is that an infusion of angiotensin at a rate of 0.5 to 1.2 μg per minute for three hours in normal subjects produced a significant rise in serum calcium of up to 3.6 mg per cent in four subjects (120). This could not be neglected in considering actions on smooth muscle or the nervous system.

The mode of action of the different forms of angiotensin must be considered. In the past it has been claimed (120) that only the octapeptide has an action on smooth muscle and there have been relatively few studies on the action of octa- and decapeptide on a variety of smooth muscles. Not all the observations are in agreement. Halvorsen *et al.* (142) could not differentiate between the octa- and decapeptide in their effects on muscle flow with intra-arterial injection. Barac (5, 6) was similarly unable to distinguish the octa- and decapeptide in their vasoconstrictor action on muscular blood flow and renal blood flow in the dog. The action of various analogues of angiotensin has previously been reported in the review by Page and Bumpus (222). Helmer (149) recently has studied a few more analogues and compared their action on the aortic strip. Asparaginyl^1 valine^5 angiotensin octapeptide had 25% and *beta*-aspartic^1 valine^5 angiotensin octapeptide had 75% of the activity of natural angiotensin octapeptide. Although the preparations were all equally active in raising the blood pressure of the pithed nephrectomised cat, their purity was not described. Many analogues were equally active on the blood pressure and rat colon (236).

V. INTERRELATIONS OF THE KIDNEY, RENIN, ANGIOTENSIN, AND ALDOSTERONE

The major work relating the kidneys to the suprarenals in various states arose from the studies of Gross (131) and Davis (89). The former showed, for example, that the amount of renin present in the kidney and probably in the circulation of the rat was markedly diminished by administration of DOCA (131, 133, 134, 135). Davis and his colleagues (87, 88, 89) investigated the stimulus to aldosterone production in many situations and found that the kidney was essential even in hypophysectomised animals and found, like Ganong and Mulrow (118), that extracts of kidney injected intravenously caused secretion of aldosterone (70, 90). In an impressive series of experiments, they showed that the stimulus to the kidney to produce aldosterone-stimulating hormone still occurred when the vena cava was constricted above the liver if the sole kidney was transplanted to the neck (71, 90). There is little doubt that the substance they were studying was renin and that stimuli such as severe bleeding and constriction of the vena cava above but not below the liver, lead to its release in amounts sufficient to cause the production of aldosterone (154, 282). Since the first discovery by Genest *et al.* (123) and Laragh *et al.* (184) that angiotensin stimulated the suprarenals in man to produce aldosterone, the physiological and pathological relation between the suprarenals and the kidneys has strengthened. Renin itself injected directly into the adrenal artery does not cause secretion of any steroid (37, 94) whereas angiotensin under the same circumstances in the sheep produces aldosterone but not corticosterone or hydrocortisone. This direct action was confirmed in the dog by Mulrow *et al.* (119, 214, 215, 216). There is obviously a close relation between

ACTH and angiotensin. Under certain circumstances in the dog, angiotensin causes the production of hydrocortisone and corticosterone, and ACTH will cause secretion of aldosterone (13, 264), and there is some evidence of potentiation between the two (165, 213). The circumstances and state of the animals in the various experiments need to be studied carefully, but the main conclusion is that angiotensin acts very early in the synthetic cycle in the adrenal cortex. While the full pathway of aldosterone production is unknown, the action of angiotensin may shortly throw considerable light upon this. It has this effect not only *in vivo* in sheep, dog and man, but also *in vitro*. The production of aldosterone from progesterone in ox suprarenal slices using angiotensin has been demonstrated (164, 166). The most interesting species difference is the failure of renin or angiotensin to stimulate aldosterone production in the rat (107). The rat is known to produce mainly corticosterone and, if it is true that aldosterone is not produced in response to angiotensin, it may give an important clue as to the metabolic pathways which are stimulated in other animals by angiotensin. In contrast, angiotensin (0.0067 to 0.9 μg per minute) given intravenously to hypophysectomised rats increased aldosterone secretion two- or three-fold (253). The other evidence relating to stimulation of aldosterone production by either renin or angiotensin in the rat is all indirect, and is mainly related to histological change in the adrenal cortex (203, 204). More evidence is needed.

A. Sensitivity of the suprarenals

Genest *et al.* (121) showed that aldosterone secretion was caused by a just subpressor dose of angiotensin in man. As discussed earlier, the pressor action of angiotensin may be markedly modified by differing conditions, and the possibility of this in relation to the suprarenal cortex exists. Blair-West *et al.* (38) showed that in the sodium-depleted sheep, angiotensin had little effect. This might have been due to maximum stimulation of aldosterone in response to sodium depletion. No other situation has been studied where the different factors shown to reduce the pressor effect have an effect on the adrenal response. Urquhart *et al.* (280, 281) showed that angiotensin given intravenously at a rate of 15 to 80 μg per kg per day produced a two- to eight-fold increase in urinary excretion of aldosterone, when infusions were carried out from four to eleven days; the aldosterone secretion was maintained without any effect on blood pressure (281). This long maintained action is very significant since it is in keeping with the action of a hormone which physiologically controls the output of aldosterone. In the sheep, Blair-West *et al.* (37) had shown a decline but not disappearance of aldosterone output when angiotensin infusion was prolonged from three to twenty-four hours. In man, Bryan *et al.* (65) injected human renin of rather crude type; this was followed by increased secretion of aldosterone. There is no evidence in the rabbit about secretion of aldosterone with angiotensin.

VI. RENIN SECRETION

Before it was possible to measure directly the amount of renin or angiotensin present in blood or other body fluids, a lot of information had been obtained by

studying the renal content of renin. In renal clip hypertension produced in the rat by unilateral clipping, renin disappears from the opposite kidney after about two weeks and increases in the clipped kidney (135). If the normal kidney is then removed, the renin in the clipped kidney returns to normal. The result indicates nothing about renin secretion rate, but it seems likely that under these circumstances a low renin content means a low secretion rate. Whatever the effect of putting a clip on one renal artery, there is a differential action on the renin content of the two kidneys. There must clearly be either something coming out of the clipped kidney or an effect produced secondarily which reduces the amount of renin in the normal opposite kidney. It would be natural to assume that this was an electrolyte or adrenal effect. Reduction of renin content by aldosterone administration has been reported, as with DOCA (131), and it will be seen that this correlates quite well with blood levels of renin and angiotensin in various animals in the presence of excess aldosterone. In the Goldblatt era following 1934 (126a), the major stimulus to renin production had seemed to lie in interference with blood flow through the kidney. Corcoran and Page (85) had suggested altered pulse pressure as the appropriate stimulus, and ultimately Tobian (276, 277, 278) suggested that the juxtaglomerular cells in the afferent glomerular arteriole were the appropriate receptors to stretch. However, bearing in mind the evidence about renal renin content, this could not be the only receptor since, in many of the situations concerned with sodium depletion, the renal arterial pressure is not altered, nor is the renal blood flow. In considering the appropriate stimulus, the question of an accurate assay for renin or angiotensin is once more introduced since measuring the effect of a stimulus means the ability to measure accurately the change in concentration. Flow through an organ must also be measured accurately if a change in concentration is to be converted into a meaningful production rate. The renin or angiotensin blood level must be carefully distinguished from production rates. The blood level of a substance depends not only on rate of production, but also on rate of removal. This may seem obvious but needs re-stating. Most of the original data about levels of renin and angiotensin and the stimulus to production, were carried out with doubtfully quantitative methods, but using semi-quantitative methods, Lever and Peart (190) were able to show that renal artery constriction in the dog increased the amount of renin in renal lymph. This was extended by Higgins *et al.* (152, 153), who showed that constricting the inferior vena cava above the liver led to an increase in the renin-like material in the thoracic duct. The absolute stimulus here is perhaps even less clear than in the previous experiments where the artery is partially occluded. Small alterations in the pulse pressure in the renal artery of the dog without flow change led to an increased concentration of renin in the renal vein blood (262). It seems likely that there is increased secretion rate in responses to changes of pulse pressure without changes in overall renal blood flow. Scornik and Paladini (247) have carried out measurements of renal venous angiotensin concentrations in the dog while varying the perfusion pressure. De Vito *et al.* (99, 110) also found little difference in concentration. Hence it seems unlikely that angiotensin is locally produced; rather, renin itself is released. Again with a semi-quan-

titative method and cross perfusion, it has been shown that clipping the renal artery in the rat did not lead to a prolonged increase of plasma renin (135). Scornik and Paladini (246) found that the application of a clip to one renal artery in the dog did not lead to a persistently raised level of angiotensin. Whether this means that the initial stimulus is transient or that the renin level is in some way then decreased by a homeostatic mechanism, is not known. It is necessary, however, to look at the possible effects on urinary composition and to consider whether the macula densa itself can be a receptor for renin production. Situated as it is on the distal convoluted tubule, it is an ideal site to measure changes in osmolality or sodium concentration in the urine, and this may well be the receptor site for those stimuli acting in this way, *e.g.*, aldosterone, DOCA, or sodium depletion or loading. It might then be argued that in the rat with renal clip hypertension and an opposite normal kidney, the decreased renin on the normal side is due to an effect on the electrolyte composition of the distal tubular urine and that the increased amounts on the clipped side are due to this stimulus being sufficient to cause increased production of renin. Brunner *et al.* (63) found that excretion of a saline load by the unclipped kidney was always more rapid than by the clipped as long as the blood pressure of the animal was high. Vander and Miller (284) produced a water and sodium diuresis in the dog kidney with chlorothiazide, urea or mannitol. Mean arterial pressure in the renal artery was varied and renin assay was performed by a semi-quantitative method. While this is not an ideal assay, since other variables could enter, they claimed that the diuretic prevented the usual rise in renin secretion caused by lowering renal artery pressure. Elevation of ureteric pressure also increased the amount of renin in renal vein blood. It is unknown whether an increased arterial pressure leads to a reduction in renin output, although the arterial pressure is higher in the normal kidney of the rat with hypertension due to unilateral renal artery clipping, in addition to any changes in electrolyte and water handling. Luetscher (196) was the first to show in man that sodium depletion led to increased aldosterone secretion. In the sheep reducing the sodium content of the blood entering the adrenal artery caused increased secretion of aldosterone, and increasing the potassium level had a similar effect (78, 94). Since lowering the plasma sodium in the whole animal or man can be associated with lowering of the renal perfusion or pulse pressure, this stimulus is perhaps not so specific as appears at first sight. Nevertheless, sodium depletion itself without change in plasma sodium or blood pressure increases plasma renin, and, conversely, salt loading leads to decrease of plasma renin (56, 58). These results have been confirmed by other estimates of renin and angiotensin (122, 286). Whatever the appropriate stimulus is here, it is certain that a change in the composition of the tubular urine must occur. Whether the change in renin or angiotensin level in the plasma is enough to account for the increased production of aldosterone with salt depletion is not yet known, but the change is in the right direction. In Addison's disease with sodium depletion, the plasma renin in two patients was between ten and twenty times the normal level and the administration of salt, cortisone and 9-*alpha*-fluorohydrocortisone led to a fall in the renin level. Deliberate induction of sodium de-

pletion led to a rise in plasma renin, which was then again depressed by restoration of treatment (56, 57). Therefore sodium deficiency alone in the absence of any adrenal steroids leads to a rise in plasma renin; this may be the converse of the experiment in the rat where DOCA administration led to disappearance of renal renin (135). In Addison's disease with sodium depletion, the glomerular filtration rate is usually decreased, and the sodium content of the urine would be inappropriately high for the level of plasma sodium. This is therefore not like the normal subject with sodium depletion. The possibility of altering tubular urine composition by other means has to be considered. Thurau (273) has proposed that an important method of regulating sodium excretion is by variations in tubular osmolarity acting on the macula densa and inducing changes in afferent arteriolar calibre, perhaps by local renin release. A reciprocal relation would then exist. The induction of renin in the juxtamedullary glomeruli produced by renal artery clipping may mean that the blood flow is usually higher to the juxtamedullary glomeruli and renin appears only when pressure or flow is reduced (54, 57a). Nevertheless, the stimulus might still be through changes in urinary composition secondary to clipping. If, as seems likely, there is a reciprocal relation between aldosterone and its effects and renin production, then all the other factors which play upon aldosterone independently of their effect on renin may be expected to have their effect, including all the factors which alter the sodium balance and renal excretion. The way in which sodium depletion raises plasma renin level in a normal subject, is unknown. There seems little doubt that under many circumstances enough renin and angiotensin are liberated to stimulate aldosterone production. The linking of the stimuli already discussed is obviously very complicated since, for example, when the inferior vena cava is constricted and aldosterone secretion increased (90), similar experiments with constriction of the hepatic veins leaving the vena cava untouched give similar results (221). Presumably some new stimulus is produced within the liver or splanchnic territory, capable of stimulating aldosterone or of releasing renin from the kidney to produce aldosterone. In the dog a definite increase in angiotensin levels occurred with severe bleeding (17 to 58 ml per kg body weight) and reduction in blood pressure. The rise was relatively slow and occurred half to one hour after bleeding (247). In man, bleeding up to 500 ml without pressure drop did not affect renin levels (59). Selkurt (249) showed that when the pressure was reduced to 40 mm Hg by bleeding in the dog, there was a ten-fold decrease in renal blood flow and a three-fold increase in renal vascular resistance, so the renal stimulus was great and effects on renin production may be multiple.

VII. CLINICAL AND EXPERIMENTAL CORRELATIONS OF RENIN AND ANGIOTENSIN LEVELS

Much of the evidence in this field is of a correlative type in that a particular level of renin or angiotensin has been measured in different circumstances. In some of these a relation to aldosterone secretion rates has been made, or more commonly to aldosterone excretion rates.

A. Addison's disease

This has been partly discussed above. All the evidence seems to point to elevated renin and angiotensin levels in circumstances where sodium depletion occurs. As is pointed out, the stimulus may be nevertheless through reduced glomerular filtration, reduced renal arterial pressure, or some other aspect related to sodium depletion.

B. Cirrhosis of the liver

Brown *et al.* (56, 57) found very high levels of plasma renin in patients with ascites as compared with those without. With ascites, the levels of aldosterone secretion are very high indeed (105, 296) and urinary sodium excretion is low. Some but not all of the low sodium excretion is related to the actions of aldosterone and can be partly blocked with spironolactone (171). The question is whether renin is here the major stimulus to aldosterone production. During accumulation of ascites, the plasma volume and the glomerular filtration rate drop. Whether either of these factors is the appropriate stimulus or whether it is changes in tubular urine composition, is not known. The experiments where constricting the hepatic veins led to increased aldosterone production in the dog without the formation of ascites suggest an additional hepatic factor (221). It would be tempting to correlate a low circulating volume in both Addison's disease and cirrhosis with ascites to increased production of renin; in one there is marked loss of extra-cellular fluid with reduced plasma volume, and in the other, increased extra-cellular fluid with reduced plasma volume in certain cases. In cirrhosis with ascites the drive to renin production must be powerful and predominant since the effects of aldosterone in excess would be expected to depress renin production.

C. Conn's syndrome

The possibility that in the presence of a tumour of the suprarenal cortex producing excess aldosterone the plasma renin would be low, was first raised by Brown *et al.* (56, 57) in describing a patient with hypertension and gross hypokalaemia due to hyperaldosteronism, in whom the plasma renin was low and was brought up to normal by spironolactone, which corrected both the potassium depletion and the hypertension. Reduction of the effects of aldosterone was associated with the rise in the plasma renin, even though the secretion rate of aldosterone went on at a high level (over 1000 μg per 24 hours). Removal of the adrenal tumour produced effects like those of spironolactone but the aldosterone secretion returned to normal (55). These subjects usually have increased extracellular fluid and total body sodium but, in contrast to patients with cirrhosis and ascites, the plasma volume is either normal or elevated. Does this mean that the lowered sodium and increased potassium in the distal convoluted tubule inhibit renin production? This seems unlikely since in cirrhosis with ascites, the urinary electrolytes are very like those in Conn's syndrome in certain circumstances. Why one group has hypertension and the other does not, is difficult to see, but the presence of liver disease must be important.

D. Heart failure

Uncomplicated heart failure is not an easy subject for discussion since in man treatment has so often been instituted. There is reasonable evidence that secretion rates of aldosterone are not elevated in heart failure unless diuretics are given (268). The increased plasma volume in congestive heart failure might be expected to decrease the secretion of aldosterone but hepatic factors and raised pressure in the inferior vena cava may play their part (88, 89, 90, 221). Raised renal venous pressure does not increase renin concentration in renal plasma (263). There have been comparatively few studies made on plasma angiotensin levels in heart failure but they seem to be variable and very much dependent on treatment. They have been claimed to be high in the oedematous phase (122, 286). In the dog with heart failure induced by experimental pulmonary stenosis (87) aldosterone secretion rises; renin or angiotensin levels are not available. If renal factors are important, then reduction of glomerular filtration rate and of sodium excretion are characteristic and cannot be explained by the actions of aldosterone alone.

E. Pregnancy

The renin level rises and is usually at its highest in early pregnancy. It is then usually, but not always, at higher than normal levels until delivery, when it falls rapidly to normal (51, 59). The source of this activity and whether it is correlated with the early appearance of renin-like activity in amniotic fluid, is unknown. Aldosterone secretion is probably increased from early in pregnancy but it could not be claimed at present that there is a causal relationship (251a).

VIII. ANTIRENIN

For such a seemingly simple concept as antirenin, the experimental data provide some of the most controversial matter. After the preparation of an antirenin to hog renin in the dog (126), a full study on the best methods of producing renin antibody was carried out (139). The work has recently proceeded along a number of lines: attempts to localise renin by use of the fluorescent antibody; the attempt to correlate the presence of a renin antibody in the circulation with a given physiological or pathological state of the animal; and the use of antirenin, usually heterologous, to nullify the effects of circulating renin. Hartroft and Hartroft (144, 145) claimed that a serum with a high titre of anti-pig antirenin increased sodium excretion when injected intravenously in the dog. Haas and Goldblatt (138), Schmid *et al.* (244, 245) and Deodhar *et al.* (96) claimed that the blood pressure of the dog made hypertensive by renal artery clip could be brought down to normal by injection of antirenin. No convincing assay for antirenin has been presented. Particularly where heterologous serum has been used, the fall in blood pressure observed does not seem to be necessarily related to levels of circulating antibody. The effect of foreign proteins has to be considered most carefully. For example, foreign albumin attaches to the glomeruli of the kidney (146); clearly, effects other than those claimed to be due to antirenin are possible. Schmid and Graham (244) and Schmid *et al.* (245) attempted to correlate renin

concentration of kidneys with antirenin titres induced by injecting hog kidney extracts. There seemed to be little correlation between the amount of renin in the kidneys and antirenin titres in the plasma. Deodhar *et al.* (96), using homologous renin, attempted to render this antigenic by chemical alterations in the molecule, especially acetylation. The pressor activity was reduced to about a third. That in fact the acetylation leads to an antigen which cross-reacts with the natural renin, is a vital claim. The investigators claimed that a dog with renal clip hypertension had its high blood pressure reduced by immunisation with acetylated dog renin and that the fall in pressure was correlated with a progressive rise in the titre of antirenin. After the immunisation was discontinued, the blood pressure rose as the antirenin titre dropped. It seems odd that, if there is such a cross-reaction, the renin spontaneously produced in the animal by the renal clip is so ineffective as a stimulator of antibody once the process has been started. Similar experiments were carried out by Frank (117). Until a more convincing body of data applying to more animals with really adequate and clear demonstration of the antirenin effect is brought forward, this aspect of renin physiology and pathology should be regarded with caution.

IX. HYPERTENSION AND THE RENIN-ANGIOTENSIN SYSTEM

It is fitting perhaps to discuss last the situation which thirty years ago was readily believed to be due to the actions of renin and angiotensin. The rise of blood pressure produced when a clip was put on the renal artery seemingly needed no further explanation than the release of the vaso-constrictor angiotensin. The question now resolves itself into whether there is enough renin and angiotensin circulating to cause hypertension by one or more of various mechanisms: as a direct vaso-constrictor, by acting on the nervous system, both centrally and peripherally, to cause increased vaso-constriction, or by alterations in sodium metabolism directly or *via* aldosterone.

As discussed earlier, a high aldosterone production and a high blood level of renin can be found without hypertension, as, for example, in cirrhosis with ascites. It would be compatible to have a normal blood pressure with a high angiotensin level if the sensitivity of the pressor response were depressed (see previous section). This might also account for the children described with gross hypertrophy of the juxtaglomerular apparatus and hyperaldosteronism (14). These children were not hypertensive and the pressor response to angiotensin was somewhat depressed. It should be pointed out that hypokalaemia cannot explain the lack of hypertension since many patients with severe hypertension have hypokalaemia. It may be considered whether the sodium retention caused by the direct renal action of angiotensin, with or without added aldosterone production, could lead to hypertension. Since Borst and Borst-De Geus (46) proposed that the basic trouble in hypertension is a kidney which retains sodium and which will excrete sodium only when the perfusion pressure is raised, then angiotensin in causing sodium retention might be a candidate. It is postulated that sodium retention leads to increased extra-cellular fluid volume with a rise in venous pressure, an increase of cardiac output, a secondary rise in peripheral resistance

in response to this, and a rise in pressure. The relation between the blood volume, the extra-cellular volume and cardiac output has been further studied (114, 189), and it was found that increases of plasma volume, extra-cellular fluid volume and cardiac output occurred in the early stages of renal clip hypertension. Borst's experimental examples mainly depend on substances causing sodium retention and potassium loss (*e.g.*, liquorice) and it has never been shown that a substance acting in this way can lead to a rise of blood pressure without an abnormality being discovered in one or other of these parameters. These abnormalities are not found in most cases of hypertension unless clearly due to adrenal overactivity. Since Conn (79) described the hypertension associated with the cortical adrenal adenoma producing aldosterone in excess, there have been attempts to make aldosterone a major cause of elevated blood pressure in many patients. It was the observation of increased excretion of aldosterone and its metabolites in the urine of patients with hypertension that led Genest *et al.* (123) to investigate the action of angiotensin in stimulating the suprarenal and, with Laragh *et al.* (184), to show increased secretion rates in subjects with severe or malignant hypertension. Genest *et al.* (121, 122) have always found a higher rate of excretion of aldosterone and its metabolites even in subjects with so-called benign hypertension (122). Since angiotensin is an effective stimulator of aldosterone, the question is whether all these patients with low potassium and often elevated plasma sodium are examples of hypertension secondary to hyperaldosteronism. The answer is probably not. The hyperaldosteronism may exacerbate the basic condition but in most cases it is not the sole explanation, as it is in the patient with a Conn's tumour. Lowering the blood pressure with various drugs restores the lowered plasma potassium to normal (155) and the increased aldosterone secretion is either not very marked in patients with hypertension other than the malignant phase, or is not necessarily very elevated even then (84, 184a). Nevertheless, particularly in patients with stenosis of the renal artery, the hyperaldosteronism leading to low potassium may be due to stimulation by renin and angiotensin. An example of this is a subject reported recently (228) who had a renin level about one hundred times normal, aldosterone secretion of 1000 μg per 24 hours and hypokalaemia; all these abnormalities returned to normal on removal of a stenosis of the renal artery. The plasma renin is usually low in the presence of a Conn's tumour (55, 56, 57, 59, 80) and with our present knowledge a safe statement is that the presence of a normal or high plasma renin level is not compatible with the presence of a secreting Conn's tumour. This may be used to discriminate in cases of hypertension. The evidence in man can be summarised by saying that in terms of angiotensin, cases of hypertension are reported, ranging from those without much change in circulating angiotensin levels (122, 211) to benign hypertension or mild hypertension where levels are claimed to be elevated, and most surprisingly, to malignant hypertension where the levels are often lower than in benign hypertension (122). The assay for angiotensin, as discussed earlier, is not easy and it is doubtful whether it will consistently measure amounts of angiotensin present in the normal situation. Morris *et al.* (211) claimed that in cases in which stenosis of the renal artery is definitely the cause

of the high blood pressure, more renin-like material is present in the blood from the affected renal vein than there is in the periphery, and that a confident forecast as to the results of surgery may be based on this. Some of the difficulties in the situation are brought out by the work of Mulrow and Ganong (214) in the dog where, with full sodium depletion, they were unable to detect any rise in plasma angiotensin using a different method from that of Genest *et al.* (122). In man, Genest *et al.* (122) have claimed that sodium depletion does indeed lead to a rise in plasma angiotensin. Most arguments in this field are about methodology, and this is as it should be since the results entirely depend upon it. Because of the small amounts of angiotensin involved and the difficulty of concentrating this for adequate assay, there will be difficulties in following blood levels by current techniques. In the matter of renin, there seems to be much more agreement, whether a really quantitative technique is being employed (60, 61, 192) or whether a method of measuring renin activity is used (47, 113, 122, 150, 162, 286). Bearing in mind the various factors which have been discussed in relation to variation of plasma renin level, it would be surprising if in hypertension it were possible to forecast a high or low level of renin. As shown by Brown *et al.* (56, 57, 228), the presence of renal artery stenosis may be associated either with a high level of renin if malignant hypertension is present, or with a normal or low level. The same applies to persons without obvious renal disease, and even in those with a low plasma potassium level and probably hyperaldosteronism the level may be low without the presence of a Conn's tumour. Boucher *et al.* (47) were unable to detect renin activity in three normal subjects, but in several subjects with hypertension, the level was raised. Based on the investigation of three patients with hypertension thought to be due to reno-vascular disease, Conn *et al.* (80) have claimed that the level is always high but this is not so. It has been claimed in some patients that the level of plasma renin is high even when the level of plasma angiotensin is not (122). This could mean an interference with the renin-substrate reaction and needs substantiation. Helmer and Judson (148, 150) believe it is possible to predict, like Morris *et al.* (211), the presence of stenosis of the renal artery and the results of surgery for the relief of hypertension by assays of renal vein blood. The question of validity of assay must be raised at this point since in discussing high or low levels of renin, they must be given a meaning. There is only one method in current use which quantitatively measures plasma renin (60, 61, 192). The others may even give misleading results since other uncontrolled factors can enter into the final assay. This is why it is likely that there will be discrepancies in published data for some time to come. "High" levels of renin in the cases of moderate or even severe hypertension are never very high and usually within the range of twice to three times normal. This may be compared with the situation in cirrhosis with ascites or pregnancy, or Addison's disease, where the levels may be ten to twenty times normal (51, 56, 57). This means that there can be no simple relation between the level of renin and the level of blood pressure. Other factors govern the level of renin and they are operating at different strengths in different patients with hypertension; still other factors affect the whole chain down to the angiotensin response. In the experimental

field with hypertension due to renal artery clip, the results do not show very great elevation in plasma renin or angiotensin. Using cross-circulation between the experimental rat and the nephrectomised rat, which is a sensitive indicator of the presence of renin, Gross *et al.* (135, 242) showed that there was more renin activity present in the rat with hypertension due to renal artery clip but that when the opposite normal kidney was removed, there was no detectable difference from normal, even though the blood pressure of the rat was still elevated. This is in line with their earlier observations (233, 234) that the renin content of the clipped kidney is increased whereas that of the opposite normal kidney is reduced towards undetectable levels, and that when the opposite normal kidney is removed, the renin level in the clipped kidney returns to normal after a short time. Bing (21) attempted to determine whether renal hypertension could be developed in rats treated with DOCA and salt to deplete the kidneys of renin. Some of the rats did not develop hypertension after three weeks on DOCA and salt and this group had a clip applied to their renal artery. Despite the fact that the renin content of the kidney was only 2 to 4% of normal, the rise in blood pressure was similar to that obtained in normal rats with a clip applied to the artery. While this result does not give information about circulating renin, it is like the findings of Gross *et al.* (135) and it would suggest that the development of hypertension due to the renal artery clip does not require renin. It was shown that even aldosterone was not necessary for the development of persistent hypertension in that, in the rat with a clip on one artery and an opposite normal kidney, the aldosterone secretion rate was increased. After removal of the normal kidney, though the blood pressure remained elevated, the aldosterone secretion rate dropped to normal (252). In the rabbit, in which it was shown that renin intravenously would elevate the blood pressure for three weeks (36) and in which Brown *et al.* (50) maintained hypertension with angiotensin for three months, earlier studies (229) had failed to demonstrate pressor amounts of renin in renal vein blood from animals with hypertension due to a clip on the renal artery. Using a more sensitive assay, Lever and Robertson (191) found moderate increases in such animals, but the overlap with the normal was considerable and some animals with renal clip which did not develop hypertension also had values of plasma renin within the hypertensive range. This has to be contrasted with earlier experiments in the dog, in which it was claimed that with malignant hypertension induced by renal artery clipping, the amount of circulating angiotensin was increased (127, 256). Scornik and Paladini (246) in similar investigations on the conscious dog with hypertension could not find increases which could be attributed to the level of pressure. The overall impression is that with reliable quantitative methods in these forms of experimental and human hypertension, where there is a slight increase in renin or angiotensin, its role in relation to the raised blood pressure is doubtful, and it seems possible to develop renal clip hypertension in the absence of much circulating renin. The terms renin level and angiotensin level have repeatedly been used here and it might be helpful to look at the dynamics of the situation. The unhindered activity of the renin-angiotensin system must depend upon the following factors: there must be sufficient sub-

strate present to allow the reaction to proceed; there should be no inhibitors blocking the action of renin on its substrate; the angiotensin produced should not be destroyed rapidly by an excess of angiotensinases; its action on blood vessels should not be modified by electrolyte changes or other undefined actions; its action on the suprarenals should not be modified, *e.g.*, by sodium or potassium changes or other unknown factors; and its action on the kidneys should not be modified, *e.g.*, by hypertension itself. It can be seen that there are numerous points in the reaction at which the end result can be modified from the time at which renin is released into the circulation, and that a high renin would be quite compatible with the production either of much angiotensin in the blood which is modified or blocked in its expected action, or of small amounts of angiotensin in the blood because of its increased removal. There have been claims that substrate varies in various situations, including hypertension, but the assay for this is not satisfactory. Similarly, angiotensinase has been claimed to vary in hypertension (151) but Biron *et al.* (34) could not confirm this observation. It would be equally possible for a low level of angiotensin to be present in the plasma if it were being taken up at an increased rate on its smooth muscle receptors so that its biological activity in terms of its end organ stimulation could be very high. We need to know more about angiotensin production rate since this is the final step.

X. CONCLUDING REMARKS

The renin-angiotensin system has emerged as a truly hormonal controlling system intimately concerned with electrolyte balance by its stimulating effect on aldosterone production and possibly by its own direct renal actions. A distinct intra-renal role in relation to vascular tone and tubular function is hinted at but not completely substantiated. The system has its seat in the juxtaglomerular apparatus but the precise site of production and storage is still uncertain. This new position of knowledge has been reached only because methods for accurate measurement of components of the system have been developed. It seems unlikely that the system usually operates as a direct pressor system, but the various ways in which the pressor effects can be modified by electrolyte changes, by aldosterone, and by interaction with the nervous system would allow widely varying quantitative relations in different physiological and pathological states. The stimuli acting on the system have not been completely elucidated but certainly include renal perfusion pressure and sodium balance. The most important effects (for the system's activation) seem to be concerned with renal changes produced by alterations in perfusion pressure, filtration rate, or tubular function. The best substantiated receptor sites are the afferent arteriolar wall and the macula densa, but much still remains to be done in this field. Like many biological problems, deeper exploration leads to greater complexity, and the renin-angiotensin system will not be less complex than that of blood clotting.

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