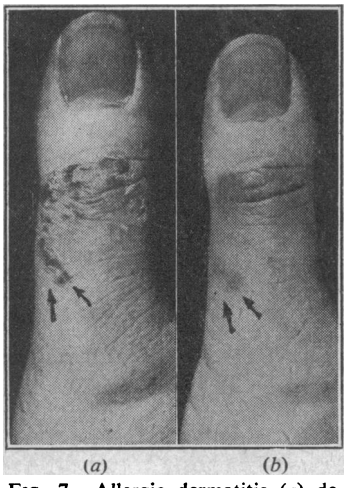
improvement after applying citrate for four days and complete disappearance of the skin lesions in less than a week. A man who had developed an extremely severe dermatitis extending over both arms and thighs on handling insecticides was advised to apply the citrate lotion intermittently. When he started this treatment the skin lesions were heavily contaminated by scratching and had a bullous erythematous aspect. After one week's application of the citrate solution subsidence of the skin lesions was almost complete, and after two weeks only a few small scattered bullae could be seen in the wrist and cheeks.

Some other minor cases of allergic dermatitis were completely relieved after a few days' application of the 3.8% solution of trisodium citrate. I had also the opportunity of applying the citrate therapy to an allergic dermatitis that had developed in my own thumb, following all the steps of citrate action. The thumb presented extensive bullae (indicated by arrows in Fig. 7*a*), as well as extensive desquamation extending over an area of 4 to 5 cm.² These lesions were the result of a slow process that took about three months to reach the stage presented in Fig. 7*a*. After applying the citrate lotion for three days a definite improvement could be observed. At the points where the process was developing more acutely, small haemorrhages took place, followed by an almost immediate relief of the subjective and objective symptoms. Instead of itching, a rather reassuring burning sensation was felt. In one week the whole process had completely stopped and cicatrization of the bullae was apparent. Erythrodermia was intensive, this probably being explained by the fact that the skin was much thinner at the region where the intensive desquamation took place before. Fig. 7*b*, showing only slight erythrodermia, was taken 14 days after the start of citrate therapy. Two cases of severe facial acne, as well as some other minor cases of possible skin allergy, were relieved by the local application of an isotonic solution of trisodium citrate.



(*a*) (*b*)

FIG. 7.—Allergic dermatitis (*a*) developed in three months, apparently as the consequence of the contact with laboratory animals; (*b*) same 14 days after the intermittent application of an isotonic solution of sodium citrate (3.8%). The arrows indicate a deep bullous process in (*a*) and its cicatricial spot in (*b*).

Conclusion

Much more work should be done by experienced dermatologists and allergists in extending the use of this citrate therapy, not only locally but as sprays, to cases of asthma and to other manifestations of allergy. The idea of employing citrate resulted from the experimental evidence of its potent effect as an inhibitory agent in the process of histamine release. Its therapeutic effect in allergic dermatitis is further evidence in favour of the participation of the factor of histamine release present in normal blood in an inactive state—a state that can be changed into an active one by treatment with the antigen–antibody complex, agar, inulin, starch, etc.—in the so-called anaphylatoxin or serotoxin. Since citrate appears to stop the whole process of activation it would constitute a potentially more efficient instrument than symptom-relieving agents such as the antihistamines. Future research and clinical trials will decide whether the credit here given to citrate is justified.

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THE ENZYME AT SYMPATHETIC NERVE ENDINGS*

BY

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At the endings of the vagus and of other nerves, where acetylcholine is liberated to transmit the impulse to the end organ, the enzyme cholinesterase is found. The importance of cholinesterase in controlling the response to the nerve impulse is well known from the action of neostigmine. Knowledge of the working of sympathetic nerves has been much behind that of cholinergic nerves until recently, when it received a great impetus from the discovery of *nor*adrenaline in sympathetic nerves by Von Euler (1946). Other workers, first Peart (1949), and later Mann and West (1950, 1951), have shown that *nor*adrenaline is released when sympathetic nerves are stimulated.

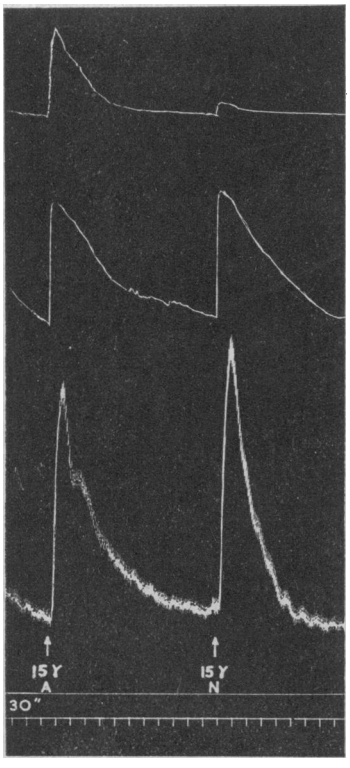
*Nor*adrenaline being established as the transmitter, the question arises what enzyme destroys it. Evidence has been accumulating that it is amine oxidase, an enzyme discovered by Hare (1928). Attention was first drawn to amine oxidase as an enzyme concerned with the destruction of adrenaline by the work of Blaschko, Richter, and Schlossmann (1937) on the one hand, and by the work of Gaddum and Kwiatkowski (1938) on the other. The former showed that adrenaline, among other amines, was a substrate of the enzyme, but that ephedrine was not, and that ephedrine indeed inhibited the action of the enzyme on adrenaline. Gaddum and Kwiatkowski demonstrated that ephedrine potentiated the action of adrenaline in causing constriction of the vessels of the rabbit ear, and they suggested that this

*A lecture delivered at Cornell Medical College on April 23, 1951.

potentiation could be explained if amine oxidase were present in the vessels, and if its action there on adrenaline was inhibited by ephedrine.

A *Nor*adrenaline Hypothesis

So the matter rested until 1949, when an approach was made from a different direction. In work on the nictitating membrane of the cat's eye Bülbring and Burn (1949) found that *nor*adrenaline was much weaker in causing contraction than adrenaline, though if the membrane was denervated both *nor*adrenaline and adrenaline were of similar and greater potency, as shown in the accompanying illustration.



Record taken from spinal cat. The upper tracing shows contractions produced in the normal nictitating membrane. The middle tracing shows contractions in the denervated nictitating membrane. The lower record is the blood pressure. The first injection is 15 μg. of adrenaline. The second injection is 15 μg. of *nor*adrenaline.

Burn and Hutcheon (1949) observed that the same relations existed for dilatation of the pupil. *Nor*adrenaline when injected intravenously had much less effect than adrenaline in dilating the normal pupil: a dose of *nor*adrenaline 12–15 times as great was necessary to produce the same effect as a dose of adrenaline. The denervated iris, however, which was more sensitive than the normal iris to adrenaline, responded to *nor*adrenaline very much as it did to adrenaline.

On the blood vessels of the rabbit ear the constrictor effect of *nor*adrenaline was shown by Luduena *et al.* (1949) and by Gaddum *et al.* (1949) to be weaker than that of adrenaline. Burn and Robinson (1951a) compared the effect of *nor*adrenaline and adrenaline in 29 experiments and obtained a mean figure of 5.9 as the amount of *nor*adrenaline which was required to produce the same constriction as a unit amount of adrenaline.

Thus in all three tissues—the nictitating membrane, the iris, and the rabbit ear vessels—*nor*adrenaline was found to be much weaker than adrenaline, a conclusion which was surprising, since *nor*adrenaline was known to be the sympathetic transmitter, and therefore likely to be at least as potent as adrenaline, if not more potent. The fact that after denervation of the nictitating membrane and iris this weakness of *nor*adrenaline largely disappeared suggested that the weakness was only apparent. Burn and Hutcheon therefore supposed that there might be an enzyme at the sympathetic nerve ending which decreased in amount after denervation; it is known, for example, that cholinesterase diminishes in the superior cervical ganglion after the preganglionic fibres have been cut (Brücke, 1937). This enzyme at the sympathetic nerve ending, being presumably designed to destroy the transmitter *nor*adrenaline, might have a greater affinity for it than for adrenaline, and, if so, might destroy *nor*adrenaline given by intravenous injection more readily than adrenaline. The presence of the enzyme might thus create the appearance that *nor*adrenaline was a much weaker substance.

Amine Oxidase

The foregoing hypothesis has now been tested in various ways, and the results have supported it. The first question which arose was the identity of the enzyme. In view of the work of Blaschko and of Gaddum, already described, it was natural to think of amine oxidase. This idea was given strong support when Thompson and Tickner (1951) published their paper showing that this enzyme is present in all the blood vessels of the rabbit. Miss Robinson (1951) then showed that amine oxidase is also present in the nictitating membrane and in the iris of the cat.

The second question was whether amine oxidase destroys *nor*adrenaline more rapidly than it destroys adrenaline. The earlier observations of Blaschko, Richter, and Schlossmann (1937) did not suggest this. The rate of oxygen uptake measured in the Warburg apparatus when amine oxidase acted on adrenaline was similar to the rate when it acted on *nor*adrenaline. Accordingly, experiments were carried out in which amine oxidase was allowed to act on a mixture of equal amounts of adrenaline and *nor*adrenaline, and the proportion of the two amines in the mixture was determined as the oxidation continued. It was found that *nor*adrenaline disappeared more rapidly than adrenaline and that the percentage of adrenaline in the unoxidized portion of the mixture steadily rose (Blaschko and Burn, 1951; Burn and Robinson, 1951). Thus evidence was obtained that amine oxidase destroys *nor*adrenaline more rapidly, as the hypothesis required. Recently Bain and Batty (1952) have made a careful statistical study of the rate of oxidation of adrenaline and of *nor*adrenaline (separately) in blood in the presence of liver slices. They found that, in these conditions also, the rate of oxidation of *nor*adrenaline is more rapid than that of adrenaline, and their findings have enabled them to calculate what changes would occur during the oxidation of a mixture of equal parts of the two substances. The calculation so obtained agreed fairly well with the results already described.

Effect of Denervation

The next step in the investigation was to test the effect of denervation on the amount of amine oxidase, to see if the amount declined. Such a decline would explain the greater effect of adrenaline and the much greater effect of *nor*adrenaline in the denervated nictitating membrane and iris.

Denervation of the nictitating membrane and iris was effected by removing the right superior cervical ganglion. Denervation of vessels was effected by removing the right stellate ganglion, which supplied the sympathetic fibres to the vessels of the foreleg. These operations were performed aseptically at various intervals before the cats were killed. The changes in the nictitating membrane were examined in 57 cats, and the mean results are shown in Table I. During

TABLE I.—*Amine Oxidase in Denervated Nictitating Membrane as Percentage of Normal*

Period of Denervation	No. of Cats	Mean Amount of Amine Oxidase
8–9 days	9	71
10–12 ,, ...	7	66·5
16–19 ,, ...	20	73
21 ,, ...	12	88
25–28 ,, ...	7	92
33 ,, ...	2	101

the first period, up to 10–12 days after denervation, the amine oxidase fell to a mean figure of 66% of the normal. Thereafter the amine oxidase rose steadily, to regain the normal figure in four to five weeks.

There was, however, much variation in the fall in the amine oxidase in different cats. In some it fell to 40% or less, while in others there was little or no fall. At first it seemed that the variation was so great that the increased sensitivity of the denervated membrane to *nor*adrenaline could not be accounted for by the fall in amine oxidase. A

comparison was therefore made of the increase in sensitivity of the denervated membrane with the fall in amine oxidase. The increase was found to be significantly correlated with the fall in amine oxidase in 10 cats examined within 8–12 days after denervation (Burn and Robinson, 1952).

For the periods after denervation longer than 12 days the correlation between increased sensitivity of the membrane to *nor*adrenaline and fall in amine oxidase was not significant. The increased sensitivity of the denervated membrane persisted (though it declined), while the amine oxidase returned towards normal.

In the blood vessels, also, a loss of amine oxidase after denervation was observed; the results were on the whole similar to those in the nictitating membrane in suggesting that the loss of amine oxidase occurred during the early days of denervation, and that the amine oxidase was then gradually restored. The results are shown in Table II. In

TABLE II.—*Percentage of Amine Oxidase in Vessels of Right Foreleg in Terms of Amount in Left Foreleg Vessels*

No. of Cats	Days Denervated	Right Leg	
		Normal	Denervated
6	—	103	—
4	—	86	—
5	—	105	—
5	9	—	22
6	21	—	71
4	28	—	57

making these experiments vessels from several cats were pooled. Table II shows that the agreement between figures for normal right leg vessels and normal left leg vessels was satisfactory, while the denervated right leg vessels contained much less enzyme.

In the iris no observations were made earlier than 15 days after denervation, and it is possible that the point of greatest fall in amine oxidase was missed. The mean figure for the period 15–26 days after denervation was 77% of the amount in the corresponding normal pupil. This was the mean figure for 36 cats.

Enzyme Inhibitors

The foregoing evidence of changes in amine oxidase produced by denervation is further strengthened by evidence from the effect of ephedrine and cocaine, which inhibit the enzyme *in vitro*. As already described, Gaddum and Kwiatkowski (1938) found that ephedrine potentiated the effect of adrenaline and of sympathetic stimulation in the rabbit ear vessels and in the nictitating membrane. Burn and Robinson (1951) found that the constrictor effect of *nor*adrenaline in rabbit ear vessels became much stronger in relation to that of adrenaline when ephedrine was added to the perfusing fluid; this change was consistent with the view that the relative weakness of *nor*adrenaline was due to its more rapid destruction by amine oxidase.

Cocaine increases the response of the normal nictitating membrane to *nor*adrenaline, but has relatively little effect on the response of the denervated membrane. When 8 mg. of cocaine was given by intramuscular injection to spinal cats, after making observations similar to those shown in Fig. 1, the contraction of the normal nictitating membrane in response to *nor*adrenaline increased and became about equal in size to the contraction of the denervated membrane. Whatever the ratio of the contraction in the denervated membrane to that in the normal membrane before cocaine was given, the ratio was approximately $=1$ after cocaine was given. Observations in some respects parallel have been made by Innes and Kosterlitz (1951) on the effect of cocaine and of denervation on the increase in heart rate produced by *nor*adrenaline. Almost the only findings which are inconsistent with the view that the effect of cocaine is to inhibit amine oxidase are that certain other substances which inhibit amine oxidase *in vitro*, such as cinchocaine and stilbamidine have not been found to act like cocaine in the body. A careful study of these anomalies has not yet been made, but, as Albert (1951) has pointed out, for a substance which acts *in vitro* to act also *in vivo* its structure must confer the correct adsorption and partition properties both during transit to the site and at the site itself.

Thyroid Activity and Amine Oxidase

The Liver.—Amine oxidase has long been known to be present in the liver, and its importance for glycogenolysis has recently been demonstrated. In hyperthyroidism the sugar tolerance is low, and in animals fed with thyroid the effect of adrenaline in raising the blood sugar is abnormally great. Burn and Marks (1925) showed that rabbits given 0.2 g. of thyroid daily for two weeks responded to the subcutaneous injection of adrenaline with a much greater hyperglycaemia than before. Spinks and Burn (1952) have now found that when rabbits are thus fed with thyroid there is a significant fall in the amount of amine oxidase in the liver (calculated per milligram liver nitrogen). Likewise there is a rise in amine oxidase in the liver after thyroidectomy, which, though not statistically significant in the rabbit, is significant in the rat. The abnormal rises in blood sugar seen in hyperthyroidism, therefore, are probably explained by a fall in amine oxidase in the liver, and the results further emphasize the importance of amine oxidase in controlling the magnitude of sympathetic effects.

The Blood Vessels.—In hyperthyroidism another abnormal response to adrenaline has long been known—namely, an abnormally great rise in blood pressure. This increased effect is the basis of the Goetsch test (1918) for hyperthyroidism. Spinks (1952) has recently examined the amine oxidase present in the aorta of eight thyroid-fed rabbits, comparing it with the amount in eight controls. He found that the amine oxidase was significantly lower in the thyroid-fed animals. He also found that the blood pressure when measured in the carotid artery after anaesthetizing the rabbits by a standard procedure was significantly higher in the thyroid-fed animals, and, moreover, that the amount of adrenaline required to produce a certain rise of blood pressure was lower in the thyroid-fed animals. (The significance here was given by $P=0.06$.) These results require extension, but they make it highly probable that changes in blood pressure occurring as a result of changes in thyroid activity are effected through changes in the amount of amine oxidase in the blood vessels.

The use of sodium or potassium thiocyanate to lower blood pressure is still common in the United States. This substance was shown by Astwood (1943) to diminish thyroid activity, and there are reasons for thinking (Burn, 1948) that it lowers blood pressure by its effect on the thyroid gland. Probably its administration is followed by a rise in amine oxidase in blood-vessel walls.

Discussion

The combined effect of the different pieces of evidence is to present a very strong, if not conclusive, argument that in the neighbourhood of the sympathetic nerve endings in the blood vessels, the nictitating membrane, and the iris there is an enzyme—amine oxidase—which exerts the same function as cholinesterase at the endings of cholinergic nerves. Amine oxidase is certainly present in these organs; it is an enzyme which destroys *nor*adrenaline more rapidly than adrenaline, and so its activity would explain the otherwise puzzling observation that *nor*adrenaline reaching the nictitating membrane and iris in the blood stream or the blood vessels in the perfusing fluid is so much weaker than adrenaline. The amount of amine oxidase falls in these organs after denervation. This observation is alone sufficient to prove that amine oxidase has some connexion with the sympathetic innervation. But when, in addition, the evidence shows that during the first ten days after denervation of the nictitating membrane the fall in the amount of enzyme is correlated with the increase in the sensitiveness of the membrane to *nor*adrenaline, the part played by the enzyme in

modifying the response of the organ to the sympathetic transmitter seems to be finally established. It is pleasant to point out that these observations are in full agreement with the evidence and views of Blaschko and his colleagues put forward in 1937 and of Gaddum in the following year. The action of cocaine seems to be similar to that of ephedrine and to consist also in an inhibition of amine oxidase.

The effect of thyroid feeding in diminishing the amine oxidase in the liver furnishes an explanation for old observations not only on the physiological but also on the clinical side. In the thyroid-fed animal adrenaline produces a greater hyperglycaemia than in the normal animal, and the work of Marks (1925) brought this fact into relation with the diminished sugar tolerance of hyperthyroid patients. Because of the fall of amine oxidase in the liver, the extent of the glycogenolysis which occurs as a result of a given sympathetic stimulus is supernormal.

Perhaps of greater import is the evidence that thyroid feeding causes a fall in the amine oxidase in the blood vessels, and that this fall is accompanied by a raised blood pressure and a raised sensitivity to the pressor action of adrenaline. These observations implicate amine oxidase in the control of blood pressure in the clearest manner, and show that changes in the amount of this enzyme or in the factors which modify its activity must be carefully considered in relation to hypertension.

Summary

At the vagus nerve endings and at motor nerve endings in skeletal muscle the enzyme cholinesterase is present. Evidence is shown that there is a corresponding enzyme, amine oxidase, around the sympathetic nerve endings in the blood vessels, the nictitating membrane, and the iris of the cat. This enzyme destroys the sympathetic transmitter, *nor*adrenaline, more rapidly than it destroys adrenaline. The enzyme declines in amount after denervation, and in the nictitating membrane during the first ten days the decline is proportional to the increase in sensitivity to *nor*adrenaline. Later the amount of enzyme is restored both in nictitating membrane and in the blood vessels.

The amount of amine oxidase in the liver is reduced by thyroid feeding and increased by thyroidectomy. These changes probably explain the greater hyperglycaemia which adrenaline produces in the thyroid-fed animal.

The amount of amine oxidase in the blood vessels is reduced by thyroid feeding. This is correlated with a rise in the blood pressure and an increase in the pressor effect of adrenaline.

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THE ANAEMIA OF HYPOPITUITARISM

BY

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The purpose of this paper is to present observations on some of the haematological changes which occur in hypopituitarism. A normochromic anaemia of moderate severity and refractory to treatment is usual in this syndrome (Sheehan and Summers, 1949), but examples of both hypochromic and hyperchromic anaemia are also recorded. In few of the recorded cases are details of absolute values given, and accurate classification of the anaemia is therefore impossible. One case of aplastic anaemia has been reported (Bloom and Bryson, 1948), and Addisonian pernicious anaemia has also been described (Witts, 1932). The diagnosis of severe hypopituitarism in these cases is, however, doubtful.

All the cases in the present study, with one exception, were typical examples of hypopituitarism due to postpartum necrosis of the anterior lobe, and had had amenorrhoea since their last delivery, absent pubic and axillary hair, cold-sensitivity, lowered basal metabolism, low 17-ketosteroid excretion, and typical responses to the insulin-sensitivity test. The exception was a case occurring in a man who had typical clinical and biochemical changes, but in whom the aetiology of the hypopituitarism was uncertain. Subsequent necropsy in one of these cases confirmed the clinical diagnosis.

Methods

The observations presented were made on 10 patients. All were observed as in-patients in the early stages, and the subsequent follow-up was done as out-patients. All the haematological investigations were made by me.

Blood for red-cell counts was obtained by venepuncture from an antecubital vein, using a dry sterilized syringe and needle. The blood was collected into a 5-ml. bottle containing Wintrobe's anticoagulant mixture (1.2 g. ammonium oxalate and 0.8 g. potassium oxalate dissolved in 100 ml. neutral distilled water). To prevent deterioration 1 ml. of 40% formalin was added to this mixture; 0.5 ml. was run into each bottle from a burette and allowed to dry. The blood was diluted in a Thoma haemocytometer pipette using Hayem's fluid (sodium chloride 1 g., sodium sulphate 5 g., corrosive sublimate 0.5 g., and distilled water 200 ml.).

The haemoglobin was estimated by means of a Spekker photo-electric absorptiometer, using a yellow-green filter with an optical density of 0.475; 100% by this method corresponds to 14.8 g. of haemoglobin per 100 ml.

Reticulocyte counts were made using equal parts of blood and a brilliant cresyl blue solution (consisting of one part of a saturated alcoholic solution of brilliant cresyl blue to five parts of a normal saline solution to which 0.4 g. of sodium citrate was added). After mixing of the blood and cresyl blue, smears were made, dried, and counterstained with Leishman's stain. The number of reticulocytes in 1,000 red cells was counted.

The mean corpuscular volume (M.C.V.), the mean corpuscular haemoglobin (M.C.H.), and the mean corpuscular haemoglobin concentration (M.C.H.C.) were determined by the methods of Price-Jones, Vaughan, and Goddard (1935).

The methods available for determination of the red-cell diameter are open to many inaccuracies. The value of the diffraction method of Pijper (1919, 1947) in the determination of red-cell diameter has recently been assessed by Hird (1949), who thought the method unreliable in esti-