THE METABOLISM OF ADRENALINE

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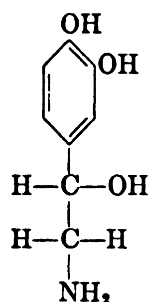
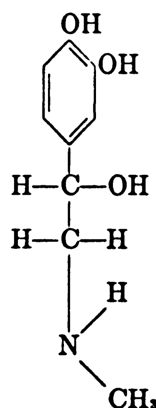
The purpose of this review is to bring together the useful information recently published on the normal metabolism of adrenaline in the body (see also other reviews, 1–4b).

It should be called to mind that adrenaline is physiologically liberated in the circulation in small amounts (a few μg per kg.); that it does not pass through the liver before reaching the lungs where very little, if any, is inactivated (5); that it is distributed to all the tissues by the arterial blood; and that most of its effects do not last longer than a few minutes.[1]



Noradrenaline



Adrenaline

The structure of the adrenaline molecule shows that there are four points of attack for enzymes or oxidizing agents: 1) the methyl group attached on the nitrogen; 2) the hydroxyl of the secondary alcohol; 3) the aminated two-carbon chain itself; and 4) the two phenolic hydroxyls.

[1] The presence in the tissues and in the blood of noradrenaline (arterenol-aminoethanol-catechol) is becoming increasingly probable (6, 7, 8, 9, 10, 11, 12) and the separation of its two optical isomers (13) is a useful step in the elucidation of its physiological importance (14).

It seems that the properties of sympathin E (excitatory) postulated by Cannon and Rosenblueth (15) are those of noradrenaline and that sympathin I (inhibitory) is adrenaline. Thus the idea of a combination of adrenaline with specific, hypothetical cellular substances E and I is abandoned. One comes back to the first concept of Cannon and Bacq (16); sympathin is the substance, or mixture of substances, liberated by adrenergic nerve action, and not adrenaline modified by contact with cellular elements.

One should speak, as suggested by von Euler (9), of sympathin N (noradrenaline) and sympathin A (adrenaline) to describe the two sympathicomimetic substances synthesized by the tissues other than the adrenal medulla, the parotid gland of tropical toads and some modified nerve cells of Annelids, which are known to synthetize pure *l*-adrenaline (17). The metabolism of arterenol has many points in common with that of adrenaline, but it is not necessarily the same.

A primary question is whether adrenaline is excreted unchanged or taken up by the tissues and retained in the cells in some type of loose biologically inactive combination, as is known for acetylcholine or histamine.

I. Excretion of Unmodified Adrenaline. It has not been reported that adrenaline when given in physiological amounts passes the kidney barrier. None of the many hypertensive substances found in the non-hydrolyzed urine of normal men or hypertensive patients exhibits properties corresponding to those of adrenaline or any catechol derivative. Surprisingly, rather high concentrations (up to 5×10^{-6}) have been found in the urine of anesthetized dogs given large amounts of *dl*-adrenaline (1 to 10 mg.) by intravenous injection subsequent to the administration of an adrenolytic (22) substance (933 F, yohimbine); but the quantities recovered were always small (23).[2] Naturally, because the quantity of adrenaline injected is enormous, the animal is often prostrate and one cannot consider its condition as physiological; but the presence of adrenaline was also observed in some samples of normal urine, in several instances, when the dog was particularly excited or the blood pressure was low before injection.

II. Possibility of Tissue Storage of Active Adrenaline. Phenolic substances rapidly leave the circulating fluids and accumulate in the cells, and it seems that adrenaline follows this rule (24, 121). A considerable proportion of adrenaline added *in vitro* to oxalated or defibrinated cat's blood enters the red cells, until equilibrium is reached. It stays active in the red cell for at least ten hours at 38° C; it can be liberated simply by laking the blood (25). There is nothing in the plasma of mammals to destroy adrenaline; on the contrary, the blood contains many substances (proteins, amino acids, ascorbic acid, glutathione, etc.) which inhibit its autoxidation. Adrenaline and all the catechol derivatives are more stable in body fluids or even in diluted plasma than in water or in sodium chloride solution of the same pH.

There is a good deal of evidence in favor of an increase in the adrenaline (or sympathin) extractable from the tissues after injection of adrenaline. It is known that adrenaline-like substances can be extracted from sympathetic nerves, the heart and various other tissues; chemical evidence corroborates physiological assays (7, 28–32). Sympathetic denervation decreases this tissue store of adrenaline-like substances (7, 32–34); but it is not yet clear whether this decrease occurs in the effector cells or is merely the result of degeneration of the postganglionic adrenergic fibers in the extracted tissues.

There is good agreement between these observations and those showing that the acetylcholine stores of various tissues innervated by cholinergic nerves disappear after section of these nerves (35–37). The concentration of acetylcholine or adrenaline-like substances in smooth muscle, heart, glands and ganglion cells appears to be a chemical constant controlled by the nervous system.

This epinephrine-like material, or absorbable chromogens (A.C.), as it is called by certain authors for technical reasons (31, 32, 34), increases after adrenaline

[2] The same phenomenon has been observed in cats injected with adrenaline (D. Richter and F. C. Mac Intosh, personal communication).

injections (38). There is also some evidence that adrenaline in the arterial blood may, in certain conditions, be stored by the adrenal medulla itself (39). Unfortunately, the chemical method of Shaw (30), even when modified (31), is not suitable for differentiating adrenaline from noradrenaline at the normal concentrations of these substances in tissue extracts (40, 41). With this method, only heart extracts appear to give clear indication of the presence of adrenaline as such. Biological titration is certainly much more sensitive. A sharp differentiation between adrenaline and noradrenaline can be obtained by simultaneously recording in the cat the responses of the sensitized nictitating membrane and of the non-pregnant uterus (10); but highly purified extracts are required and a variable proportion of the active material is lost during the process of purification. Thus, when one is confronted with the problem of the titration of small amounts of adrenaline in tissue extracts, the chemical method of Shaw is found *qualitatively* unsuitable, and the physiological method *quantitatively* unreliable. A more recent attempt to solve this problem is interesting. By the combined use of a polyphenoloxidase from *Atropa belladonna* and the filter paper adsorption technic (so useful for amino acids), James has found it possible to differentiate adrenaline in the presence of arterenol (42). The fluorescence method is difficult and, so far, has given contradictory results (43–46).

Despite these difficulties, there is little doubt that the store of adrenaline-like substances is increased by adrenaline injections, that this phenomenon is limited, and that it may have significance in certain pathological conditions (47). It would be desirable, however, to have more quantitative data on how much adrenaline, circulating in physiological concentrations, can leave the blood and be stored in tissues in a loose, inactive combination. It has been shown recently that certain tissues can store large amounts of histamine (118).

Unexpected confirmation of this storage of active adrenaline by the tisues has come from recent observations on the elimination of free adrenaline in the urine when anesthetized dogs are injected intravenously with large amounts of this amine. During the injection, when renal vasoconstriction is maximal, there is little or no secretion of urine, and such urine as is secreted does not contain adrenaline. The compound appears in the urine after the injection is completed, when the cardiovascular effects have disappeared; it may be present in the urine an hour after the end of the intravenous injection. The peak of the excretion is reached in 30 to 40 minutes.

It is a well-known fact that injected adrenaline disappears rapidly from the blood (48, 121); indeed, in certain conditions, it may disappear from the blood at a time when some physiological effects are still visible (49). The fact that free adrenaline appears in the urine for a long time after the injection appears to imply that some of the free adrenaline stored by the tissues or the blood cells is slowly released and eliminated, provided the kidney is unable to hydrolyze sulfoconjugates (see page 8 to 12).

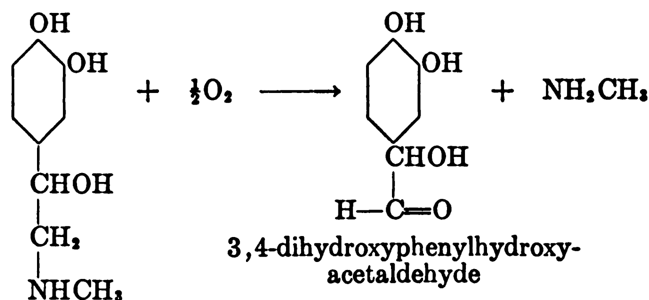
III. The Methyl Group on the Nitrogen. The source of the —CH_3 attached to the nitrogen is not known, but it is not improbable that it comes from methionine, the methyl donor "par excellence" (3, 50); the use of modern tech-

nics employing methyl groups labelled by deuterium or radioactive carbon may settle this point. Indeed, it has already been observed in du Vigneaud's laboratory that radioactive carbon concentrates in the adrenals of a rat fed with methionine containing C^{14} in its methyl radical (51). On the other hand, there is no evidence that adrenaline is demethylated in the body; noradrenaline seems to be the precursor of adrenaline and not the first step in its inactivation. It should be important, however, to eliminate every uncertainty since caffeine, dimethylxanthines and other methylated substances are demethylated in the body (52) and since monomethylaminoethanol, the side-chain of adrenaline is an excellent precursor of choline, as was shown by the aid of monomethylaminoethanol labeled with deuterium in the methyl group (53). The possibility cannot be excluded that adrenalin, or some of its oxidation derivatives in the series of adrenochrome, might be a methyl donor.

The presence of a single methyl group on the nitrogen is a basic factor in the balance between excitatory and inhibitory actions of the catecholamines (see page 21).

IV. The Secondary Alcohol Group. This group is less important at first sight; but its rôle is not negligible in the oxidation to adrenochrome and beyond adrenochrome (see page 20), and in the determination of the relative potencies of the inhibitory and excitatory actions of the amines derived from catechol.

V. Deamination of the Side-Chain. An enzyme called monoamine-oxidase, which is concentrated in the liver, the intestine and the central nervous system, inactivates adrenaline *in vitro*. Schematically, the reaction is as follows (54, 55):



This reaction is accompanied by complete physiological inactivation; methylamine, like all the aliphatic amines, has only a negligible action. The aldehyde (dihydroxyphenylacetaldehyde) resulting from amine-oxidase action on oxytyramine has vasodilator properties (56, 57), but the amounts of aldehyde eventually liberated from adrenaline by amine-oxidase in the body are too small to be of physiological interest (58). Furthermore these aldehydes are rapidly oxidized *in vivo*.

Amine-oxidase is not inactivated by cyanide, glutathione or sulfhydryl substances (54). Adrenaline is not a specific substrate for this enzyme which oxidizes (and deaminates) all amines, whether aromatic or aliphatic, which

possess an aminated two carbon chain $\left(\equiv \overset{\beta}{C}—\overset{\alpha}{C}H_2—N\langle\right)$. If the α-carbon is substituted (for example, by a methyl radical, $\equiv C—CH(N\langle)—CH_3$), the amine is not oxidized and becomes an inhibitor of the enzyme (59,60). Blaschko, Richter and Schlossman (54) re-described under the name of "*adrenaline-oxidase*" the tyramine-oxidase or the aliphatic amine-oxidase of Hare (61) and of Pugh and Quastel (62); the identity of these enzymatic systems is unquestionable (59). Accordingly this enzymatic system was correctly named "*amine-oxidase*" or monoamine-oxidase; its activity allowed satisfactory interpretation of older experiments on the metabolism of tyramine (63) and of the amines resulting from putrefaction (64), which have been confirmed in man (65).

Indirect arguments in favor of a physiological action of amine-oxidase were found in the fact that ephedrine and amphetamine, which are not oxidized by this enzyme, are excreted unchanged by the kidneys (65) and that ephedrine, presumably by its inhibitory effect on amine-oxidase, increases the amount of sympathin (in this case, very probably adrenaline) liberated by sympathetic stimulation of the perfused rabbit's ear (66).

Nevertheless, it seems that the presence of this enzyme does not provide an adequate explanation for the rapid disappearance of low concentrations of adrenaline in the circulating blood. The main arguments are the following:

1) Amine-oxidase is concentrated in the liver, the intestine and central nervous system. In these strategic positions, it protects the organism and the nerve centers against the toxic action of the amines originating from food digestion and bacterial action (67).

2) Amine-oxidase is absent (67), or is present only in very small concentration (68), in tissues which, like the rabbit's ear, inactivate adrenaline perfused through them.

3) Liver amine-oxidase requires 12 minutes to inactivate *in vitro* 50% of 10^{-7} M adrenaline (69). It can be calculated that the concentration of adrenaline which saturates 50% of the enzyme is greater than 1.5×10^{-2} M, or 40 times that of tyramine. Even if one assumes the enormous adrenaline concentration of 10^{-4} M, the enzyme would act at only 1% of its optimal activity; the physiological concentration of adrenaline in the blood is about 10^{-9} M. Thus amine-oxidase is more prone to inactivate tyramine or aliphatic amines than to oxidize adrenaline (69). Philpot (77) correctly points out that conditions of enzyme activity *in vivo* are quite different from those *in vitro*.

4) Evisceration, or temporary arrest of the circulation in the liver and the intestine where amine-oxidase is concentrated, does not increase either the intensity or the duration of action of physiological amounts of adrenaline injected intravenously in the dog (70) or in the cat (71). There is a slow process of sensitization to adrenaline after evisceration, but this phenomenon is simply

due to a drop in body temperature; it does not occur if the eviscerated animal is kept warm (73).

5) It is unfair to compare the effects of adrenaline injected in the portal vein with the actions of an equal dose given intravenously. In order to obtain, by the method of adrenaline injection, a true idea of the inactivating power of the tissues *in situ*, one must compare, on a distant test object, the results of intraportal injection with injection of the same physiological amount in an artery (the femoral, for example) irrigating a mass of tissue approximately the same size as the liver. The amount injected must be small in order to avoid complete arrest of circulation by arterial constriction. With the denervated nictitating membrane and the non-pregnant cat's uterus as test objects, it can be shown that from 75 to 90% of 2 to 5 μg of adrenaline disappear in the hind limb and that the same percentage is inactivated by the liver (72).

6) The argument put forward by Gaddum and Kwiatkowski (66) that ephedrine sensitizes to adrenaline by virtue of its inhibitory action on amine-oxidase does not seem to be valid for at least three reasons: a) ephedrine sensitizes tissues deprived of amine-oxidase (67); b) ephedrine sensitizes in very weak concentrations which *in vitro* do not inhibit amine-oxidase (67); c) cocaine abolishes completely and rapidly the sympathomimetic action of ephedrine, while it simultaneously increases the actions (mainly *excitatory*) of adrenaline (74). A detailed study of the effects of ephedrine and adrenaline on various smooth muscles and under various physiological and pharmacological conditions shows beyond question that ephedrine acts directly on the cells and not indirectly by way of a sensitization to the actions of adrenaline or sympathin. For example, ephedrine normally contracts the chronically denervated nictitating membrane of the anesthetized and even the adrenalectomized cat; thus it acts on a tissue deprived of its store of adrenaline-like substances, in an animal which has no adrenaline or sympathin in the circulation (74). It cannot be argued that cocaine suppresses the action of ephedrine because of its own inhibitory action on amine-oxidase; the fact that cocaine rapidly inhibits the long-lasting contraction of the nictitating membrane caused by the prior injection of ephedrine is not compatible with the hypothesis of Gaddum, supported by Tripod (75) and MacGregor (76).

7) The argument that cocaine and the local anesthetics which sensitize to adrenaline also inhibit amine-oxidase (77) seems at first sight more difficult to refute. But there is no parallelism between the degree of enzyme inhibition and the sensitizing power. For example, nupercaine was found by Philpot (77) to be the best inhibitor of amine-oxidase, yet it potentiates only slightly the action of adrenaline on the nictitating membrane "*in situ*"; in contrast procaine, a weaker inhibitor, sensitizes markedly (78).

Furthermore, Philpot's hypothesis does not explain why cocaine, instead of increasing, completely abolishes the action of tyramine and phenylethylamine (74) which *are* oxidized *in vitro* by amine-oxidase (63, 64).

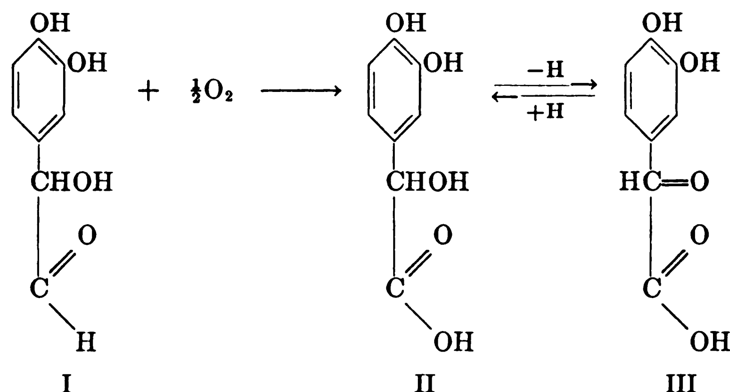
The phenomena of sensitization and desensitization to sympathomimetic compounds are very complex in the case of cocaine and local anesthetics, and at present cannot be interpreted on the basis of a theory of enzyme inhibition.

8) Efficient inhibitors of amine-oxidase such as ethylurethane (54) do not sensitize *in vivo* to adrenaline. Sulfoconjugated adrenaline is found in the urine of man and animals given large doses of adrenaline (80, 86, 87). Although the direct experiment has not been tried, there is *a priori* no reason why amine-oxidase should not deaminate and oxidize the side-chain of the sulfuric ester of adrenaline or epinine.

9) According to Weinstein and Manning (79), the urine of rabbits given large doses of adrenaline shows the reaction for protocatechuic acid $(OH)_2C_6H_3COOH$ which should be the final product of the oxidation *in vivo* of the aldehyde resulting from amine-oxidase action on adrenaline. Unfortunately, as pointed out by Richter (80) and by Bernheim (1), these authors treated the urine with alkali and adrenaline, if present, would be converted in part to protocatechuic acid. Inasmuch as the presence of free and conjugated adrenaline has been demonstrated in the urine, the evidence brought forward by Weinstein and Manning is not conclusive. Richter (80) tried without success to find, by means of a sensitive color reaction, protocatechuic acid in his own urine after ingestion of large doses of epinine (61 mg.) or adrenaline (*d* or *l*, from 10 to 55 mg.); even after acid hydrolysis, which should liberate protocatechuic acid from its sulfoconjugate, the test was negative.

Florkin and Bacq (81), employing Baumann's method which avoids the use of alkali, have observed the presence of phenolic acid in the urine of a dog which received 150 mg. of epinine by intraperitoneal injection; but epinine is a better substrate for amine-oxidase than is adrenaline, and the amount injected was enormous.

Confirmation of the negative results of Richter is given by the fact that up to 70% of tyramine perfused through the rabbit's liver (63) was recovered as 4-oxyphenylacetic acid. Large quantities of the same acid were found in the urine of dogs and rabbits after large doses of tyramine were given (63, 64). The assumption of Weinstein and Manning that protocatechuic acid $(OH)_2C_6H_3COOH$ must be the final product of adrenaline oxidation by amine-oxidase is open to question. If one considers the probable fate of dihydroxyphenyl-hydroxy-acetaldehyde (I) in the body, it appears that two flavoproteins (xanthine oxidase and a specific aldehyde oxydase) will oxidize it to the corresponding acid (II),



which is the 3,4-hydroxymandelic acid. If this acid follows the metabolism of mandelic acid ($C_6H_5 \cdot CHOH \cdot COOH$), it should be mainly excreted with the two-carbon side-chain unaltered. It might be partly sulfoconjugated at the level of at least one of the phenolic groups. An equilibrium might also occur between the acid and its ketone (III) (for bibliography, see ref. 82, page 115).

It is not probable that the presence of the phenolic hydroxyls alters the fate of the side-chain since para-hydroxyphenylacetic acid, $OH \cdot C_6H_4 \cdot CH_2 \cdot COOH$, passes through the body unchanged (83). This last observation is interesting because it shows that in this particular case the addition of a two-carbon side-chain to the phenol molecule inhibits the sulfoconjugation (see section V). Thus a careful review of the literature does not indicate how 3,4-oxymandelic acid could lose a carbon atom and be changed into protocatechuic acid.

Even if one accepts the view that protocatechuic acid is formed from adrenaline, one should find in the urine not only the sulfuric ester of this acid, but also some free acid and sulfoconjugated catechol. It is known that these three substances are found in the urine of the dog fed with protocatechuic acid (84).

VI. ESTERIFICATION OF THE PHENOLIC GROUPS. The two phenolic hydroxyls of adrenaline are essential in the determination of the quality and the intensity of sympathomimetic action of the aromatic amines. The OH in the *meta* position is more important than the hydroxyl in the *para* position, but only catechol derivatives may be considered as true sympathomimetic amines (see ref. 85). Thus oxidation or esterification of these two phenolic groups results in complete inactivation of the molecule, as far as the classical sympathomimetic effects are concerned.

Curiously, it was not before 1940 that the first contribution appeared on the possibility of detoxification of adrenaline by the mechanism of sulfoconjugation, a process so well known for phenolic substances. The evidence given by Richter (80) in favor of this mechanism is the following:

1) Richter ingested large amounts of *d*- or *l*-adrenaline (15 to 55 mg., per 76 kg.) with glycine and acetic acid (to avoid, as far as possible, oxidation in the digestive tract). A substance was excreted in the urine which had the characteristics of an adrenaline sulfoconjugate: it was inactive before hydrolysis; after acid hydrolysis, it gave all the chemical reactions (specific and non-specific) of adrenaline[3] and inhibited the isolated rabbit's intestine.

2) By the use of a method accurate within 10%, it was shown that the excretion of this conjugated adrenaline began 3 hrs. after the ingestion, was maximal in about 5 hrs. and then slowly decreased in the course of 24 hrs. The amount of adrenaline recovered as the sulfoconjugate varied from 30 to 70%.

3) An increase in blood pressure indicated that, between 1 and 4 hrs. after the ingestion, some active adrenaline reached the tissues, but certainly the greater part of the amine was detoxified in the intestine and the liver.

[3] Green color reaction with ferric chloride, formation of adrenochrome and iodoadrenochrome, reduction of arsenomolybdic acid with a marked increase in color with NaOH, as described by Shaw (30).

4) Glycuronide tests were negative; tests for sulfuric esters were positive. The sulfoconjugate has not been isolated in pure state; its structure is tentatively given as $\bar{O}\text{-}SO_2\cdot O\cdot(OH)\cdot C_6H_3\cdot CHOH\cdot CH_2\cdot \overset{+}{N}HCH_3$.

5) Similar observations were made by Richter after ingestion of *dl*-corbasil, $(OH)_2C_6H_3\cdot CHOH\cdot CH\cdot(NH_2)\cdot CH_3$, and epinine, $(OH)_2C_6H_3\cdot CH_2\cdot CH_2\cdot NH\text{-}CH_3$. Confirmation of Richter's observations has been provided by Richter and MacIntosh (86). After hydrolysis, adrenaline has been adequately identified pharmacologically, but only 39% of the ingested adrenaline was accounted for. Beyer and Shapiro (87) have also confirmed Richter's observations and added interesting facts. In dogs, 15.9 and 22.3% of 25 mg. of ingested adrenaline were recovered in 8 hours as the sulfoconjugate. A much higher amount (50 to 78%) of epinine was recovered in this form in 8 hours and the percentage rose to 83% when the urine was collected for 24 hours. If 30 mg. of epinine were injected subcutaneously in dogs, 40 to 50% was recovered in 8 hours as the sulfoconjugate; after ingestion of an equal amount, 50 to 79% was recovered in the same period. An average of 65% of ingested cobefrine (3,4-dihydroxyphenylisopropanolamine), which is not oxidized by amine-oxidase, was recovered in 24 hours as the sulfoconjugate.

Florkin and Bacq (81), quite independently from Richter, tried to find evidence for the excretion of the sulfoconjugate with a different technic. In dogs weighing 6 to 7 kg., 100 mg. of catechol (injected subcutaneously or intraperitoneally) are necessary to obtain a decrease below unity of the ratio $\frac{\text{inorganic S}}{\text{ester S}}$ in the urine collected during the 24 hrs. following the injection. A dose of 50 mg. does not provoke the so-called "reversal" of the ratio. If equimolecular amounts of adrenalone (the ketone of adrenaline) or epinine are injected intraperitoneally in these dogs, the ratio $\frac{\text{inorganic S}}{\text{ester S}}$ remains unchanged.

It cannot be concluded from these experiments that adrenalone and epinine are not esterified. Part of these catechol derivatives (50% or less) still may be detoxified by sulfoconjugation. The presence of an aminated side-chain has undoubtedly modified the metabolism of the catechol nucleus.[4] A more extensive use of Florkin and Bacq's method might show with fair approximation what proportion of the amines derived from catechol is excreted as sulfoconjugate; but, at first sight, these results do not contradict the observations of Richter and of Beyer and Shapiro.

Deichmann (88) has observed no increase in glycuronides, but a marked decrease of the ratio inorganic/total sulfates in the urine of rabbits following oral, subcutaneous or intravenous administration of adrenaline. Some of his results are in contradiction with the carefully controlled observations of Florkin and Bacq (81), and a far greater quantity of organic sulfates was excreted than could be accounted for by the conjugation of the administered adrenaline. Dogson,

[4] It has already been mentioned (page 8) that para-oxyphenylacetic acid, $HO\cdot C_6H_4\cdot CH_2\cdot COOH$, is excreted unchanged in the urine.

Garton and Williams (201) failed to confirm Deichmann's observations; in rabbits given 200 to 250 mg./kg. of *d*-adrenaline orally, they found insignificant amounts of sulfoconjugate in the urine, whereas 21% was excreted as a glucuronide in 24 hours. The question whether *l*-adrenaline also forms a glucuronide has yet to be investigated because *l*-adrenaline suppresses glucuronic acid conjugation in liver slices (202).

The great objection to all these experiments is that very large, unphysiological amounts of sympathomimetic compounds are needed. One cannot extrapolate from these results what happens to a few micrograms of adrenaline in the arterial blood. A further objection to Richter's work is that the adrenaline was taken orally and consequently only a very small fraction reached the heart and the general circulation.

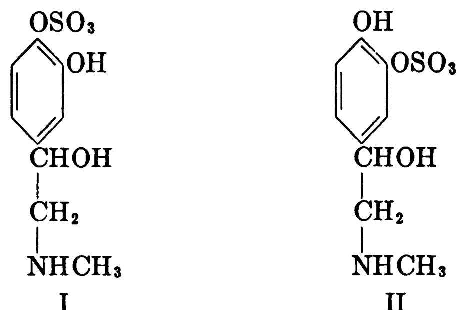
Although the ability of the liver and intestine to detoxify adrenaline is definitely established, it is beyond question (in contrast to common belief) that the liver is not the main site of detoxification of adrenaline circulating in physiological concentrations. The experiments of Bacq (72) show that, if one uses very small amounts (2 to 5 μg), the cat's hind limb can inactivate as much adrenaline as the liver (see also ref. 5).

The enzymatic system called sulfosynthase which catalyzes the esterification of phenolic substances seems to be localized in the liver and in the intestinal mucosa (89, 90, 104), although it has been reported that eviscerated animals still conjugate phenols (91). It has already been mentioned that evisceration does not increase or prolong the action of adrenaline injected in physiological amounts; thus it seems that the action of this liver sulfosynthase is not a predominant factor in the physiological inactivation of adrenaline.

Bernheim (1) points out that Richter's observations leave certain important facts unexplained: "Esterification does not require oxygen, yet perfused liver does not inactivate adrenaline unless oxygen is present and the same is true of isolated liver slices." It appears, however, that the conjugation requires the energy produced by reactions coupled with oxygen consumption (87). The adrenaline ester recovered from the human urine is pharmacologically inactive in small doses (86); from what we know of the relation between chemical structure and physiological activity in the series of sympathomimetic amines, ". . . it is also not entirely clear why esterification of one hydroxyl group should make adrenaline pharmacologically inactive, even though the sulfate ester of tyramine has been reported to be inactive in the rabbit" (92).

Trimethoxyphenylethylamine (mescaline) at a dose of 10 mg./kg. is not sympathomimetically inactive (74); the monosulfuric ester of adrenaline still possesses a phenolic hydroxyl which is known to increase (markedly, if in the *meta* position) the action of aromatic amines.
Of the two possible isomers of Richter's ester, ester II should be less active than I, but not completely inactive in large doses.

The suggestion that esterification of adrenaline occurs only if the amine is ingested has not been supported by the experiments of Bacq, Lecomte and Fischer (93). In the urine of chloralosed dogs given intravenous injections of large



amounts of adrenaline, these investigators found an inactive substance which gave the physiological actions of adrenaline after acid hydrolysis; it was presumably the same sulfuric ester as that reported by Richter. The percentage recovered in 8 hours was about 5%. The only objection remaining is that the quantity injected (5 to 10 mg.) was unphysiological. With the available physiological and biochemical technics, it would appear possible to make conclusive observations with smaller amounts of adrenaline.

Holtz *et al.* (94), in confirmation of earlier observations, found a pressor substance "urosympathin" in the urine of normal man, subjected to acid hydrolysis; a closer analysis showed that this urosympathin is a mixture of adrenaline and its two postulated precursors: arterenol and oxytyramine. The daily excretion, as tested on the cat's blood pressure, was equivalent to 0.1–0.15 mg. of adrenaline or 2–3 mg. of oxytyramine. The excretion of urosympathin is increased by muscular work and in some cases of arterial hypertension (see also ref. 2). Although Holtz *et al.* do not mention the fact, their urosympathin is probably in the urine as an inactive sulfoconjugate. Richter (80) and those who have confirmed his views in men and animals, did not find a sulfoconjugate of a sympathomimetic amine in normal urine, but the small amount normally present may have escaped their attention.

Torda (95) attempted to explain the actions of cocaine, ergotamine and yohimbine by their effect *in vitro* on a very weak preparation of liver sulfo-esterase; for many reasons, Bernheim (1) is correct in his statement that these experiments are of little value, although cocaine increases slightly the excretion of free phenol in the cat after phenol injection (96).

Experiments of Bacq (97) have shown that many phenols sensitize markedly to adrenaline and to sympathetic stimulation. This sensitization, quite different from that following cocaine injection, had been related to the well-known antioxidant power of these unstable phenols (catechol, hydroquinone, pyrogallol) and it had been considered as an argument in favor of the oxidation (through adrenochrome) of adrenaline *in vivo*. Richter (80) suggests that these phenols compete with adrenaline for the inactivating system and act to augment and prolong the effects of adrenaline by inhibiting its sulfoconjugation; he draws an analogy between the inhibition of cholinesterase by eserine and the inhibition of "sulfosynthase" by phenols. There is the following objection to Richter's interpretation: resorcinol, which is sulfoconjugated as readily as catechol or

pyrogallol but which is not an antioxidant because it is stable, does not sensitize to adrenaline even if injected in an amount three times that of catechol (97).

Thus substantial amounts of inactive sulfoconjugated catecholamines are found in the urine of dog and man after oral ingestion or subcutaneous or intravenous injections. After injection, the amounts recovered are less than after ingestion. Quantitive data indicate that the sulfoconjugation of the catecholamines is not as complete as that of catechol, and other possible pathways of adrenaline inactivation must be sought. This conclusion is consistent with the fact that adrenaline disappears in tissues which are not known to conjugate phenols.

VII. Oxidation to Quinone. *A. Introduction.* *In vitro*, aqueous solutions of adrenaline are rapidly oxidised by molecular oxygen with the formation of a red color. This oxidation is accelerated by the presence of traces of copper (Fe, Hg and other heavy metals), by an alkaline pH and by light (ultraviolet rays). Many substances inhibit this oxidation, for example, strong reducing agents such as glutathione (103), cysteine, ascorbic acid (26, 103) and dimercaptopropanol (BAL) (27); amino acids (98, 99); the so-called "antioxygens," such as phenols and thyroxin (97, 100). The presence of some of these substances in the blood and the tissues has undoubtedly a physiological significance. In the adrenals of mammals and in the parotoid glands of tropical toads where adrenaline is concentrated without any tendency to oxidize, one finds large concentrations of ascorbic acid and glutathione.

Thyroxin is beyond question one of the most important chemical agents which normally regulate the sensitivity of the tissues to adrenaline (48, 101, 102, 97). Physiologists and pharmacologists are aware of the fact that, when secretion of adrenaline is considered, not only is the quantity secreted important but also the sensitivity of the effector cells, which may vary considerably (for discussion, see ref. 97). The so-called "sympathetic hyperexcitation" state of hyperthyroid patients is probably a purely peripheral phenomenon. It seems probable that these variations in the sensitivity to adrenaline are due to changes in the metabolism of the hormone. For example, after pyrogallol injection, the denervated nictitating membrane reacts to adrenaline by a long-lasting contracture similar to that obtained with ephedrine in the normal cat, although adrenaline disappears from the blood (49).

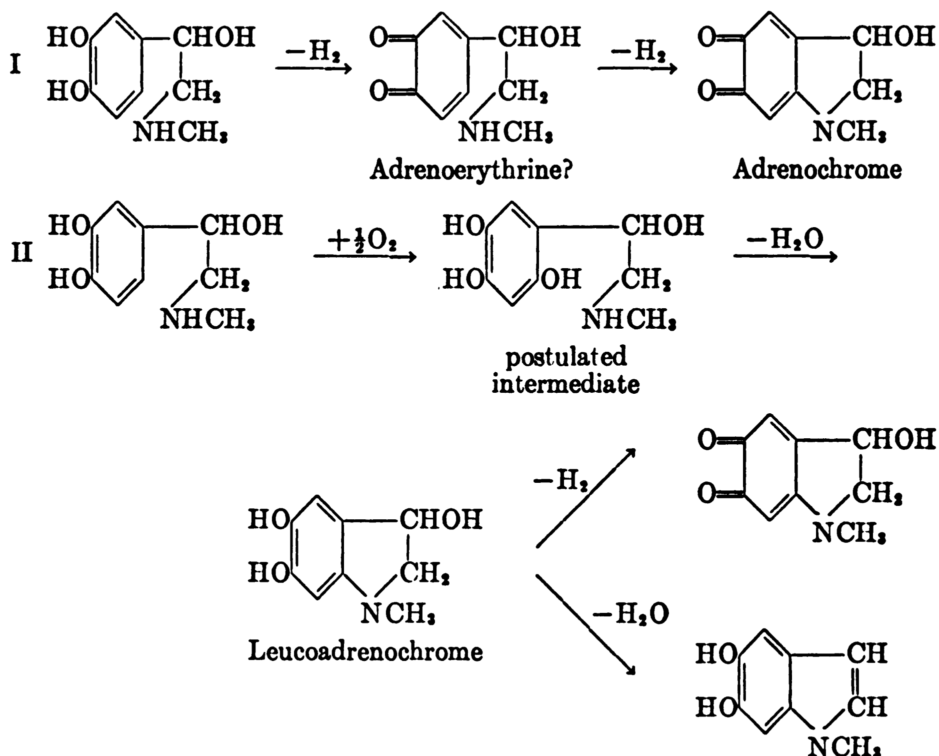
Before discussing the properties of adrenochrome, three questions should be answered.

1) Is the slow oxidation *in vitro* (autoxidation) comparable to the oxidation by catecholoxidase? The answer is presumably yes. Oxidation of adrenaline by silver oxide gives the same adrenochrome as that by catecholoxidase. The catalytic action of Cu^{++} ions is known to be similar to that of the phenolases, the active metal of which is copper.

One should not too strongly emphasize the fact that a red solution of autoxidized adrenaline cannot be considered as adrenochrome, because adrenochrome is very unstable and is rapidly transformed into many different substances. The

complete oxidation of adrenaline to melanin requires 8 to 9 atoms of oxygen per molecule of adrenaline, and some CO_2 is evolved (103); only 2 atoms of oxygen are needed to obtain adrenochrome. A careful review of the literature shows that several melanins requiring different amounts of oxygen may be obtained from adrenaline.

2) Is it possible to conceive the existence of a substance intermediate between adrenaline and adrenochrome? Schematically, two mechanisms are possible to account for the transition from adrenaline to adrenochrome. In sequence I, the phenolic groups are first oxidized, the side-chain being open; in sequence II, the side-chain reacts prior to the oxidation of the hydroxy groups.



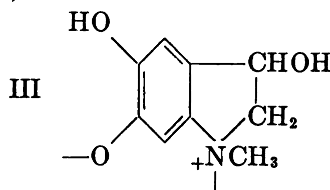
These two possibilities have been much discussed (105, 106, 107, 108, 109); it is not within the scope of this article to enter into the details of the chemical reactions.

If sequence I is correct, one should expect the quinone with open chain to retain some sympathomimetic activity and to yield adrenaline on reduction. Many authors (110, 111, 112, 113, 26) have published observations in favor of the existence of this adrenaline quinone or "adrenoerythrine" (26), but the substance has never been isolated (not even in the form of stable derivatives such as the oxime or semicarbazone).

If sequence II is correct, reduced adrenochrome, the so-called leucoadrenochrome (107, 114), is produced before adrenochrome itself. If we accept Harley-

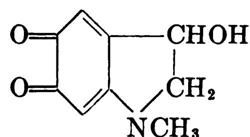
Mason's view (114), this series of reactions is impossible because the very unstable leuco derivative easily loses a molecule of water, and one should never obtain the quinone adrenochrome.

The possibility of the existence of a semiquinone (type III) has been emphasized only recently (114).

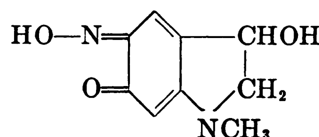


3) Does "autoxidation" of adrenaline occur in the body? The answer is that this is quite unlikely, as pointed out by Richter (80), because the cells contain many substances which stabilize adrenaline.

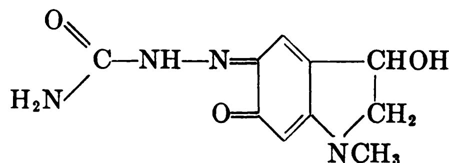
B. Chemical properties and derivatives of adrenochrome. A very important contribution by Green and Richter was the isolation and clear identification of the quinone resulting from adrenaline oxidation (107). They used a highly purified catecholoxidase from mushroom, a concentrated solution (1×10^{-2}) of adrenaline, a pH of 5 to prevent melanin formation, and bubbling O_2. Adrenochrome is now prepared more easily by the action of silver oxide on adrenaline in methyl alcohol (115, 116). Adrenochrome is very unstable even when crystallized in the cold, in the dark and in the absence of O_2. It turns to a brownish black, insoluble pigment which is by definition a melanin. Three stable derivatives of adrenochrome have been isolated: the monoxime (107, 115, 116), the mono-semicarbazone[5] (116) and the mono-*p*-nitrophenylhydrazone (115), by reacting one of the quinone oxygens with hydroxylamine, semicarbazide or para-nitrophenylhydrazine, respectively.



Adrenochrome
(according to *107*)



Monoxime

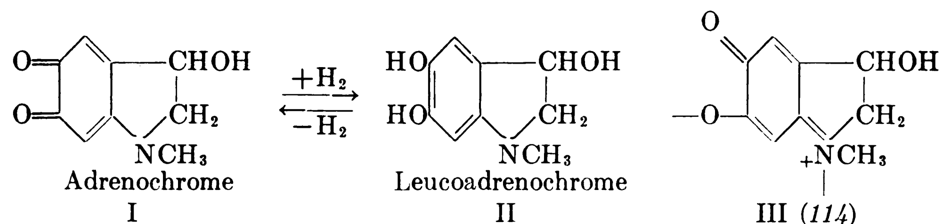


Mono-semicarbazone
(Adrenoxyl)

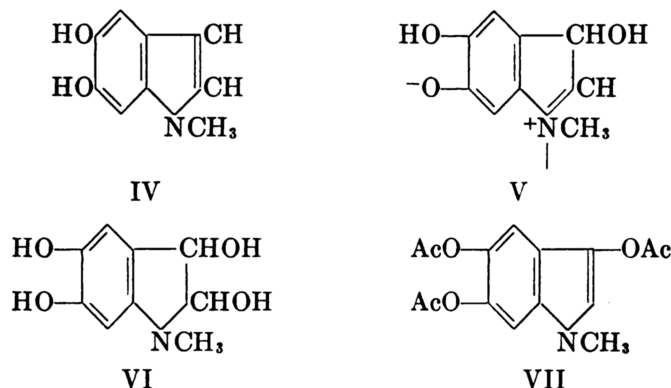
In the presence of a strong reducing agent (hydrosulfite, ascorbic acid, cysteine, Raney's nickel), the red color of adrenochrome changes to various shades of light

[5] Also known in Europe as "Adrenoxyl Labaz." Société belge de l'Azote.

green, presumably the color of the assumed leucoadrenochrome which has not been isolated. There seems to be a redox equilibrium between the two forms (I and II).



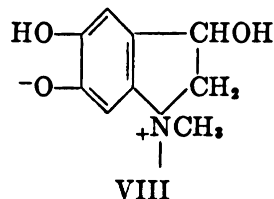
Recent investigations show that the physical and chemical properties of adrenochrome are better accounted for by the structure of a zwitterion, para-quinoneimine (III) (114). For example, the facts that only the *mono*xime and the *mono*-semicarbazone have been prepared and that all attempts to induce a reaction with a second molecule of hydroxylamine or semicarbazide have been unsuccessful show that only one true carbonyl group is present in the molecule of adrenochrome. A careful study of the reduction with hydrogen-Pd-charcoal shows that absorption of hydrogen ceases when one atom of hydrogen per molecule of adrenochrome has been absorbed[6] and two products have been obtained; none of them has the supposed structure of leucoadrenochrome (II). One of these substances is the 5,6-dihydroxy-N-methyl-indole (IV) which has been isolated in colorless needles.



The other (V) could not be isolated as such because it decomposes on concentration, but two stable derivatives were obtained: VI after treatment with alkali and VII after acetylation. The compound VI is yellow and exhibits a strong fluorescence in ultraviolet light; it is probably responsible for the color appearing in the test of Gaddum and Schild (117), the intensity of which is specific for adrenaline in the series of sympathomimetic amines; these authors observed a marked green fluorescence when adrenaline is oxidized (for example, at an

[6] Unpublished observations by Beaudet show that the same absorption of a single H atom occurs when adrenochrome is reduced by Raney's nickel.

alkaline pH). According to Utevsky (112), the fluorescence of oxidized adrenaline is due to the supposed leucoadrenochrome (II); this contention seems to be untenable in view of the observations of Harley-Mason (114). Substance IV results from the immediate, irreversible dehydration of the so-called leucoadrenochrome (II) and the redox equilibrium postulated should be impossible. Substance V is isomeric with adrenochrome. The existence of a common intermediate for substances IV and V is postulated; it should be the semiquinone VIII.



VIII

If one accepts these views, how is it possible to explain the action of adrenochrome as a hydrogen carrier in the aerobic, malic and lactic dehydrogenase systems (107)? The opinion of Harley-Mason is that, since compounds IV and V cannot be reconverted to adrenochrome, "the redox system involves *one* electron transfer between adrenochrome and the semiquinone (VIII)." The short note of Harley-Mason does not answer several questions;[7] one must wait for the complete report.

Adrenochrome and its derivatives are certainly the most interesting oxidized derivatives of adrenaline. It is a great error to consider adrenochrome as an inactive substance because it has lost all its classical sympathomimetic actions (107, 77, 119). Beyer (2) discusses at length the inactivation of sympathomimetic amines in his 1946 review but simply ignores adrenochrome.

C. Physiological properties of adrenochrome and its stable derivatives. Adrenochrome was isolated by Green and Richter; it was found to play the role of an efficient hydrogen carrier, a property common to many quinoid substances (122, 123). If adrenaline is added to a lactic acid or malic acid dehydrogenase system, there is a latent period for its oxidation to adrenochrome. A measurable effect is obtained even with concentrations as low as 6×10^{-7}. "The general experience with oxygen carriers such as cytochrome and lactoflavine is that far higher concentrations are required to obtain an oxygen uptake measurable *in vitro* than are necessary in the living cell. It can therefore be concluded that the carrier action of adrenochrome may come within the range of physiological concentrations" (Green and Richter, 107, p. 615). Recent work indicates that the adrenaline-adrenochrome system is present in mammalian skeletal muscle at a concentration of approximately 1×10^{-7} (125). Older observations by Kisch and collaborators (120) had shown that oxidized adrenaline (the so-called "omega substance" which is probably adrenochrome and various unidentified substances beyond adrenochrome) catalyzes the oxidation of certain amino-acids and increases oxygen consumption of isolated tissues. Blix (124) has also found an action of oxidized adrenaline on amino-acids.

[7] For example, it is difficult to understand how substance VI is colored when IV is colorless.

Adrenochrome and its oxime and semicarbazone are excellent hemostatic substances as far as capillary hemorrhages are concerned (126, 127, 128, 129); the semicarbazone (*adrenoxyl*) has been widely used with success in Belgium and France in human therapeutics. The lack of toxicity and the absence of any interfering sympathomimetic action are obvious advantages (130). Large amounts of the semicarbazone have weak nicotinic actions (131). They increase capillary resistance (vitamin P action) and this increase is parallel to the hemostatic action (132, 133); iodo-adrenochrome also increases capillary resistance (134). The action of the many substances possessing vitamin P properties has been interpreted as a sensitization phenomenon related to the adrenaline-adrenochrome action (135).

The hemostatic action of minute amounts of adrenaline (1 μg in the rabbit) has a latent period of four minutes, is maximal after 7 minutes and lasts for hours; these facts led Roskam and Derouaux to suspect that some oxidized derivative might be responsible (126, 136). Adrenochrome is as good a hemostatic as adrenaline and its action is maximal three minutes after injection (136). Thus the hypothesis seems justified that this particular long-lasting action of adrenaline is due to adrenochrome or the products of adrenochrome metabolism in the body.

Adrenochrome possesses many properties of the other quinones. It oxidizes reversibly the — SH groups of glutathione, proteins and enzymes, and by this mechanism inhibits many enzymes of the glycolytic cycle: hexokinase, phosphohexokinase, etc. (137, 138). The physiological expression of this inhibition is the Lundsgaard contracture (contracture and inexcitability after work) observed in amphibian (139) and mammalian (140) muscles. Like many quinones, adrenochrome inhibits mitosis (141, 142); and the literature shows an increasing tendency to link anti-mitotic action with inhibition of carbohydrate metabolism (175).

Thus adrenochrome, by virtue of its quinone function or functions, joins the vast group of "thioloprive" substances, a term created by Bacq (143) in order to designate toxic substances of widely different chemical structure which deprive the tissues of their thiol groups, inhibit the same enzymatic systems and possess common pharmacological properties. An important question is whether adrenochrome is one of the chemical factors which control the mitotic activity of cells *in situ*.

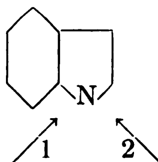
The semicarbazone, which is not in itself a thioloprive substance, seems to be hydrolyzed by the tissues and to liberate adrenochrome (139). Adrenoxyl has the unexplained ability to increase, sometimes markedly, the response of the isolated rat's diaphragm to maximal phrenic nerve stimulation (140); this potentiation is not exhibited by adrenochrome and is quite different from the Orbeli effect of adrenaline (140).

Adrenochrome like other quinones catalyzes the inactivation of catecholamines and may play a role in chronic hypertension (144, 145); this last observation has not been confirmed. There is a controversy concerning the action of adrenochrome, its derivatives or unidentified oxidation products of adrenaline on blood sugar; some investigators obtained hyperglycemia, many observed hypoglycemia and an increased effect of insulin (146, 147) and others were unable to find any change (148, 149). Adrenochrome increases glycogen formation (150, 147) and

induces the formation of verdohaemochromogen from haemochromogen (115). Oxidized adrenaline also catalyzes the oxidation of glyceraldehyde (200). The action of adrenochrome on the circulation is discussed by Marquardt and Oettel (203).

D. Adrenochrome as mother-substance of sympathin. The theory of chemical transmitters postulates the existence, at the endings of adrenergic nerves, of preformed sympathin easily liberated by the nerve impulse. It cannot be questioned that this store exists, but the problem arises whether adrenochrome can be resynthetized to the active amine; in other words, is adrenochrome the "mother-substance" of sympathin?

The perfusion with saline solution of the isolated amphibian heart (33, 151) or the rabbit's ear (136), combined with frequent stimulation of the corresponding adrenergic nerves, results in the progressive disappearance of the cardioaccelerator and vasoconstrictor effects. This is interpreted as being due to the loss of the peripheral store of sympathin or of its precursor. If small amounts of adrenochrome or oxidized "inactive" adrenaline are added to the perfusion fluid, the normal effects of nerve stimulation are re-established. Does this mean that adrenochrome is the precursor of adrenaline? *In vitro* as well as *in vivo*, not a single observation exists showing that indole or a molecule with an indole nucleus breaks between the nitrogen and the nucleus, as shown by arrow 1; when it does break, it is at the position shown by arrow 2 (152). Thus, chemically speaking, the oxidation of adrenaline to adrenochrome is irreversible (see also ref. 114).



The synthesis of adrenaline proceeds from phenylalanine to dioxyphenylalanine, then to oxytyramine by decarboxylation, arterenol (noradrenaline) probably being the immediate precursor of adrenaline (153–157, 10).

E. Occurrence and metabolism of adrenochrome in the body. The presence of adrenochrome in mammalian tissues or blood has never been unequivocally established. The observations of Green and Richter and of Roskam and Derouaux already referred to provide only indirect evidence. One may hope that, as a result of Harley-Mason's recent contribution, specific and accurate methods will be developed for the quantitative detection of adrenochrome and its derivatives in tissue extracts.

The argument of Richter (80) that after adrenaline is ingested the urine never gives the reactions of adrenochrome has little weight. When large doses of adrenochrome (up to 18 mg./kg.) are injected subcutaneously into rabbits, adrenochrome is not excreted in the urine and there is no increase in the excretion of indolic substances titratable with the ninhydrin reaction. When injected intravenously in dogs, adrenochrome (50 to 100 mg. of pure crystallized substance) is excreted in the urine; this excretion ends after about 45 minutes and

the amount recovered does not exceed 25% (158). The fate of adrenochrome in the body needs further investigation.

When adrenoxyl (semicarbazone of adrenochrome) is orally administered to fasting persons, a large part (20 to 25%) is excreted unchanged by the kidneys; there is an increased excretion of indolic substances, but this seems to be due to the activity of intestinal bacteria because it does not occur if a sulfonamide has been ingested in adequate amounts (158). When injected in dogs and rabbits, adrenoxyl is rapidly excreted unchanged in the urine, but there is also an increased excretion of other indolic substances (158). When *dl*-adrenaline is injected in large, anesthetized dogs (subsequent to the injection of an adrenolytic substance such as 933F or yohimbine), there is an increased excretion of indolic substances equivalent to 10–20% of the adrenaline injected. This increased excretion of indolic substances roughly parallels the excretion of the adrenaline sulfoconjugate. One may argue that the large amounts of adrenaline injected are unphysiological (0.5 to 20 mg.), and that an increased protein catabolism may result in the excretion of larger amounts of indolic substances from tryptophane or tyrosine. However, simultaneous determinations of urinary urea show that this second objection is not valid (23).

In vitro, the oxidation of adrenaline to adrenochrome is catalyzed not only by catecholoxydase, the existence of which is questionable in mammalian tissues,[8] but also by the cytochrome-indophenol-oxidase system *present in all tissues* (107, 162, 164) and by a cyanide-insensitive enzymatic system present in the heart and skeletal muscles (107). Green and Richter (107) have probably been dealing with the same powerful enzymatic system described by Schütz in liver extracts (165). There is no reason to suppose that these enzymes are not active *in vivo*, and the increased excretion of indolic substances after adrenaline injection may be considered as the result of their activity. The combination of the rapid disappearance of small amounts of adrenaline in all tissues and of their long-lasting hemostatic action is thus satisfactorily explained. Clark and Raventos (167) observed a prolonged action of adrenaline on the frog's auricle after the administration of ascorbic acid. The heart muscle contains a high concentration of the cytochrome system which is inhibited by ascorbic acid (164).[9]

Bennet and Hausberger (166) have elegantly demonstrated that adrenaline is

[8] The literature on this point is confusing; many authors claim that there is no catecholoxydase in mammalian tissues (160, 161), but some believe the contrary (162). The experiments showing that tissue extracts or slices oxidize adrenaline with production of a black pigment do not prove the presence of a catecholoxydase because 1) adrenochrome may give melanin without the aid of an enzymatic system and 2) there are other enzymes which oxidize adrenaline to adrenochrome (163, 107).

[9] Beyer and Shapiro (87) do not believe that the cytochrome system oxidizes adrenaline *in vivo*, although the fact is demonstrated *in vitro*. Their main argument is that "hydroquinone, catechol and homogentisic acid, which are also oxidized readily by cytochrome *in vitro*, escape this oxidation *in vivo* and appear in the urine as such or in conjugated forms." But this fact does not exclude the possibility that a certain fraction of catechol is oxidized to quinone; catechol and other phenols have the same antimitotic action as the quinones and it is difficult to give an interpretation of this fact without assuming the formation of a certain amount of quinone in the body from these phenols (204). The possibility of several simultaneous metabolic pathways of the phenylamines must not be overlooked.

the substrate for melanin formation in the iris. In the young rabbit, sympathetic denervation of the eye results in the lack of pigmentation; thus, one can reproduce experimentally the heterochromia known in human patients to be associated with congenital absence of sympathetic innervation. If adrenaline solutions are instilled in the conjunctival sac, one succeeds not only in producing normal pigmentation but sometimes hyperpigmentation. Thus it is the adrenaline (or adrenaline-like substance) constantly liberated in the iris by the adrenergic nerves which is the substrate for the phenolase of that organ. To be sure, only a negligible fraction of adrenaline is metabolized to melanin; albino rabbits exhibit the same response and sensitivity to adrenaline as do black rabbits (168).

F. Oxidized derivatives, beyond adrenochrome, obtained in vitro. With biological tests, one can obtain the most contradictory results, because solutions of more or less "oxidized adrenaline" are mixtures of many substances in variable proportions. The best demonstration of this basic fact is the following. If one oxidizes a 1×10^{-4} solution of adrenaline at room temperature and neutral pH with a catecholoxydase, one obtains precipitate of melanin at a time when the red, clear solution still gives the physiological effects of adrenaline. Thus, in this solution all the intermediates are present together, with some of the unaltered substrate and the end-product. Under well-defined conditions, weaker solutions oxidize more uniformly.

Some authors obtained acetylcholine-like effects[10] with oxidized derivatives of adrenochrome (170, 171), but if one adds cysteine these effects are inhibited. Fresh solutions of adrenochrome and cysteine should have an adrenaline-like action. Gijon (172) states that adrenaline solutions which are autoxidizing *in vitro* have a powerful contracting effect on isolated uteri, and that this effect is inhibited by adrenaline.

The pertinent literature is very confusing and it may be hoped that Harley's recent contribution will clarify the subject. Chemically speaking, it is possible that the secondary alcohol group is oxidized to an "oxoadrenochrome" which has not been isolated (173); it has been known for a long time that *l*-adrenaline oxidizing at an alkaline pH loses its optical activity (165) which adrenochrome still keeps (107). Melanin should be a polymer of this oxoadrenochrome (174).

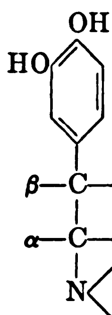
A series of investigations by Bacq and Heirman has linked the inhibitory action of adrenaline with the production of a powerful inhibitory substance beyond adrenochrome (58, 177, 178, 179, 180, 181, 182).

This concept is based on the following facts: 1) There are special pharmacological features of the inhibitory effects of adrenaline and allied substances. For example, denervation and cocaine increase only slightly the inhibitory actions of adrenaline, but potentiate markedly the excitatory actions. Ergot alkaloids, yohimbine and many synthetic adrenolytic substances inhibit specifically the excitatory actions of adrenaline; in the same series of aminomethylbenzodioxane derivatives, some compounds (1081 F or methoxy-2-iodo-5-phenoxyethyldiethyl-

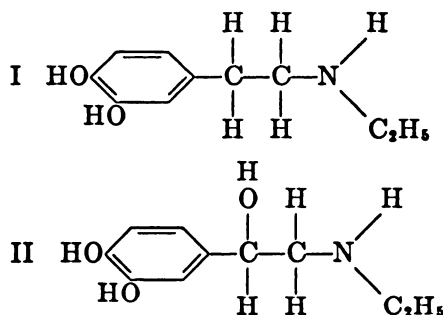
[10] A regrettable confusion of terms exists in the literature; one finds "cholinergic" or "adrenergic" instead of choline-like or adrenaline-like. According to Dale (169), who coined these words, only a nerve can be cholinergic or adrenergic.

amine, for example) abolish the inhibitory effects of adrenaline, but do not inhibit the excitatory actions (58, 176).

2) In the series of the catechol derivatives, there is a subtle correlation between chemical structure and intensity of inhibitory action. Barger and Dale (183) had demonstrated that the presence of a single methyl group on the nitrogen is a



determinant factor for true inhibitory activity (for example, on the non-pregnant cat's uterus, the pure test for inhibition[11]); noradrenaline (without a methyl group) and methedrine (with two $-CH_3$ radicals) have little inhibitory power. Recent investigations have confirmed this law, but the importance of hydroxyl group on the β-carbon of the side-chain has also been stressed (185, 186). The best demonstration is the following: N-ethyloxytyramine (I) is about twenty times less active than adrenaline on the basis of tests for excitation (nictitating membrane, blood pressure) and has almost no inhibitory actions (non-pregnant cat's uterus, blood pressure after 933 F); on the contrary, N-ethylarterenol (II), which also is much less active than adrenaline in the test for excitation, has *qualitatively* and *quantitatively* the same powerful inhibitory effects as adrenaline (186).



This must be correlated with differences in their chemical behavior: arterenol (very weak inhibitor) does not oxidize to an "arterenolochrome" corresponding

[11] It might be useful to call attention to the fact that the isolated mammalian or birds' intestine is not a pure test for inhibition because adrenaline acts on the neurones which regulate the automatism of this smooth muscle (184). A great deal of the discrepancies found in the literature as regards the inhibitory action of sympathomimetic amines is due to the use of the intestine as the test for inhibition. Cushny, Dale and Cannon have always chosen for this purpose the faithful uterus of the non-pregnant cat.

to adrenochrome (187) and does not give the marked fluorescence of adrenaline after addition of NaOH (117). The same phenomenon occurs with the N-ethyl derivatives: substance II gives the Gaddum and Schild fluorescence test, like adrenaline; substance I behaves like arterenol. It is clear from the investigations of Cohen (173, 174) and Harley-Mason (114) that the secondary alcohol group does play an important rôle in the oxidation and reduction of adrenochrome.

3) When an isolated amphibian heart is impregnated with a phenolase preparation, the excitatory reaction to adrenaline is changed to inhibition (177) and sympathetic nerve excitation decreases the rate and weakens the strength of the heart beat (188, 189); these reversed effects are progressively changed to normal when the phenolase is washed out.

4) Dilute solutions of adrenaline at neutral pH when oxidized *in vitro* by a phenolase, irregularly exhibit, after an initial phase of complete inactivation, a very powerful inhibitory action on the heart, blood vessels (179) and various smooth muscles (182, 190, 191). This inhibitory substance, called "adrenoxine", could not be isolated because it decomposes on concentration and is not produced from strong solutions of adrenaline. Many physiologists failed to reproduce Bacq and Heirman's experiments (172, 192, 164), but some have confirmed them at least partially (192, 193, 194, 195, 196). Older observations of Schweitzer, with the omega substance (198), are quite consistent with the results of Heirman. Marquardt (197) quite frequently observed the depressor action of oxidized adrenaline and believes that it is a general property of phenols and indole. Starkenstein (199) has described a curious "Sauerstoffphänomen"; the inhibitory action of adrenaline on isolated intestine is abolished by lack of oxygen.

In 1940, Bacq and Heirman (58) summarized the facts in favor of and the objections to the adrenoxine theory.

VIII. Other Possible Mechanisms of Inactivation. Aldehydes (formaldehyde, acetaldehyde or methylglyoxal) which inactivate adrenaline *in vitro* (18, 19, 20) do not seem, for many reasons, to take part in its inactivation *in vivo* (21); but this opinion has been contested (4). Nothing indicates that the ring of adrenaline cannot be broken just as that of tyramine seems to be broken by heart and skeletal muscle tissue (104).

SUMMARY

The statement formulated in 1905 by Elliott (5) that "adrenalin disappears in the tissues which it excites" is unquestionable. Review of the literature shows 1) that adrenaline may be excreted unchanged in small amounts by the kidneys and stored in the tissues and red blood cells; 2) that its deamination by amine-oxidase in the body is unlikely; 3) that an important fraction is sulfoconjugated; 4) that another important fraction is simultaneously oxidized to indole substance; 5) that adrenochrome and its derivatives have important biochemical and physiological properties, entirely different from those of adrenaline, and deserve further study.

ADDENDUM*

The presence of arterenol (noradrenaline) in some adrenal extracts (206, 208, 209), in adrenal medullary tumors (207) and in the secretion of the adrenals stimulated by the splanchnics (206) seems well established. The suggestion of Bacq and Fischer (10) that the tissues synthesize a mixture in variable proportions of adrenaline and noradrenaline is discussed by Goldenberg *et al.* (211). U. S. von Euler and his pupils have actively continued their work on adrenaline and noradrenaline in tissue extracts (213 to 217). A method is given for purification and titration of noradrenaline in presence of adrenaline (213); a paper on the same subject by Gaddum, Peart and Vogt is in press in the Journ. Physiol.

Further data have been published on adrenochrome, its derivatives and adrenaline ascorbate (212). B. Kisch has summarized the pre-war work on the catalytic effects of oxidized adrenaline (205). Herve and Lecomte have shown that the semicarbazone of adrenochrome (*adrenoxyl* Labaz) inhibits in the mouse the cutaneous purpura which follows a heavy dose of X-ray (218).

Fischer (219) has isolated, in confirmation of Harley-Mason's statement, a crystalline, yellow, highly fluorescent substance (M.P. 235° C.) from adrenochrome in alkaline medium. This substance (formula VI, p. 15) seems to be responsible for the fluorescence of adrenaline in alkaline solution. Many experiments of Utevsky have been confirmed. The relation between fluorescence and inhibitory action of catecholamines has been strengthened (220, 221).

REFERENCES

1. Bernheim, F.: The interaction of drugs and cell catalysts, Burgess Publishing Co., Minneapolis (1942).
2. Beyer, K. H.: Sympathomimetic amines: the relation of structure to their action and inactivation, Physiol. Rev., **26**: 169, 1946.
3. Bacq, Z. M.: Métabolisme et synthèse de l'adrénaline, Bull. Acad. Méd. Suisse, **4**: 127, 1948.

4a. Malafaya Baptista, A.: Inactivacão da adrenalina no organismo, Thesis, Lisbon, 1938.

4b. Hartung, W. H.: Annual review of Biochemistry, **15**: 593, 1946.

5. Elliott, T. R.: Journ. Physiol. **32**: 401, 1905.
6. Euler, U. S., von: Journ. Physiol., **105**: 38, 1946.
7. Euler, U. S., von: Acta Physiol. Scand., **11**: 168, 1946 and **12**: 73, 1946.
8. Schmiterlöw, C. G.: Acta Physiol. Scand., **15**: 47, 1948.
9. Euler, U. S., von: Science, **107**: 422, 1948.
10. Bacq, Z. M., and Fischer, P.: Arch. Internat. Physiol., **55**: 73, 1947.
11. Bacq, Z. M.: Science, **108**: 135, 1948.
12. Gaddum, J. H., and Goodwin, L. G.: Journ. Physiol., **105**: 357, 1947.
13. Tainter, M. L., Tullar, B. F., and Luduena, F. P.: Science, **107**: 39, 1948.
14. Verly, W.: Arch. Internat. Physiol., **55**: 409, 1948.
15. Cannon, W. B., and Rosenblueth, A.: Amer. Journ. Physiol., **104**: 557, 1933.
16. Cannon, W. B., and Bacq, Z. M.: Amer. Journ. Physiol., **96**: 392, 1931.
17. Bacq, Z. M.: Biol. Rev., **22**: 73, 1947.
18. Bayer, G., and Wense, Th.: Arch. exper. Path. Pharm., **188**: 114, 1937.
19. Toscano, Rico J., and Malafaya, Baptista A.: Compt. rend. Soc. Biol., **120**: 545, 1935.
20. Wense, Th.: Arch. exper. Path. Pharmakol., **191**: 358, 1939.
21. Bacq, Z. M.: Arch. Internat. Physiol., **46**: 125, 1938.
22. Bacq, Z. M., et Fredericq, H.: Arch. Internat. Physiol., **40**: 454, 1935.
23. Bacq, Z. M., Lecomte, J., and Fischer, P.: Arch. Internat. Physiol., 1949, in press.
24. Bacq, Z. M.: Arch. Internat. Physiol., **44**: 15, 1936.
25. Bain, W. A., Gaunt, W. E., and Suffolk, S. F.: Journ. Physiol., **91**: 233, 1937.
26. Verly, W.: Arch. Internat. Physiol., **56**: 1, 1948.
27. Bacq, Z. M., Fischer, P., and Lecomte, J.: Arch. Internat. Physiol., **56**: 25, 1948.
28. Loewi, O.: Pflüger's Arch., **237**: 504, 1936.

* Added in galley.

29. CANNON, W. B., AND LISSAK, K.: Amer. Journ. Physiol., 125: 765, 1939.
30. SHAW, F. H.: Biochem. Journ., 32: 19,1938.
31. RAAB, W.: Biochem. Journ., 37: 470, 1943.
32. RAAB, W., AND HUMPHREYS, R. J.: Amer. Journ. Physiol., 148: 460, 1947.
33. BACQ, Z. M.: Arch. Internat. Physiol., 36: 167, 1933.
34. RAAB, W., AND MAES, J. P.: Amer. Journ. Physiol., 148: 470, 1947.
35. ENGELHART, E.: Pflüger's Arch., 227: 220, 1931.
36. CHANG, H. C., AND GADDUM, J. H.: Journ. Physiol., 79: 255, 1933.
37. MACINTOSH, F. C.: Arch. Internat. Physiol., 47: 321, 1938.
38. RAAB, W., AND HUMPHREYS, R. J.: Journ. Pharm. exper. Ther., 88: 268, 1946.
39. BÜLBRING, E., BURN, J. H., AND DE ELIO, F. J.: Journ. Physiol., 107: 222, 1948.
40. WEST, G. B.: Journ. Physiol., 106: 418, 1947.
41. VERLY, W.: Arch. Internat. Physiol., 55: 397, 1948.
42. JAMES, W. O.: Nature, 161: 851, 1948.
43. KALAJA, L., AND SAVOLAINEN, H.: Nord. Med., 12: 3562, 1941.
44. LEHMAN, G., AND MICHAELIS, H. F.: Arbeitsphysiol., 12: 52 and 265, 1942.
45. JØRGENSEN, K. S.: Acta Pharmacol. et Toxicol. (Copenhague), 1: 225, 1945.
46. PORAT, B., VON, Acta Med. scandin., 123: 317, 1946.
47. RAAB, W.: Amer. Heart Journ., 33: 707, 1947.
48. TRENDELENBURG, P.: Die Hormone, Springer, Berlin, 1929.
49. BACQ, Z. M.: Arch. Internat. Physiol., 44: 15, 1936.
50. WEST, G. B.: Journ. Physiol., 106: 426, 1947.
51. MACKENZIE, C. G., CHANDLER, J. P., KELLER, E. B., RACHELE, J. R., CROSS, N., MELVILLE, D. B., AND DU VIGNEAUD V.: Journ. Biol. Chem., 169: 757, 1947.
52. KRÜGER, M., AND SCHMIDT, J.: Arch. exp. Path. Pharm., 45: 259, 1901.
53. VIGNEAUD, V. DU, CHANDLER, J. P., SIMMONDS, S., MAYER, A. W., AND COHN, M.: Journ. Biol. Chem., 164: 603, 1946.
54. BLASCHKO, H., RICHTER, D., AND SCHLOSSMANN, H.: Journ. Physiol., 90: 1, 1937.
55. RICHTER, D: Bioch. Journ., 31: 2022, 1937.
56. HOLTZ, P., AND HEISE, R: Arch. exper. Path. Pharm., 190: 178, 1938.
57. HOLTZ, P., REINGOLD, A., AND CREDNER, K.: Zeitsch. Physiol. Chem., 261: 278, 1939.
58. BACQ, Z. M., AND HEIRMAN, P.: Arch. Internat. Physiol., 50: 153, 1940.
59. BLASCHKO, H., RICHTER, D., AND SCHLOSSMANN, H.: Bioch. Journ., 31: 2187,1937.
60. ORZECHOWSKI, G.: Arch. exper. Path. Pharm., 198: 27, 1941.
61. HARE, M. L. C.: Bioch. Journ., 22: 968, 1928.
62. PUGH, C. E. M., AND QUASTEL, J. H.: Bioch. Journ., 31: 286 and 2036, 1937.
63. EWINS, A. J., AND LAIDLAW, P. P.: Journ. Physiol., 41: 78, 1910.
64. GUGGENHEIM, M., AND LÖFFLER, W.: Bioch. Zeitsch., 72: 325, 1915.
65. RICHTER, D.: Bioch. Journ., 32: 1763, 1938.
66. GADDUM, J. H., AND KWIATKOWSKI, H.: Journ. Physiol., 94: 87, 1938 and 96: 385, 1939.
67. RICHTER, D., AND TINGEY, A. H.: Journ. Physiol., 97: 265, 1939.
68. SCHAPIRA, G.: Compt. rend. Soc. Biol., 139: 36, 1945.
69. KOHN, H. I.: Biochem. Journ., 31: 1693, 1937.
70. MARKOWITZ, J., AND MANN, F. C.: Amer. Journ. Physiol., 89: 176, 1929.
71. BACQ, Z. M.: Arch. Internat. Physiol., 45: 1, 1937.
72. BACQ, Z. M.: Arch. Internat. Physiol., 44: 112, 1936.
73. PETRILLO, L. M., AND BACQ, Z. M.: Compt. rend. Soc. Biol., 141: 839, 1947.
74. BACQ, Z. M.: Arch. Internat. Pharm. Thér., 55: 190, 1937.
75. TRIPOD, J.: Journ. Physiol., 97: 289, 1940.
76. MACGREGOR, D. F.: Journ. Pharmacol. exper Ther., 66: 393, 1939.
77. PHILPOT, F. J.: Journ. Physiol., 97: 301, 1940.
78. BACQ, Z. M., AND LEFREBVRE, F.: Arch. Internat. Pharm. Thér., 49: 363, 1935.
79. WEINSTEIN, S. S., AND MANNING, R. I.: Science, 86: 19, 1937.
80. RICHTER, D.: Journ. Physiol., 98: 361, 1940.
81. FLORKIN, M., AND BACQ, Z. M.: Arch. Internat. Physiol., 53: 247, 1943.
82. WILLIAMS, R. T.: Detoxication mechanisms, Chapman and Hall, London, 1947.
83. THIERFELDER, H., AND SHERWIN, C. P.: Zeitsch. physiol. Chem., 94: 1, 1915.
84. PREUSSE, C.: Zeitsch. physiol. Chem., 2: 324, 1878.
85. BACQ, Z. M.: Annales Physiol., 10: 467, 1934.
86. RICHTER, D., AND MACINTOSH, F. C.: Amer. Journ. Physiol., 135: 1, 1941.
87. BEYER, K. H., AND SHAPIRO, S. H.: Amer. Journ. Physiol., 144: 321, 1945.
88. DEICHMANN, W. B.: Proc. Soc. exper. Biol. Med., 54: 335, 1943.
89. ARNOLT, R. I., AND DEMEIO, R. H.: Rev. Soc. Argent. Biol., 17: 570, 1941.
90. MARENZI, A. D.: Compt. rend. Soc. Biol., 107: 737 and 743, 1931.
91. BARAC, G.: Arch. Internat. Pharm. Thér., 56: 13, 1937.
92. LOEPER, M., LOEPER, J., LEMAIRE, J., COTTET, A., AND PARROT, J.: Compt. rend. Soc. Biol., 128: 1050, 1938.
93. BACQ, Z. M., LECOMTE, J., AND FISCHER, P.: Arch. Internat. Physiol., 1949, in press.

94. HOLTZ, P., CREDNER, K., AND KRONENBERG, G.: Arch. exper. Path. Pharmakol., 204: 228, 1947.
95. TORDA, C.: Journ. Pharmacol. exper. Ther., 77: 123, 1943.
96. TORDA, C.: Journ. Pharmacol. exper. Ther., 77: 274, 1943.
97. BACQ, Z. M.: Arch. Internat. Physiol., 42: 340, 1936.
98. ABDERHALDEN, E., AND GELLHORN, E.: Pflüger's Archiv., 203: 42, 1924.
99. EKERFORS, H.: Compt. rend. Soc. Biol., 93: 1162, 1925.
100. DE CARO, L.: Zeitsch. physiol. Chem., 219: 257, 1933.
101. MACKAY, SAWYER M. E., AND BROWN, M. G.: Amer. Journ. physiol., 110: 620, 1933.
102. DE VISSCHER, M.: La régulation hormonale du métabolisme et la vitamine A, Masson, Paris, 1946.
103. WELSCH, A. D.: Amer. Journ. Physiol., 108: 360, 1934.
104. BERNHEIM F., AND BERNHEIM, M. L. C.: Journ. Biol. Chem., 153: 369, 1944.
105. GUGGENHEIM, M.: Die biogenen Amine, Karger, Basel, 1940.
106. RAPER, H. S.: Biochem. Journ., 21: 89, 1927.
107. GREEN, D. E., AND RICHTER, D.: Biochem. Journ., 31: 596, 1937.
108. DEROUAUX, G.: Arch. Internat. Pharmac. Thér., 69: 205, 1943.
109. POLONOVSKI, M.: Biochimie Médicale, Masson, Paris, 1948.
110. BALL, E. G., AND CHEN, T. T.: Journ. Biol. Chem., 102: 691, 1933.
111. MARQUARDT, P.: Enzymologia, 12: 166, 1947.
112. OUTEVSKY, A. M.: Nouvelles biologiques soviétiques, 18: 145, 1944.
113. GIJON, J. R.: Trabajos Inst. Cajal, 3: 73, 1945.
114. HARLEY-MASON, J.: Experientia, 4: 307, 1948.
115. VEER, W. L. C.: Recueil trav. chim. Pays-Bas, 61: 638, 1942.
116. BRACONIER, F., LE BIHAN, H., AND BEAUDET, C.: Arch. Internat. Pharm. Thér., 69: 181, 1943.
117. GADDUM, J. H., AND SCHILD, H.: Journ. Physiol., 80: 9, 1934.
118. PARROT, J. L.: Compt. rend. Soc. Biol., 142: 631, 1948.
119. BACQ, Z. M., LECOMTE, J., AND BEAUDET, C.: Unpublished observations, 1947-1948.
120. KISCH, B., AND COLL.: Numerous papers in Bioch. Zeitsch., 220 to 271: 1930 to 1934.
121. CAHEN, A.: Compt. rend. Soc. Biol., 139: 22, 1945.
122. FRIEDHEIM, E. A. H.: Compt. rend. Soc. Phys. Hist. Nat. Genève, 50: 20, 1933; Bioch. Zeitsch., 259: 257, 1933; Schweitz. Med. Wochensch., 65: 256, 1935.
123. EVANS, W. C., AND RAPER, H. S.: Biochem. Journ., 31: 2162, 1937.
124. BLIX, G.: Skand. Archiv. Physiol., 56: 131, 1929.
125. WAJZER, J.: Bull. Soc. Chim. Biol., 28: 341, 1946.
126. DEROUAUX, G.: Arch. Internat. Pharmac. Thér., 66: 202, 1941.
127. DEROUAUX, G.: Arch. Internat. Pharmac. Thér., 69: 142, 1943.
128. ROSKAM, J., DEROUAUX, G., MEYS, L., AND SWALUÉ, L.: Arch. Internat. Pharmac. Thér., 74: 162, 1947.
129. ROSKAM, J., AND DEROUAUX, G.: Arch. Internat. Pharmac. Thér., 69: 12, 1943.
130. BACQ, Z. M.: Presse Médicale, 175, March 15, 1947.
131. BACQ, Z. M.: Compt. rend. Soc. Biol., 141: 536, 1947.
132. PARROT, J. L., AND LAVOLLAY, J.: Compt. rend. Acad. Sciences, 218: 211, 1944.
133. PREVOST, H., COTEREAU, H., AND PARROT, J. L.: Compt. rend. Soc. Biol., 141: 1043, 1947.
134. PARROT, J. L., AND COTEREAU, H.: Compt. rend. Soc. Biol., 139: 902, 1945.
135. JAVILLIER, M., AND LAVOLLAY, J.: Helvetica Chimica Acta, 29: 1283, 1946.
136. ROSKAM, J., AND DEROUAUX, G.: Bull. Acad. Roy. Méd. Belg., 6th series, 10: 68, 1945.
137. WAJZER, J.: Bull. Soc. Chim. Biol., 28: 345, 1946.
138. MEYERHOF, O., AND RANDALL, L. O.: Archiv. Biochem., 17: 171, 1948.
139. LECOMTE, J., AND FISCHER, P.: Arch. Internat. Physiol., 56: 35, 1948.
140. GOFFART, M.: Compt. rend. Soc. Biol., in press, 1949.
141. LETTRÉ, H.: Chemiker Zeitung, 67: 52, 1943.
142. LETTRÉ, H., AND ALBRECHT, M.: Zeitsch. Physiol. Chem., 271: 200, 1941.
143. BACQ, Z. M.: Experientia, 2: 349 and 385, 1946.
144. SOLOWAY, S., AND OSTER, K. A.: Proc. Soc. exper. Biol. Med., 50: 108, 1942.
145. OSTER, K. A., AND SOBOTKA, H.: Journ. Pharmacol. exp. Ther., 78: 100, 1943.
146. QUIVY, D., AND CLUNY, L.: Compt. rend. Soc. Biol., 141: 1173, 1947.
147. HALSE, TH., AND MARQUARDT, P.: Enzymologia, 12: 246, 1948.
148. MARTIN, F. N.: Proc. Soc. exper. Biol. Med., 59: 1, 1945.
149. SNYDER, F. H., LEVA, L., AND OBERST, F. W.: Journ. Amer. Pharm. Assoc., 36: 253, 1947.
150. WAJZER, J.: Bull. Soc. Chim. Biol., 29: 237, 1947.
151. CALDEYRO-BARCIA, R.: Arch. Soc. Biol. Montevideo, 13: 183, 1946.
152. NEUBERGER, A.: Annual reports of Chem. Soc., 41: 237, 1944.
153. BLASCHKO, H.: Journ. Physiol., 101: 337, 1942.
154. POLONOVSKI, M., GONNARD, P., AND PELOU, A.: Bull. Soc. Chim. Biol., 26: 449, 1944.
155. VINET, A.: Bull. Soc. Chim. Biol., 22: 559, 1940.
156. HOLTZ, P., CREDNER, K., AND LUEDTKE, K.: Archiv. exper. Path. Pharmakol., 191: 87, 1938.
157. GURIN, S., AND DELLUVA, A. M.: Journ. Biol. Chem., 170: 545, 1947.
158. LECOMTE, J., AND FISCHER, P.: Arch. Internat. Physiol., 1949, in press.
159. NIHOUL, E., LECOMTE, J., AND FISCHER, P.: Compt. rend. Soc. Biol., 1949, in press.
160. BHAGVAT, K., AND RICHTER, D.: Bioch. Journ., 32: 1397, 1938.

161. Duchateau-Bosson, and Florkin, M.: Compt. rend. Soc. Biol., **132:** 47, 1939.
162. Keilin, D., and Hartree, E. F.: Proc. Roy. Soc. B., **119:** 114, 1936.
163. Keilin, D., and Hartree, E. F.: Proc. Roy. Soc. B., **125:** 171, 1938.
164. Blaschko, H., and Schlossmann, H.: Journ. Physiol., **98:** 130, 1940.
165. Schütz, F.: Bioch. Zeitsch., **265:** 282, 1933.
166. Bennett, G. A., and Hausberger, F. X.: Archiv. exper. Path. Pharmak., **188:** 40, 1938.
167. Clark, A. J., and Raventos, J.: Quart. Journ. exper. Physiol., **29:** 159, 1939.
168. Heirman, P.: Compt. rend. Soc. Biol., **126:** 1267, 1937.
169. Dale, H. H.: Journ. Physiol., **80:** 10 P., 1933.
170. Minz, B., and Plotka, C.: Compt. rend. Acad. Sciences, **225:** 697, 1947.
171. Plotka, C., and Jequier, R.: Compt. rend. Soc. Biol., **141:** 1190 and 1191, 1947.
172. Gijon, J. R.: Trab. Instit. Cajal., **3:** 193, 1945.
173. Cohen, G. N.: Bull. Soc. Chim. Biol., **28:** 104, 1946.
174. Cohen, G. N.: Bull. Soc. Chim. Biol., **28:** 107, 1946.
175. Friedmann, E., Marrian, D. H., and Simon-Reuss, I.: Brit. Journ. Pharmacol. Chemoth., **3:** 263, 1948.
176. Bovet, D., and Simon, A.: Arch. Internat. Pharmac. Thér., **55:** 223, 1937.
177. Heirman, P.: Arch. Internat. Physiol., **46:** 404, 1938.
178. Heirman, P.: Arch. Internat. Physiol., **49:** 449, 1939.
179. Heirman, P., and Bacq, Z. M.: Arch. Internat. Physiol., **50:** 100, 1940.
180. Heirman, P.: Arch. Internat. Physiol., **50:** 115, 1940.
181. Bacq, Z. M., and Heirman, P.: Arch. Internat. Physiol., **50:** 129, 1940.
182. Bacq, Z. M.: Arch. Internat. Physiol., **50:** 141, 1940.
183. Barger, G., and Dale, H. H.: Journ. Physiol., **41:** 19, 1910.
184. Nolf, P.: Mélanges J. Demoor, Masson, Paris, 1937, p. 378.
185. Marsh, D. F., Pelletier, M. H., and Ross, C. A.: Journ. Pharmac. exper. Ther., **92:** 108, 1948.
186. Bacq, Z. M., Lecomte, J., and Fischer, P.: Arch. Internat. Physiol., in press, 1949.
187. Beaudet, C.: Personal communication, 1948.
188. Bacq, Z. M.: Arch. Internat. Physiol., **46:** 417, 1938.
189. Caldeyro-Barcia, R.: Arch. Soc. Biol. Montevideo, **13:** 67, 1946.
190. Goffart, M.: Compt. rend. Soc. Biol., **130:** 1372, 1939.
191. Heirman, P., and Goffart, M.: Compt. rend. Soc. Biol., **132:** 84, 1939.
192. Lissak, K.: Science, **87:** 371, 1938.
193. Sanders, E.: Arch. exper. Path. Pharm., **193:** 572, 1939.
194. Veil, C.: Compt. rend. Soc. Biol., **132:** 860, 1939.
195. Ungar, G., and Parrot, J. L.: Compt. rend. Soc. Biol., **131:** 62 and 1165, 1939.
196. Parrot, J. L., and Verliac, F.: Compt. rend. Soc. Biol., **136:** 323, 1942.
197. Marquardt, P.: Enzymologia, **12:** 166, 1947.
198. Schweitzer, A.: Pflüger's Archiv, **228:** 268, 1931.
199. Starkenstein, E.: Arch. Internat. Pharm. Thér., **65:** 423, 1941.
200. Seitz, W.: Zeitschr. exper. Med., **105:** 559, 1939.
201. Dodson, K. S., Garton, G. A., and Williams, T. R.: Bioch. Journ., **41:** L, 1947.
202. Lipschitz, W. L., and Bueding, E.: Journ. Biol. Chem., **129:** 333, 1939.
203. Marquardt, P., and Oettel, H.: Arch. Internat. Pharm. Thér., **77:** 160, 1948.
204. Dustin, P.: Compt. rend. Soc. Biol., 1949, in press.
205. Kisch, B.: Exper. Med. Surg. **5:** 70, 1947.
206. Bülbring, E., and Burn, J. H.: Nature, **163:** 363, 1949.
207. Holton, P.: Nature, **163:** 217, 1949.
208. Holtz, P., and Schümann, H. J.: Naturwiss., **35:** 159, 1948.
209. Holtz, P., Schümann, H. J., Langenbeck, W., and Le Blanc, H.: Naturwiss., **35:** 191, 1948.
210. Holtz, P., and Schümann, H. J.: Arch. exper. Path. Pharm., 1949, in press.
211. Goldenberg, M., Pines, K. L., Baldwin, E. de F., Greene, D. G., and Roh, C. E.: Amer. Journ. Med., **5:** 792, 1948.
212. Beauvillain, A., and Sarradin, J.: Bull. Soc. Chim. Biol., **30:** 472 and 478, 1948.
213. Euler, U. S. von: Arch. Internat. Pharm. Thér., **77:** 477, 1948.
214. Euler, U. S. von: Schweitz. Med. Wochschr., **78:** 777, 1948.
215. Euler, U. S. von: Nature, **162:** 570, 1948.
216. Euler, U. S. von: Acta Physiol. Scand., **16:** 63, 1948.
217. Schmiterlöw, C. G.: Acta Physiol. Scand., **16:** suppl. 56, 1948.
218. Herve, A., and Lecomte, J.: Arch. Intern. Pharm. Thér., 1949, in press.
219. Fischer, P.: Unpublished observations.
220. Bacq, Z. M., and Lecomte, J.: Arch. Intern. Pharm. Thér., **78:** 369, 1949.
221. Bacq, Z. M., and Fischer, P.: Compt. rend. Soc. Biol., 1949, in press.