DRUGS AFFECTING THE BLOOD PRESSURE AND VASOMOTOR TONE[1]

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Attention will be concentrated chiefly on the topics of blood pressure and vasomotor tone since this field has seen very great advances in the last year. Study of the mode of action of the sympathetic nervous system in its postganglionic path has revealed much that is new and important in understanding vasomotor control and the action of drugs used clinically to control high blood pressure.

The effects of electrical stimulation of sympathetic postganglionic vasomotor nerves seemed to have been well understood up to about two years ago, following the demonstration of the presence of norepinephrine in the fibers by von Euler (1) and the subsequent release, mainly of norepinephrine, into the blood [Peart (2)]. However, the work of Burn & Rand in the last few years, which has been admirably summarized by Burn (3), has suggested a new mechanism which is rather revolutionary. They believe that sympathetic stimulation first liberates acetylcholine at or near the nerve ending. This substance subsequently releases norepinephrine from stores either in or close to the nerve terminals. The norepinephrine is then liberated into the blood or is free to attach to its tissue receptors. This important claim has such far reaching consequences for vasomotor control that the evidence must be marshalled and assessed. As Burn points out, a sympathetic cholinergic nerve is not a novelty. The nerves to the sweat glands are the outstanding examples [Dale & Feldberg (4)]. However, these fibers seem to be entirely cholinergic, and there is good evidence that the type of nerve now considered, e.g., the splenic nerves, contain much norepinephrine [von Euler (1); Iggo & Vogt (5)]. Iggo & Vogt found about 2.5 μg/g of norepinephrine in splenic nerves of the cat, rabbit, and dog; and Schümann (6) found an equal amount of dopamine with norepinephrine in the splenic nerves of cattle. The starting point of the new concept was the observation by Burn (7) that stimulation of the postganglionic sympathetic nerves to the perfused vessels of the dog's hind limb led to vasodilatation unless epinephrine was previously added to the perfusion fluid. This led him to the idea that a store of epinephrine, then thought to be the sympathetic transmitter, was required at the nerve endings, ready for release. This seemed to fall into place when cholinergic fibers were found in the sympathetic chain [Bülbring & Burn (8)], but the effect of the epinephrine perfusion was not then understood. The new evidence on the main thesis falls into two groups, indirect and direct.

[1] The survey of the literature pertaining to this review was concluded in May 1961.

Indirect Effects

A large number of drugs are known to affect postganglionic sympathetic nerves and free use has been made of these to analyze the underlying basis of sympathetic activity.

Direct Effects

In some instances studies have been made on the nature of the substances appearing in the venous effluent from organs following sympathetic stimulation. Drugs which theoretically interfere with release or synthesis of substances in sympathetic nerves have been incorporated in these studies.

Reserpine.—The now well-known effect of this drug in depleting tissues of norepinephrine, whether arterial wall [Burn & Rand (9)], brain [Holzbauer & Vogt (10)], or postganglionic sympathetic fibers [Muscholl & Vogt (11)], has given a lead. Burn & Rand (12) showed that previous reserpine treatment of the dog led to a dilator response in the vessels on sympathetic stimulation and that this could be blocked by atropine. Addition of norepinephrine to the perfusion fluid now led to the usual vasoconstriction on stimulation. Moreover, norepinephrine perfused through vessels not treated with reserpine enhanced the effects of sympathetic stimulation [Burn & Rand (13)] as if some store were being filled that was essential for the greatest response to sympathetic stimulation. The evidence for such a store is based on firm ground which recent work has supported. Although initially von Euler (14) was unable to show an uptake of norepinephrine perfused through various sympathetically innervated organs of the cat, recent work by Pennefather & Rand (15) and the elegant use of ^{3}H epinephrine and norepinephrine by Axelrod, Weil-Malherbe & Tomchick (16) and Whitby, Hertting & Axelrod (17) have clearly shown that sympathetically innervated organs like the spleen and heart do take up these amines when perfused. Even tissues isolated in an organ bath, like the colon with intact nerves [Gillespie & Mackenna (18)] or the atrium [Burn & Burn (19)], can be similarly shown to take up norepinephrine. The precise region of storage is not known.

Tyramine.—In animals treated with reserpine the pressor action of tyramine disappeared [Carlsson *et al.* (20)] and returned on intravenous infusion of norepinephrine [Burn & Rand (12)]. Further work has shown that dopamine, dopa, *m*-tyrosine, and phenylalanine, all precursors of norepinephrine, will also restore the response [Burn & Rand (21)]. Although there is some evidence which suggests that tyramine merely requires the presence of norepinephrine to exert its effects [Kuchinsky *et al.* (22); Nasmyth (23)], the most convincing evidence of release of norepinephrine by tyramine is presented by Lockett & Eakins (24) from the aortic wall in cats and by von Euler & Lishajko (25) and Schümann & Weigmann (26) from chromaffin granules in splenic nerves, separated by differential centrifugation. Recently Burn & Burn (19) showed that tyramine increased the outflow of ^{14}C-labelled norepinephrine from atria suspended in an organ bath. All this perhaps makes clear the older observations of Burn (7, 27) that, following ganglionic

sympathectomy, the vasoconstrictor action of tyramine was lost but not that of epinephrine. Postganglionic sympathectomy has been shown to lead to disappearance of norepinephrine from the spleen and other organs [von Euler & Purkhold (28)], and the natural conclusion is that without norepinephrine available for release, tyramine is inactive.

Cocaine.—The action of this substance has been clarified but bears on the main theme rather more indirectly. Macmillan (29) suggested that it prevented the uptake of norepinephrine by tissues, and this was in line with Trendelenberg's (30) observation that the rate of disappearance of norepinephrine from the blood was much slower in the cocainized animal. Whitby, Hertting & Axelrod (17), using tritiated norepinephrine in a similarly treated animal, demonstrated directly a reduced uptake by heart, spleen, and suprarenals, as did Muscholl (31). Muscholl further showed that the endogenous concentration of norepinephrine and epinephrine was unchanged by cocaine and that there was a quantitative competitive block between cocaine and norepinephrine. Burn & Burn (19) confirmed the effect of cocaine on the isolated atrium, so at least one of its modes of action seems clear.

The reason for its action on the intact vascular system is less clear, however. The increased response of the cocainized animal to sympathetic stimulation and injected norepinephrine may be attributable to this block in uptake of norepinephrine so that more adrenergic receptors are left free to cause an increased response [Muscholl (31)]. This argument sounds rather circular, especially since this worker showed that tissue stores of the amine were not affected by cocaine. Burn & Rand (12) had previously likened the behavior of cocainized vessels to those treated with reserpine. In both there is supersensitivity to epinephrine and norepinephrine and decreased sensitivity to tyramine. Again, this is like the effect of denervation, and it is possible that cocaine is preventing the release of norepinephrine from its store in the vessel wall, thus effectively blocking the action of tyramine, which seems to depend upon release of norepinephrine.

Nicotine.—Previous observations on the peripheral actions of nicotine, which resemble sympathetic stimulation, have been extended by Burn *et al.* (32), and the conclusion that the vasoconstrictor action is caused by release of norepinephrine from the artery wall [Kottegoda (33); Burn & Rand (34, 35)] is supported. General denervation and treatment with reserpine reduced the action of nicotine. This is convincing for reserpine, where, for example, the skin content of norepinephrine was greatly reduced, but not so for denervation where the effects seem much more variable and the skin content of norepinephrine was not affected. The vascular effects are not therefore so readily interpreted as, say, the effects on the nictitating membrane. However, nicotine may act in part in the way suggested. Further studies on the isolated atria were made by Azarnoff & Burn (36), who found that nicotine and tyramine increased the rate of beating if norepinephrine had been previously added to the bath. Reserpine abolished the effect of both drugs. The effect of norepinephrine itself after reserpine was also reduced, and Muscholl (37)

has shown that reserpine blocks certain effector sites and storage sites in addition to its depletion effects. It could be argued therefore that both nicotine and tyramine liberated norepinephrine, whose action was blocked by reserpine. This evidence is, however, too indirect for confidence. The site of the store from which nicotine or tyramine might release norepinephrine is not known; however, norepinephrine has been extracted from vessel walls [Schmiterlöw (38)], and there are chromaffin cells present in the skin quite close to vessels [Adams-Ray & Nordenstam (39); Burch & Phillips (40)], though their content and function are unknown and hypothetical.

Acetylcholine.—Burn *et al.* (32) and Burn & Rand (41) supplemented the observations of Kottegoda (33). Kottegoda showed that sympathetic stimulation to the rabbit's ear released not only a norepinephrine-like substance, but also an acetylcholine-like substance. In particular, in the reserpine-treated animal, acetylcholine loses its effects on the blood vessels and atria. Further, de Burgh Daly & Scott (42) showed that intraarterial acetylcholine caused splenic contraction which was not blocked by atropine but was blocked by dibenzyline (phenoxybenzamine). Since dibenzyline blocks the receptor sites for norepinephrine, it was concluded that the norepinephrine released by acetylcholine was thereby blocked. Perhaps too many assumptions are made here about the specificity of dibenzyline. In the reserpine-treated animal, acetylcholine caused dilatation of blood vessels [Burn & Rand (41)]. The sympathomimetic actions of acetylcholine disappeared after degeneration of the nerves.

Much more satisfying evidence has been presented by Brandon & Rand (43) who have studied the sympathetic innervation of the spleen and showed that an acetylcholine-like substance appeared in the venous effluent following electrical stimulation, as in the case of the rabbit's ear vessels [Burn & Rand (41)]. Further, following denervation, the acetylcholine content of the spleen fell *pari pasu* with the norepinephrine. That this acetylcholine is not just a chance consequence of the presence of some cholinergic fibers in the postganglionic sympathetic trunk will be considered later.

Bretylium.—This interesting drug will only be discussed here as it bears on the new concept of sympathetic vasomotor action; it will be considered from other aspects later in the chapter. Having seen that there is evidence for release of norepinephrine from a hypothetical store, the action of bretylium gave further clues. It was shown to block the action of acetylcholine on the isolated atropinized atria [Hukovic (44)] and on atropinized rabbit ear vessels [Burn & Rand (41)]. The pressor effect of eserine in the rat was blocked without affecting the response to norepinephrine [Lesic & Varagic (45)], as was the pressor response to tyramine [Lesic & Varagic (46)]. These latter two observations are not so clear-cut as they seem when the evidence is considered carefully. Since bretylium blocks acetylcholine and since the vasoconstriction on sympathetic stimulation of rabbit ear vessels was also blocked, the real mode of action of the sympathetic vasomotor nerves comes into question. If the evidence for tyramine as a norepinephrine releaser is

accepted, the fact that the effect of tyramine was enhanced when bretylium blocked the sympathetic vasomotor nerves suggests that bretylium prevented the release of acetylcholine, so that the norepinephrine stores in the vessel wall were intact or even larger [Burn & Rand (41)]. The presence of a type of cholinergic fiber in the peripheral sympathetic pathway seemed to be established, and, as the bretylium block was total, it would seem to be a fundamental part of the functioning system of all the fibers.

Hemicholinium.—The evidence from the bretylium work was very suggestive, but it was considerably strengthened by the important observations of Chang & Rand (47) and Brandon & Rand (43). Hemicholinium interferes with the synthesis of acetylcholine and therefore causes a failure of response following cholinergic nerve stimulation. This effect can be reversed by administration of choline [MacIntosh, Birks & Sastry (48)]. Chang & Rand first showed that the effect of sympathetic nerve stimulation was antagonized by hemicholinium and restored by the addition of choline. Brandon & Rand completed the story by showing that an acetylcholine-like substance appeared in the venous effluent from the spleen of a reserpine- and eserine-treated cat when the sympathetic nerves were stimulated.

The evidence that stimulation of the adrenergic nerves releases acetylcholine, which in turn releases norepinephrine from the endings or a store in the vicinity, seems complete, but it is well to consider gaps or weaknesses since this is such an important claim. If the sympathetic nerve fibers release acetylcholine from within or close to nerve endings, then the possible anatomical sites should be considered, and so far no very special structures near the end of sympathetic nerve fibers have been demonstrated. This awaits more refined methods of examination. It is possible to show acetylcholine in extracts of postganglionic sympathetic fibers and in the superior cervical ganglion of the cow [Loewi & Hellauer (49)]. Koelle (50) demonstrated histochemically the presence of acetylcholine esterase.

The content of norepinephrine is relatively much higher in the organs supplied by sympathetic nerves than in the postganglionic fibers themselves. For example, as von Euler (51) discovered, the splenic nerves of the cow, which consist almost entirely of C fibers, contain about 15 μg/g of norepinephrine, whereas the spleen contains 3 μg/g. If an assumption is made about the proportion of nerve endings to other splenic tissue, then the concentration in the nerve endings would be 3 to 30 mg/g of nerve. This is roughly of the same order as in the adrenal medulla so that it is within the known capacity of some cells. However, there is no direct evidence as to the localization of such a store within or without the nerve endings. At such concentrations it should be possible to demonstrate norepinephrine by relatively simple histochemical techniques. Alternatively, the use of tritiated norepinephrine, which could be taken up in the nerve endings, followed by autoradiography of tissue slices, might solve the problem in sympathetically innervated tissues.

There is no doubt that there is a biochemical difference as far as extract-

able substances are concerned between, say, the vagus and the splenic nerves; the vagus contains 0.1 μg/g of norepinephrine, compared with 15 μg/g in the splenic nerves [von Euler (51)]. Further, in addition to norepinephrine, dopamine and dopa decarboxylase have also been shown in sympathetic fibers [Schümann (52)]. What is also very important, however, is that the norepinephrine is found along the length of the nerve away from the innervated organ [Iggo & Vogt (5); von Euler (51)], and it may be asked how this fits into the new concept.

Although the high acetylcholine content of the spleen has been known for a long time, the exact location of all these substances, in particular, their anatomical relation one to another, is now of the greatest importance. However attractive the new hypothesis of sympathetic action is, these doubts still exist because the evidence is not always conclusive and in some cases is contradictory. A great deal seems to depend upon specificity of action of blocking drugs; before too much weight is put upon the action on isolated tissues of substances like bretylium and hemicholinium, complete evidence about other possible modes of action is needed. This is brought out in one of the main conclusions in the hemicholinium story. Brandon & Rand (43) showed that stimulation of the sympathetic nerves was blocked by hemicholinium and they concluded that this was attributable to an initial block of formation of acetylcholine which was not then available to release norepinephrine. It would have been desirable to show that under these circumstances norepinephrine itself was not liberated following stimulation of the nerves. Because many of these drugs are probably not unifunctional in action, this may lead to too much weight being placed on the reduction in an effect (for example, reserpine on acetylcholine-induced contraction of the dog's spleen), while leaving unexplained the potentiation of norepinephrine in similar circumstances by bretylium.

Hypotensive Agents

In the last two years many new drugs that lower blood pressure have been introduced for the treatment of hypertension. Some have already been discussed, but further points should be made since, as is so often the case, study of human pharmacology and therapeutics reveals facets of drug action unsuspected in animals or organ baths. This is often a result of dosage level. An example is reserpine, which produces its hypotensive effects in man with repeated doses of the order of 5 to 10 μg/kg. Many experiments with reserpine in animals employ much larger doses, for example, in dog 500 μg/kg, in rabbit 3000 μg/kg [Burn & Rand (12)], or in organ baths where, if the same concentrations were reached in the whole body fluids, enormous doses would have to be given. For example, Azarnoff & Burn (36) suspended rabbit's atria in a concentration of reserpine at 4 μg/ml. If reserpine were distributed freely in the extracellular fluid, this would represent a dose of about 125 mg to a normal man, although, because of other effects, it is usually not desirable to use a single dose above 0.5 mg. This is a most important principle to

understand when far reaching conclusions are being drawn from the use of drugs about physiological or pathological mechanisms. The results of organ bath experiments and animal experiments cannot be transferred uncritically to explain the mode of action of the drug, either in man or in the many animals being considered.

Reserpine.—The above remarks are particularly apposite with respect to this drug and its effect in depleting norepinephrine in the vascular system. Some human experiments point to other actions which require further explanation. Parks *et al.* (53) have infused reserpine into the brachial artery of man and shown a considerable vasodilating action, which could be prevented by ordinary therapeutic doses of reserpine given previously. The vasodilating action was not affected by antihistamines, atropine, or by sympathetic denervation. During the dilatation, there was no increase in the direct response to norepinephrine or to reflex sympathetic activity. Among other possibilities, it suggests that reserpine releases norepinephrine from vessel wall stores which may be essential for a given state of tone, and that these stores are not depleted by sympathectomy, nor, in fact, affected by sympathetic stimulation. In practice it is still uncertain as to how reserpine administered therapeutically lowers the blood pressure and whether it is a central action on the brain where the release or depletion of norepinephrine or 5-hydroxytryptamine occurs. Also, whether it has a similar effect on the peripheral sympathetic neurone or the vessel wall remains to be seen. Adequate analysis of reserpine's action in man has not been undertaken. Its effects, mainly from a therapeutic angle, have been well reviewed by Krogsgaard (54).

Bretylium.—There are some further points to be made about the action of this substance, first described by Boura & Green (55). The localization in sympathetic ganglia and postganglionic fibers using radioactive bretylium was shown by Boura *et al.* (56). They further showed that, apart from blocking sympathetic discharge and the subsequent release of norepinephrine, there was a distinct increase in the sensitivity of the innervated organ to added norepinephrine or epinephrine. This has been amplified by Aviado & Dil (57), Vernikos-Danellis & Zaimis (58), and Gokhale & Gulati (59). This particular supersensitivity is, of course, part of the larger problem of increased sensitivity in organs denervated by surgery or by drugs, which is well reviewed by Emmelin (60) as "pharmacological denervation." The use of bretylium therapeutically has not been as successful as initially hoped and the supersensitivity induced may, in part, have something to do with this.

Guanethidine.—This substance was first described by Maxwell, Mull & Plummer (61) and the actions further reported by Maxwell *et al.* (62, 63). Depletion of norepinephrine stores at or near the nerve endings seems to be the fundamental mode of action, and it seems to cause the same sort of supersensitivity as bretylium [Vernikos-Danellis & Zaimis (58); Maxwell *et al.* (64); Cass, Kuntzman & Brodie (65)]. There are certainly other effects

and other modes of action, but a complete analysis has not been made. In patients, clues to certain of these other effects may be given by the sleepiness and depression which sometimes occur. Investigation of guanethidine's actions on circulation are reported by Richardson *et al.* (66), Taylor & Donald (67), Dollery, Emslie-Smith & Milne (68), Gaffney (69), and Mc Cubbin, Kaneko & Page (70). It seems likely that the postural hypotensive action is closely related to a reduction of cardiac output. This brings up the point, as with the ganglion-blocking drugs, that the effect on veins and venous distensibility and the reflex vasomotor action on veins have been rather neglected compared with the studies and effects on the peripheral arterioles.

Chlorothiazide and congeners.—These substances are of great interest therapeutically as hypotensive agents. They only seem to be effective in lowering high arterial pressure, and controversy has arisen over whether the action is purely caused by a reduction in blood volume as was thought initially, or whether other effects are also present. Certainly the blood volume does drop, as does the body sodium, due to renal excretion. This subject has been ably reviewed by Freis (71), and the conclusions are that this initial change in blood volume can be reversed by dextran infusion [Dollery, Harrington & Kaufman (72)] and that in this early phase the cardiac output decreases on standing. However, after some weeks the plasma volume returns to normal and the effect of the drug on blood pressure is not decreased [Wilson & Freis (73)]. This raises the possibility of other modes of action, and an effect on peripheral resistance has been suggested by Varnauskas *et al.* (74) who showed that the cardiac output rose less in response to standard exercises than before the drug. They suggested a drop in the arteriolar resistance that could not be restored by returning the plasma volume to its previous levels. This view is supported by Conway & Lauwers (75).

A lessened response of the vessel wall to local norepinephrine is suggested by diminished pressor response to infused norepinephrine in normal treated subjects [Wanko & Freis (76); Merrill, Guinand Baldo & Giordano (77)], although, of course, the blood volume or cardiac output effect may be important here. A more direct approach to the peripheral vessels and their responses was made by Mendlowitz *et al.* (78) who showed a decreased response to intraarterial norepinephrine in treated subjects. Winer (79) looked at other factors and found that, even when the plasma volume was restored to normal after the first weeks of treatment, sodium depletion was still present and, if this were corrected, the blood pressure returned to its pretreatment level. This seems a very likely explanation of the long-term effects. An investigation of the effect of sodium depletion on the local vascular response to norepinephrine would be of interest.

Activity of the Sympathetic Nervous System and Circulation

Besides the primarily indirect studies reported above, another approach for assessing the degree of activity of the sympathetic nervous system has

been made by studying norepinephrine and epinephrine metabolism in the animal. Clearly it is difficult in the intact animal to assess the degree of such activity by electrophysiological methods, but because of increased knowledge of the metabolic pathways of catechol amines, it has become possible to obtain quantitative data about their rate of production and excretion.

Since most of the norepinephrine liberated in the body is probably from sympathetic nerve endings in blood vessel walls, an adequate measure of this substance and its metabolites gives some idea of the degree of sympathetic activity. A start on this was made by the early studies of von Euler and his co-workers (51), who observed differences in the rate of urinary excretion of free norepinephrine between lying and standing and in patients with orthostatic hypotension where the sympathetic discharge was low. Some interesting studies were carried out by Munro & Robinson (80) who found that the blood levels of norepinephrine were low in patients with high transection of the spinal cord. In other patients with lower levels of transection the blood levels were higher. This would in itself clearly point to differences of sympathetic discharge and would encourage the view that measuring the metabolic products of norepinephrine was really measuring sympathetic activity indirectly. Clearly any alteration of the metabolic path of norepinephrine would render the results invalid.

Observations like those of Trendelenberg (30) and Muscholl (31), who showed that cocaine blocked the uptake of norepinephrine from the circulation, showed the need for caution, since if released norepinephrine were not taken up in tissues, the total metabolic fate might be very different. A further example is the finding of an increased rate of metabolism of epinephrine and norepinephrine following the administration of various sympathomimetic amines [Axelrod & Tomchick (81)]. This is very like the action of reserpine, and an increase occurs in the amounts of conjugated *O*-methyl. The sympathomimetic amines may interfere with tissue binding, leaving the epinephrine and norepinephrine more open to oxidation. This would mean that total metabolic studies of this sort ought to be controlled by the use of isotopic markers as in studies of aldosterone secretion [Laragh *et al.* (82)]. There is, however, no evidence that there is a very great variation in metabolic pathways among different subjects. The subject of quantitative norepinephrine metabolism and blood pressure has been dealt with by Sjoerdsma (83), who also provided an interesting discussion on the use of monoamine oxidase and decarboxylase inhibitors.

Monoamine oxidase and decarboxylase inhibitors add another tool in the analysis of sympathetic activity on the vascular system. Their usefullness lies in the fact that the pathway to norepinephrine goes from dihydroxy phenylalanine to dihydroxy phenylethylamine (dopamine) by decarboxylation, and thence to norepinephrine and epinephrine before their removal as inactive substances by oxidative deamination. Even if normal amounts of norepinephrine were formed in the arterial wall, there could be an increased vasoconstriction if it were not quickly removed, for example, by deficiency of amine oxidase or *O*-methyl transferase [Axelrod ***et al.*** (84); Labrosse,

Axelrod & Kety (85); Kopin (86)]; therefore, techniques defining this type of enzymic activity are needed to give definition to studies of over-all sympathetic vasomotor action. Tyramine is a substance which seems to be completely dependant on amine oxidase for its final metabolic path. If the metabolism of this substance is studied quantitatively, it gives information about the state of this enzyme system [Sjoerdsma *et al.* (87)]. Similar use has been made of diisoproterenol, which is metabolized exclusively by *O*-methylation. Clearly, sympathetic activity can now be studied using a large number of new techniques.

An interesting application of this knowledge has been the attempt to use monoamine oxidase inhibitors in the treatment of human arterial hypertension [Sjoerdsma (88); Horwitz & Sjoerdsma (89)], which has not been very successful due to other associated effects. The use of decarboxylase inhibitors, in this instance alpha methyl dihydroxy phenylalanine (alpha methyl dopa), has, however, been more successful [Oates *et al.* (90); Sjoerdsma (91); Gillespie (92)]. The fundamental action of this substance was first described by Sourkes (93). The proof that decarboxylase was inhibited was provided by showing that dihydroxy phenylalanine (dopa) was not converted to dopamine in the usual amounts. However, the therapeutic effects may not purely depend on this aspect [Goldberg, DaCosta & Ozaki (94)], and further analysis of the mode of action on the circulation is needed.

Bradykinin

Elliott, Lewis & Horton (95) were the first to isolate the pure substance bradykinin and determine its amino acid composition. Its biological activity was shown to be the same as the crude preparation from which it was derived [Elliott, Horton & Lewis (96)]. Initially they thought it contained eight amino acid residues and, in particular, two of proline, but synthesis of the proposed structure showed it inactive [Boissonnas, Guttmann & Jaquenoud (97); Schwyzer *et al.* (98); Nicolaides *et al.* (99)]. The facility of the synthetic chemist was shown when Boissonnas and co-workers (100, 101) synthesized a similar nonapeptide with three proline residues and with the same biological properties as natural bradykinin. The missing proline in the natural bradykinin was soon assigned to its place, and the joint publications of Konzett & Stürmer (102), Lewis (103), and Shorley & Collier (104) clarified the position. The synthetic and natural bradykinin has the structure Arg·Pro·Pro·Gly·Phe·Ser·Pro·Phe·Arg.

Studies of the actions of synthetic bradykinin on isolated tissues and organs have been made by Fox *et al.* (105), Elliott, Horton & Lewis (106), and Konzett & Stürmer (107). The major action on the circulation seems to be that of vasodilatation affecting not only arterioles, but also capillaries, where increased permeability occurs. Hence a fall in blood pressure occurs on intravenous injection; in man this is accompanied by a marked facial flush. Skin and muscle flow both increase on intraarterial injection. A full review of the vasodilator peptides derived from plasma proteins has been made by

Lewis (108), and this gives a wider view of the origin and formation of substances like bradykinin in the plasma and extracellular fluid. Their probable physiological and possible pathological role, deduced mainly from work on vasodilatation in the salivary gland [Hilton & Lewis (109)] and sweating in the skin [Fox & Hilton (110)], needs consideration.

Similar studies have been made on plasma kinins during human parturition by Armstrong, Keele & Stewart (111). There are two main difficulties in interpreting this work. The first difficulty is that any interference with the plasma may release kinin activity [Schachter (112)], and in some of this work dilution of the plasma or extracellular fluid does invoke this possibility. Even contact with glass has been shown to release kinin activity [Armstrong *et al.* (113)]. However, when the other approach is made to show directly the kinins in circulating blood, the marked lability of bradykinin emerges. It is destroyed within minutes or less by "kininases," which are difficult to remove or inhibit [Horton (114)]. Losses on extraction of the plasma are large and unpredictable and hamper advance. Nevertheless, these experiments are a striking approach to the demonstration of highly labile vasoactive substances which are released in organs and which may be responsible for local control of the circulation in response to activity in the organ. Since the clearest demonstration of vasoactive substances has been in extracellular fluid [Hilton & Lewis (109); Fox & Hilton (110)], the new idea of release into this fluid of active substances, which then act on blood vessels, needs consideration. On teleological grounds (and this must not be despised), it seems desirable that such a substance should be released into the extracellular fluid where it might have a longer time for action. In any case, since tissue enzymes, such as kallikrein, release the kinins from substrate and since time is needed for this action, it would not be very reasonable for this reaction to occur within the blood which is flowing through the organ, because the active substance would be rapidly carried away and destroyed. The situation is very like that of acetylcholine metabolism before the discovery of eserine.

Renin and Angiotensin

Major advances have been made in the investigations of renin and angiotensin, and although the exact role of the renal enzyme renin and its peptide end product, angiotensin, is still undefined, much suggestive evidence has accumulated. Some of the renin and most of the angiotensin work has been reviewed recently by Page & Bumpus (115). The earlier renin work was reviewed by Gross (116) and Peart (117, 118).

Renin still remains an enzyme of puzzling nature. Knowledge of its exact location has come closer due to the elegant work of Cook (119), who succeeded in assaying renin in the separate halves of glomeruli, and of Bing & Kazimicrczak (120), who used a different microdissection technique. From these direct approaches depending on final biological assay, it seems that renin is situated very close to the vascular pole of the glomerulus.

Some more definite idea of the role of renin would emerge if the precise cellular and ultimately intracellular location of renin were known. This awaits more precise techniques. Such a seemingly precise technique using a fluorescent antibody to renin to locate renin by ultraviolet microscopy in histological sections, has been reported in two publications by Nairn, Fraser & Chadwick (121) and Hartroft & Hartroft (122). Nairn, Fraser & Chadwick claimed that renin was present around the total circumference of the glomerulus, which conflicts with the direct evidence quoted above and further illustrates that this technique can give misleading results. In using a fluorescent antibody to reveal an antigen it is axiomatic that the antigen from which the labelled antibody is made must be pure. Neglect of this essential may lead to the production of antibodies to other contained antigens. Since neither of these reports provide evidence that the preparations of kidney used as antigen were pure, this criticism applies.

There is now a large body of work depending on argument by analogy and parallel change which shows that the renal content of renin fluctuates directly with the degree of granulation of the juxtaglomerular apparatus. Procedures causing degranulation are associated with a decrease in renin content [Hartroft & Hartroft (123)]; this subject has been specifically reviewed by Tobian (124). Gross (125) has ably reviewed the experimental background relating the kidney and the adrenal, particularly with reference to hypertension, a field in which he and his colleagues have made great contributions. This relation is brought out by such observations as the drop in renin content if deoxycorticosterone acetate or aldosterone is given to rats and an increased renin concentration after adrenalectomy. This, of course, relates to the observations on juxtaglomerular granulation, but, unfortunately, concentration is no guide to rate of secretion.

Recent attempts to show renin in the renal venous blood of rabbits with renal clip hypertension have been unsuccessful [Peart (126)] despite ready detection of infused renin. It is likely that renin as such has been demonstrated in renal vein blood only when the artery has been clamped off for a considerable time, as in the original experiments of Houssay & Fasciolo (127, 128) and Braun-Menendez *et al.* (129). This is not the same as established renal hypertension.

It would not be surprising if gross ischaemia or partial autolysis led to release of other intracellular enzymes besides renin. However, new information about angiotensin makes a new look at renin essential. This stems not only from the renal/adrenal link noted in the rat but from the fact that patients with malignant hypertension may secrete and excrete in the urine a large excess of aldosterone. This fact led Laragh *et al.* (130) and Genest *et al.* (131) to infuse angiotensin in man which caused an increased secretion rate and an increased excretion rate, respectively, of aldosterone. Such an increase immediately suggests the release of renin and the formation of angiotensin to cause this adrenal stimulation in malignant hypertension, but the demonstration of this has still not been achieved. Further evidence is provided by

Davis *et al.* (132) and Ganong & Mulrow (133) who have shown the release into the blood, after severe controlled hemorrhage in the dog, of a substance that stimulates aldosterone production. This substance may well be renin but more direct evidence is necessary. A most important question is whether this means that renin situated close to the glomerulus is intimately concerned with water and electrolyte metabolism by release into the blood, production of angiotensin, and, thereby, control of aldosterone secretion and the consequent sodium and potassium handling by the kidney. Although it would seem to be a rather cumbersome method of control, it is obviously a possibility since renin first acts on the substrate to produce a decapeptide [Peart (134)], which then is changed by a "converting" enzyme to an octapeptide [Skeggs, Kahn & Shumway (135)], which then acts on the suprarenal. Because control of aldosterone secretion has not yet been clearly defined, this new candidate arouses excitement, especially since it is a peptide, and the analogy with ACTH is obvious.

The direct effects of angiotensin on adrenal slices or after intraarterial injection into the adrenal should be soon forthcoming. At present, angiotensin seems to be the most potent known stimulator of aldosterone production. It is capable of carrying out this part of its action without raising blood pressure in man [Genest *et al.* (136)]. It may well be that the position is like that of vasopressin, which, although it has a vasopressor action in large doses, is physiologically released in small amounts as an antidiuretic substance.

Much more work is needed to clarify this interesting situation. If angiotensin is now to be looked on mainly as a stimulator of the adrenals, it raises the questions of the relation to circulation of aldosterone, as well as other steroid hormones, and, in particular, whether it could be concerned with the hypertension of renal artery stenosis.

In the nonmalignant stage of hypertension, the secretion of aldosterone seems to be normal [Laragh *et al.* (82)], and, as has just been stated, the stimulus to aldosterone production can be achieved with nonpressor infusions of angiotensin [Genest *et al.* (136)]. It would seem that in less severe or nonmalignant hypertension either smaller amounts would have to be liberated or none at all. Nevertheless, the effect of aldosterone on the whole circulation, about which little is known, is thereby raised for consideration.

The effect of aldosterone on blood pressure was considered by Gross (125). Unlike deoxycorticosterone acetate, it does not readily cause hypertension experimentally, even in the rat, which is peculiarly liable to develop hypertension with salt and various steroid administrations. In man, excessive production of aldosterone, as in the so-called secondary hyperaldosteronism, is not usually accompanied by hypertension (as in cirrhosis of the liver with ascites, or nephrosis with edema), unlike the severe hypertension associated with primary aldosteronism and a tumor in the suprarenal [Conn (137)]. Although the circulatory states as well as the water and electrolyte conditions of these groups of patients differ widely, the effects of the hormone need

clarification under any condition, since its pharmacology is still at a superficial state.

The other major effects of angiotensin on circulation can be summarized by stating that is is an over-all vasoconstrictor in skin, muscle, kidney, splanchnic area, and lung, with more action on the arterioles than on the veins [Page & Bumpus (115); Gross (116); Peart (117)]. It raises the blood pressure in man in doses of 1 to 4 μg per minute, and, as with norepinephrine, the rise in blood pressure leads to reflex vagal action and bradycardia.

A decrease in cardiac output is also associated with angiotensin in normal subjects. It is especially potent on renal circulation [Peart (117); Bock & Krecke (138)] where the plasma flow is greatly reduced in the normal subject. The antidiuretic action in the normal subject is accompanied by a marked drop in sodium, potassium, and chloride excretion and is probably attributable to a direct effect on the renal vessels [Peart (117)], but Leyssac, Lassen & Thaysen (139), using evidence derived from an *in vitro* effect on kidney slices, have suggested that it might act directly on sodium transport. There is certainly no evidence against a direct tubular effect which would have to have a very quick onset, but it seems more likely that a vascular action and the effects of ischaemia, perhaps of a selective type, on tubular function are responsible for the effects on the water and electrolyte excretion. Certainly the presence of antidiuretic hormone is not essential to the angiotensin antidiuresis since it occurs in patients with diabetes insipidus [Peart (118)].

The fact that angiotensin infused into hypertensive subjects causes diuresis and sodium loss, however, illustrates the danger of extending an argument based on the action of a substance in a normal subject to either a changed state or to a pathological state [Peart & Brown (140); Peart (141)]. This action is contrary to the usual action of aldosterone, so if increased secretion occurs, its renal action is readily overcome by the dose of angiotensin used.

The precise mode of action on the vessel wall is not known, and at present no pharmacological blocking agent has been described with any specific effect. In rather large doses, Friedman, Friedman & Nakashima (142) found that angiotensin caused sodium and water to shift into the arterial wall, and they believe that this altered ratio of intra- to extracellular sodium plays a large part in vascular tone and, therefore, in blood-pressure regulation. With this sort of experiment, it is difficult to dissociate cause from effect. Thus the role of this peptide in physiological or pathological states is still uncertain.

It is now possible to consider whether renin is a genuine hormone which exerts its action by producing angiotensin, that in larger amounts acts as a direct vasoconstrictor to raise the blood pressure or in smaller amounts has no obvious immediate action on the blood pressure. It might then secondarily cause hypertension through another mechanism, perhaps by stimulation of aldosterone secretion, which secondarily may have some action on the blood pressure or on water and electrolyte metabolism. There is no

real evidence for the former and only suggestive evidence for the latter. Another possibility is that the release of renin into the blood stream is a chance phenomenon, that only occurs under extreme damage to cell walls in the kidney, similarly to the release of other enzymes or substances into the circulation under these conditions. Renin could then be looked upon as a stored enzyme, not normally released, but with an action either within the kidney's own cells or close to them as a local hormone within the kidney [Peart (141)]. Like the enzymes which release bradykinin into the extracellular fluid of the salivary gland [Hilton & Lewis (109)] or near the sweat glands [Fox & Hilton (110)] and which are responsible for local vasodilatation, renin could release angiotensin to act directly on renal vessels or nephrons. Perhaps in acute glomerular nephritis such a local release may be responsible for some of the functional effects. An analogous situation again might be the release of sympathin into the blood stream following stimulation of the sympathetic nerves. This release is probably independent of the action of the nerves and can be regarded as a spill-over of excess hormone [Peart (143)].

The main factor holding up further advance in this field is lack of knowledge of the precise location of renin in the kidney and a real knowledge of the true production rate, or its concentration in the blood. In the case of angiotensin, there is hope that this position will be improved by the use of the method of Paladini, *et al.* (144) as modified by Scornik, Paladini & Braun-Menendez (145). A real knowledge of the quantity circulating and comparison with the known substance will bring an answer to some of these difficult questions and will remove the subject from the conjectural state it has been in for so long.

LITERATURE CITED

1. Euler, U. S. von, *Acta Physiol. Scand.*, **16**, 63 (1948)
2. Peart, W. S., *J. Physiol. (London)*, **108**, 491 (1949)
3. Burn, J. H., *Brit. Med. J.*, **I**, 1623 (1961)
4. Dale, H. H., and Feldberg, W., *J. Physiol. (London)*, **82**, 121 (1934)
5. Iggo, A., and Vogt, M., *J. Physiol. (London)*, **150**, 114 (1960)
6. Schümann, H. J., *Arch. exptl. Pathol. Pharmakol.*, **227**, 566 (1956)
7. Burn, J. H., *J. Physiol. (London)*, **75**, 144 (1932)
8. Bülbring, E., and Burn, J. H., *J. Physiol. (London)*, **83**, 483 (1935)
9. Burn, J. H., and Rand, M. J., *Lancet*, **II**, 1097 (1957)
10. Holzbauer, M., and Vogt, M., *J. Neurochem.*, **1**, 8 (1956)
11. Muscholl, E., and Vogt, M., *J. Physiol. (London)*, **141**, 132 (1958)
12. Burn, J. H., and Rand, M. J., *J. Physiol. (London)*, **144**, 314 (1958)
13. Burn, J. H., and Rand, M. J., *J. Physiol. (London)*, **150**, 295 (1960)
14. Euler, U. S. von, *Circulation Research*, **4**, 647 (1956)
15. Pennefather, J. N., and Rand, M. J., *J. Physiol. (London)*, **154**, 277 (1960)
16. Axelrod, J., Weil-Malherbe, H., and Tomchick, R., *J. Pharmacol. Exptl. Therap.*, **127**, 251 (1959)
17. Whitby, L. G., Hertting, G., and Axelrod, J., *Nature*, **187**, 604 (1960)
18. Gillespie, J. S., and Mackenna, B. R., *J. Physiol. (London)*, **147**, 31P (1959)
19. Burn, G. P., and Burn, J. H., *Brit. J. Pharmacol.*, **16**, 344 (1961)
20. Carlsson, A., Rosengren, E., Bertler, A., and Nilsson, J., *Psychotropic Drugs*, 363 (Garattini, S., and Ghetti, V., Eds., Elsevier Publishing Company, Amsterdam, the Netherlands 1957)

21. Burn, J. H., and Rand, M. J., *Brit. J. Pharmacol.*, **15**, 47 (1960)
22. Kuchinsky, G., Lindmar, R., Lüllmann, H., and Muscholl, E., *Arch. exptl. Pathol. Pharmakol.*, **240**, 242 (1960)
23. Nasmyth, P. A., *Adrenergic Mechanisms*, 337 (Vane, J. R., Wolstanholme, G. E. W., and O'Connor, M., Eds., J. & A. Churchill Ltd., London, England, 1960)
24. Lockett, M. F., and Eakins, K. E., *J. Pharm. and Pharmacol.*, **12**, 720 (1960)
25. Euler, U. S. von, and Lishajko, F., *Experientia*, **16**, 376 (1960)
26. Schümann, H. J., and Weigmann, E., *Arch. exptl. Pathol. Pharmakol.*, **240**, 275 (1960)
27. Burn, J. H., *J. Pharmacol.*, **46**, 75 (1932)
28. Euler, U. S. von and Purkhold, A., *Acta Physiol. Scand.*, **24**, 212 (1951)
29. Macmillan, W. H., *Brit. J. Pharmacol.*, **14**, 385 (1959)
30. Trendelenberg, U., *J. Pharmacol. Exptl. Therap.*, **125**, 55 (1959)
31. Muscholl, E., *Brit. J. Pharmacol.*, **16**, 352 (1961)
32. Burn, J. H., Leach, E. H., Rand, M. J., and Thompson, J. W., *J. Physiol. (London)*, **148**, 332 (1959)
33. Kottegoda, S. R., *Brit. J. Pharmacol.*, **8**, 156 (1953)
34. Burn, J. H., and Rand, M. J., *Lancet*, **274**, 673 (1958)
35. Burn, J. H., and Rand, M. J., *Brit. Med. J.*, **I**, 903 (1958)
36. Azarnoff, D. L., and Burn, J. H., *Brit. J. Pharmacol.*, **16**, 335 (1961)
37. Muscholl, E., *Arch. exptl. Pathol. Pharmakol.*, **240**, 234 (1960)
38. Schmiterlöw, C. G., *Acta Physiol. Scand.*, **16**, Suppl. 56 (1948)
39. Adams-Ray, J., and Nordenstam, H., *Lyon Chir.*, **52**, 125 (1956)
40. Burch, G. E., and Phillips, J. H., *Circulation Research*, **6**, 416 (1958)
41. Burn, J. H., and Rand, M. J., *Brit. J. Pharmacol.*, **15**, 56 (1960)
42. Daly, M. de B., and Scott, M. J., *J. Physiol. (London)*, **156**, 246 (1961)
43. Brandon, K. W., and Rand, M. J., *J. Physiol. (London)*, **157**, 18 (1961)
44. Hukovic, S., *Brit. J. Pharmacol.*, **15**, 117 (1960)
45. Lesic, R., and Varagic, V., *Brit. J. Pharmacol.*, **16**, 99 (1961)
46. Lesic, R., and Varagic, V., *Brit. J. Pharmacol.*, **16**, 320 (1961)
47. Chang, V., and Rand, M. J., *Brit. J. Pharmacol.*, **15**, 588 (1960)
48. MacIntosh, F. C., Birks, R. I., and Sastry, P. B., *Nature*, **178**, 1181 (1956)
49. Loewi, O., and Hellauer, H., *Arch. ges. Physiol.*, **240**, 769 (1938)
50. Koelle, G. B., *J. Pharmacol.*, **114**, 167 (1955)
51. Euler, U. S. von, *Noradrenaline* (Charles C Thomas, Springfield, Ill., 1956)
52. Schümann, H. J., *Arch. exptl. Pathol. Pharmakol.*, **234**, 17 (1958)
53. Parks, V. J., Sandison, A. G., Skinner, S. L., and Whelan, R. F., *Clin. Sci.*, **20**: 289 (1961)
54. Krogsgaard, A. R., *On Reserpine with Particular Reference To Its Use in Hypertension* (Munksgaard, Copenhagen, The Netherlands, 1961)
55. Boura, A. L., and Green, A. F., *Brit. J. Pharmacol.*, **14**: 536 (1959)
56. Boura, A. L., Copp, F. C., Duncombe, W. G., Green, A. F., and McCoubrey, A., *Brit. J. Pharmacol.*, **15**, 265 (1960)
57. Aviado, D. M., and Dil, A. H., *J. Pharmacol. Exptl. Therap.*, **129**, 328 (1960)
58. Vernikos-Danellis, J., and Zaimis, E., *Lancet*, **II**, 787 (1960)
59. Gokhale, S. D., and Gulati, O. D., *Brit. J. Pharmacol.*, **16**, 327 (1961)
60. Emmelin, N., *Pharmacol. Revs.*, **13**, 17 (1961)
61. Maxwell, R. A., Mull, R. P., and Plummer, A. J., *Experientia*, **15**, 267 (1959)
62. Maxwell, R. A., Plummer, A. J., Schneider, F., Povalski, H., and Daniel, A. I., *Schweiz. med. Wochschr.*, **90**, 109 (1960)
63. Maxwell, R. A., Plummer, A. J., Schneider, F., Povalski, H., and Daniel, A. I., *J. Pharmacol. Exptl. Therap.*, **128**, 22 (1960)
64. Maxwell, R. A., Plummer, A. J., Povalski, H., and Schneider, F., *J. Pharmacol. Exptl. Therap.*, **129**, 24 (1960)
65. Cass, R., Kuntzman, R., and Brodie, B. B., *Proc. Soc. Exptl. Biol. Med.*, **103**, 871 (1960)
66. Richardson, D. W., Wyso, E. M., Magee, J. H., and Carell, G. C., *Circulation*, **22**, 184 (1960)
67. Taylor, S. H., and Donald, K. W., *Lancet*, **II**, 389 (1960)
68. Dollery, C. T., Emslie-Smith, D., and Milne, M. D., *Lancet*, **II**, 381 (1960)

69. Gaffney, T. E., *Circulation Research*, 9, 83 (1961)
70. McCubbin, J. W., Kaneko, Y., and Page, I. H., *J. Pharmacol. Exptl. Therap.*, 131, 346 (1961)
71. Freis, E. D., *Essential Hypertension: An International Symposium*, 179 (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 1960)
72. Dollery, C. T., Harrington, M., and Kaufman, G., *Lancet*, I, 1215 (1959)
73. Wilson, I. M., and Freis, E. D., *Circulation*, 2, 1028 (1959)
74. Varnauskas, E., Cramer, G., Malmcrona, R., and Werkö, L., *Clin. Sci.*, 20, 407 (1961)
75. Conway, J., and Lauwers, P., *Circulation*, 21, 21 (1960)
76. Wanko, A., and Freis, E. D., *Circulation*, 18, 792 (1958)
77. Merrill, J. P., Guinand Baldo, A., and Giordano, C., *Clin. Research*, 6, 230 (1958)
78. Mendlowitz, M., Naftchi, N., Gitlow, E., Weinreb, H. L., and Wolff, R. L., *Ann. N. Y. Acad. Sci.*, 88, 964 (1960)
79. Winer, B. M., *Circulation*, 23, 211 (1961)
80. Munro, A. F., and Robinson, R., *J. Physiol. (London)*, 141, 4P (1958)
81. Axelrod, J., and Tomchick, R., *J. Pharmacol. Exptl. Therap.*, 130, 367 (1960)
82. Laragh, J. H., Ulick, S., Januszewicz, V., Deming, Q. B., Kelly, W. G., and Lieberman, S., *J. Clin. Invest.*, 39, 1091 (1960)
83. Sjoerdsma, A., *Circulation Research*, 9, 734 (1961)
84. Axelrod, J., Inscoe, J. K., Senoh, S., and Witkop, B., *Biochim. et Biophys. Acta*, 27, 210 (1958)
85. Labrosse, E. H., Axelrod, J., and Kety, S. S., *Science*, 128, 593 (1958)
86. Kopin, I.J., *Science*, 131, 1372 (1960)
87. Sjoerdsma, A., Lovenberg, W., Oates, J. A., Crout, J. R., and Udenfriend, S., *Science*, 130, 225 (1959)
88. Sjoerdsma, A., *J. Neuropsychol.*, Suppl. 2, 159 (1961)
89. Horwitz, D., and Sjoerdsma, A., *Proc. Soc. Exptl. Biol. Med.*, 106, 118 (1961)
90. Oates, J. A., Gillespie, L., Udenfriend, S., and Sjoerdsma, A., *Science*, 131, 1890 (1960)
91. Sjoerdsma, A., *Ann. N. Y. Acad. Sci.*, 88, 933 (1960)
92. Gillespie, L., *Ann. N. Y. Acad. Sci.*, 88, 1011 (1960)
93. Sourkes, T. L., *Arch. Biochem.*, 51, 444 (1954)
94. Goldberg, L. I., DaCosta, F. M., and Ozaki, M., *Nature*, 188, 502 (1960)
95. Elliott, D. F., Lewis, G. P., and Horton, E. W., *Biochem. J.* 74, 15P (1960)
96. Elliott, D. F., Horton, E. W., and Lewis, G. P., *J. Physiol. (London)*, 150, 6P (1960)
97. Boissonnas, R. A., Guttmann, S., and Jaquenoud, P.-A., *Helv. Chim. Acta*, 43, 1481 (1960)
98. Schwyzer, R., Rittel, W., Sieber, P., Kappeler, H., and Huber, H., *Helv. Chim. Acta*, 43, 1130 (1960)
99. Nicolaides, E. D., De Wald, H. A., Shorley, P. G., and Collier, H. O. J., *Nature*, 187, 113 (1960)
100. Boissonnas, R. A., Guttmann, S., and Jaquenoud, P. A., *Helv. Chim. Acta*, 43, 1349 (1960)
101. Boissonnas, R. A., Guttmann, S., Jaquenoud, P.-A., Konzett, H., and Stürmer, E., *Experientia*, 16, 326 (1960)
102. Konzett, H., and Stürmer, E., *Nature*, 188, 998 (1960)
103. Lewis, G. P., *Nature*, 188, 999 (1960)
104. Shorley, P. G., and Collier, H. O. J., *Nature*, 188, 999 (1960)
105. Fox, R. H., Goldsmith, R., Kidd, D. J., and Lewis, G. P., *J. Physiol. (London)*, 155, 28P (1961)
106. Elliott, D. F., Horton, E. W., and Lewis, G. P., *J. Physiol. (London)*, 153, 473 (1960)
107. Konzett, H., and Stürmer, E., *Brit. J. Pharmacol.*, 15, 544 (1960)
108. Lewis, G. P., *Physiol. Revs.*, 40, 647 (1960)
109. Hilton, S. M., and Lewis, G. P., *J. Physiol. (London)*, 134, 471 (1956)
110. Fox, R. H. and Hilton, S. M. *J. Physiol. (London)*, 142, 219 (1958)
111. Armstrong, D., Keele, C. A., and Stewart, J. W., *J. Physiol. (London)*, 155, 37P (1961)
112. Schachter, M., *Brit. J. Pharmacol.*, 11, 111 (1956)
113. Armstrong, D., Jepson, J. B., Keele, C. A., and Stewart, J. W., *J. Physiol. (London)*, 135, 350 (1957)
114. Horton, E. W., *J. Physiol. (London)*, 148, 267 (1959)
115. Page, I. H., and Bumpus, F. M., *Physiol. Revs.*, 41, 331 (1961)
116. Gross, F., *Klin. Wochschr.*, 36, 693 (1958)
117. Peart, W. S., *Ergeb. Physiol. biol. Chem. u. exptl. Pharmakol.*, 50, 409 (1959)

118. Peart, W. S., *Brit. Med. J.*, **II**, 1353, 1421 (1959)
119. Cook, W. F., *J. Physiol.* (*London*), **152**, 27P (1960)
120. Bing, J., and Kazimicrczak, J., *Acta Pathol. Microbiol. Scand.*, **47**, 105 (1959)
121. Nairn, R. C., Fraser, K. B., and Chadwick, C. S., *Brit. J. Exptl. Pathol.*, **40**, 155 (1959)
122. Hartroft, P. M., and Hartroft, W. S., *Brit. Med. J.*, **I**, 1171 (1961)
123. Hartroft, P. M., and Hartroft, W. S., *J. Exptl. Med.*, **97**, 415 (1953)
124. Tobian, L., *Physiol. Revs.*, **40**, 280 (1960)
125. Gross, F., *Essential Hypertension: An International Symposium*, 92 (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 1960)
126. Peart, W. S., *Polypeptides Which Affect Smooth Muscles and Blood Vessels: A Symposium*, 122 (Pergamon Press, London, England, 1960)
127. Houssay, B. A., and Fasciolo, J. C., *Bol. acad. nacl. med.* (*Buenos Aires*), 342 (September 1937)
128. Houssay, B. A., and Fasciolo, J. C., *Rev. soc. arg. biol.*, **13**, 284 (1937)
129. Braun-Menendez, E. Fasciolo, J. C., Leloir, L. F., and Munoz, J. M., *J. Physiol.* (*London*), **98**, 283 (1940)
130. Laragh, J. H., Angers, M., Kelly, W. G., and Lieberman, S., *J. Am. Med. Assoc.*, **174**, 234 (1960)
131. Genest, J., Nowaczynski, W., Koiw, E., Sandor, T., and Biron, P., *Essential Hypertension: An International Symposium*, 126 (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 1960)
132. Davis, J. O., Carpenter, C. C. J., Ayers, C. R., Holman, J. E., and Bahn, R. C., *J. Clin. Invest.*, **40**, 684 (1961)
133. Ganong, W. F., and Mulrow, P. J., *Nature*, **190**, 1115 (1961)
134. Peart, W. S., *Biochem. J.*, **62**, 520 (1956)
135. Skeggs, L. T., Jr., Kahn, J. R., and Shumway, N. P., *J. Exptl. Med.*, **103**, 295 (1956)
136. Genest, J., Biron, P., Koiw, E., Nowaczynski, W., Chrétien, M., and Boucher, R., *Circulation Research*, **9**, 775 (1961)
137. Conn, J. W., *J. Lab. Clin. Med.*, **45**, 3 (1955)
138. Bock, K. D., and Krecke, H. J., *Klin. Wochschr.*, **36**, 69 (1958)
139. Leyssac, P. P., Lassen, U. V., and Thaysen, J. H., *Biochim. et Biophys. Acta*, **48**, 602 (1961)
140. Peart, W. S., and Brown, J. J., *Lancet*, **I**, 28 (1961)
141. Peart, W. S., *Essential Hypertension: An International Symposium*, 112 (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 1960)
142. Friedman, S. M., Friedman, C. L., and Nakashima, M., *Nature*, **180**, 194 (1957)
143. Peart, W. S., *J. Physiol.* (*London*), **108**, 491 (1949)
144. Paladini, A. C., Braun-Menendez, E., Del Frade, I. S., and Massani, Z. M., *J. Lab. Clin. Med.*, **53**, 264 (1959)
145. Scornik, O. A., Paladini, A. C., and Braun-Menendez, E., *Acta Physiol. Latinoam.*, **10**, 64 (1960)

CONTENTS