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OBSERVATIONS ON THE INACTIVATION OF ADRENALINE BY BLOOD AND TISSUES *IN VITRO*

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Though knowledge relating to the chemical mediation of the effects of cholinergic nerve activity has advanced rapidly in the course of the last few years, the same progress has not been made with respect to our knowledge of adrenergic nerve activity. It appeared to us that one of the factors responsible for this latter state of affairs was a lack of information concerning the inactivation of adrenaline under experimental conditions resembling as closely as possible the conditions which obtain in the body.

The work recorded in this paper is an attempt to fill some of these gaps in our knowledge of adrenaline inactivation. It is necessarily incomplete and our interpretation of most of the phenomena described here for the first time must be considered tentative. Many fresh problems arose as the work proceeded and only a few of these have been solved: others we hope to deal with later.

We have worked for the most part from first principles. Our main object was to determine whether adrenaline is destroyed by blood and other tissues in a manner analogous to that in which acetylcholine is destroyed, and to determine whether cocaine produces its potentiating action on adrenaline effects, and on effects of adrenergic nerve stimulation, by preventing or delaying this destruction, in the same way as eserine potentiates the action of acetylcholine.

A chance observation, that the degree of inactivation of adrenaline in blood at the end of 10 hr. was no different from that at the end of 5, led us to investigate in more detail what happened to adrenaline when added to blood alone, and it is with this phase of our work that the second and major part of the present paper deals. The order of presentation of

our results may seem illogical in so far as our detailed description of what happens to adrenaline in blood follows our description of experiments with liver; but it is simpler to deal with the work in the order in which it was done and so, to this extent, we sacrifice logic to convenience.

Our experience of the bio-assay of adrenaline in the earlier experiments enabled us to develop a method of following the inactivation over periods of several hours, at such intervals, and with such a degree of accuracy, as to make the construction of inactivation-time curves possible. It is in the form of such graphs that most of our results are presented. Certain of our early and more or less qualitative experiments on the action of liver and cocaine have been repeated using this later technique, and the results are given in the same form as the others. Some of our results have been communicated to the Physiological Society [Bain & Suffolk, 1936; Bain *et al.* 1936].

The literature on adrenaline inactivation is scanty. For further information and references on the subject the reader is referred to the papers of Elliott [1905], Wiltshire [1931] and Blaschko *et al.* [1937].

Materials and methods

General details

In most of the experiments the medium in which we measured adrenaline inactivation was oxalated or defibrinated cat's blood, with or without added tissue slices or extracts. The blood was collected into clean or oxalated glass vessels from a cannula in the common carotid artery of a spinal or etherized cat. There was no apparent difference in the results with oxalated and with defibrinated blood, nor did it appear to matter whether the blood was obtained from spinal or from etherized animals. For experiments continued for 24 hr. or longer the blood was collected under conditions as nearly aseptic as possible, but usually no such precautions were taken.

The adrenaline used was the pure synthetic lævo base of British Drug Houses, Ltd. A concentrated stock solution of the hydrochloride was made by dissolving an amount of the base in a volume of $N/10$ HCl such that the solution contained 10 mg. adrenaline base per ml. From the concentrated stock solution we prepared, with distilled water, a dilute stock solution containing exactly 1 mg. of the base per ml. The experimental solutions in blood, in which inactivation was to be determined, were made up by adding measured quantities of the dilute stock solution to measured volumes of the blood. In experiments where several

different initial concentrations of adrenaline in blood were investigated, and in which therefore the volume of blood having a given concentration of adrenaline was small, the dilutions were effected by making up a certain volume of blood to an initial concentration of, say, 100γ/ml., and diluting measured portions of this to give the other desired and lower initial concentrations.

The standard solutions of adrenaline used for the assays were prepared from the dilute stock solution by further dilution with distilled water. The concentrations of adrenaline in the standard solutions were similar to the initial concentrations in blood, so that quantities of standard and experimental solutions of the same order were being measured during the assays, thus, to a large extent, obviating major errors of measurement. For injection of very small standard quantities of adrenaline in the late stages of an experiment some of the standard solution was still further diluted before use: such diluted standards were always checked against the original.

All the reactions described were carried out at a temperature of $38 \pm 1°$ C. except where otherwise stated. In most of the early experiments the experimental fluids were placed in test-tubes or hard glass flasks in a Wassermann bath and agitated at intervals to counteract sedimentation and promote full oxygenation. In all the later experiments the fluids were either in hard glass flasks or 25 ml. Jena glass vaccine bottles and were agitated continuously during the whole experiment.

Method of assay

The "adrenaline values" or "adrenaline equivalents" of the various experimental fluids were determined by blood pressure assay on cats, by what may be called the method of "continuous assay". In the earliest experiments we used both spinal and anæsthetized cats, but as the former proved much more sensitive to adrenaline all our later assays were made on spinal animals: all the results reported here refer to experiments on atropinized spinal preparations which had been "rested" for 2–3 hr. after transection of the spinal cord and destruction of the brain.

The arterial blood pressure was recorded from the left common carotid artery, and the anticoagulant fluid in the manometer system was a half-saturated solution of sodium bicarbonate. Injections were made into the right external jugular vein through a narrow metal cannula the proximal end of which fitted the nose of a record syringe: the cannula was provided with a stilette.

All the injections of experimental or standard solutions were made up to 1 ml. by dilution with Locke solution *immediately* before injection and were washed in with a further 0·5 or 1 ml. of Locke solution, making the volume of fluid injected at each observation 1·5 or 2 ml. according to the particular experiment. Control experimental fluids were treated in an exactly similar way. In some instances, where the amount of adrenaline left in the experimental fluids was small, or where the initial concentration was itself small, as much as 3 ml. of *undiluted* blood and adrenaline, or of control blood, had to be injected at an observation; but in general the total amount of undiluted experimental fluid measured out and subsequently diluted before injection was not more than 0·5 ml. and, in the early stages of an experiment, was usually less than 0·1 ml. These small amounts were measured into little conical glass dishes, and, a few seconds before the injection was due, were made up to volume with Locke solution from the injection syringe, rapidly mixed by barbotage, taken up and injected.

Injections were given at regular intervals of 3–6 min., the interval in any particular experiment depending chiefly on the time taken for the blood pressure to recover from a previous injection. After giving a number of injections of various doses of standard adrenaline, in order to determine the initial sensitivity of the animal, we continued with the injections of the standard doses within the range of greatest sensitivity. At convenient intervals a standard injection was replaced by an experimental one, the volume of experimental fluid taken up for dilution and injection being such as was calculated to bring the blood pressure rise between two of the standard rises, and as near to one of these as possible. Injections of standard and experimental fluids were continued in this way until the end of the experiment.

An important point about this continuous assay technique is that after 20 or 30 injections a large rise of blood pressure may be got with an amount of adrenaline which, at the beginning of the experiment, gave a small rise or no rise at all. This increase in sensitivity to adrenaline is always seen under the conditions of our experiments and usually occurs without any appreciable change in the resting blood pressure level. Sometimes the maximum increase may be only 80 p.c. for a given dose or it may be as much as 800 p.c. Whatever the extent of the increase it usually takes place smoothly, provided the injections are continued at regular intervals and that the heights of the blood pressure rises in response to the injections do not suddenly become much greater or much smaller, on the average, by marked alterations in the amounts of

adrenaline injected. The sensitivity generally reaches a maximum after 40 or 50 injections, may stay at this level for hours, and then decrease either gradually or suddenly. This decrease usually heralds the death of the animal, though in some instances there may be a fall after a few hours and the sensitivity persist at this lower level for a further period. The rate at which maximum sensitivity is attained seems to depend not so much on the number of injections given as on the blood pressure rises which they cause: the larger the individual rises of blood pressure the quicker is the rise of adrenaline sensitivity.

The importance of this change of sensitivity need not be stressed but it shows the absolute necessity for continued injections of standard adrenaline solutions throughout an experiment. It is also evident that if a high degree of sensitivity in the animal is desired before the assays proper are started, this can be achieved by giving a sufficient number of standard injections beforehand. For the type of work described here the increase of sensitivity is advantageous in that as the adrenaline disappears from our experimental solutions we are not compelled to increase the amount of such solutions injected to the same extent as would otherwise be necessary.

The standards to be injected are determined in the first place by the initial sensitivity of the animal and usually have to be modified later on: thus at the start of an experiment convenient standards may be 3, 3·5 and 4γ or more and, when full sensitivity is established, 0·5, 1 and 1·5γ or less.

Our method of arriving at the actual amount of adrenaline in the different volumes of experimental fluid injected is as follows: The blood pressure rises are measured and plotted on graph paper against time as abscissa. A line is drawn through each set of points representing rises for a particular dose of standard. The adrenaline value of any given experimental injection is then determined by observation or calculation.

As noted above, standards were not usually closer than 0·5γ, but in good experiments, where the sensitivity is high and the sensitivity curves are smooth, the adrenaline equivalent of the actual volume of fluid injected can be estimated to 0·1γ, especially if the position of intermediate points on the sensitivity curves is determined on the basis of a few injections and interpolated accordingly. In experiments where low concentrations of adrenaline had to be determined, standards differing by as little as 0·05γ were used.

A remarkable feature of these assay experiments is the large number of injections that can be given to a single spinal animal without causing

any appreciable change in the level of the resting arterial blood pressure, or in the accuracy of the adrenaline determinations. Most of our cats have had at least 60 injections and many well over 100, and it is very seldom that an experiment has had to be abandoned because of irregular responses at a late stage in the assaying. Bad cats are usually so from the start.

Results

Part I. Preliminary observations on inactivation by blood and tissues

In oxalated blood plasma and in blood serum at body temperature the inactivation of adrenaline proceeds to completion as it does in normal saline, Ringer or Locke solution. The inactivation proceeds in an almost

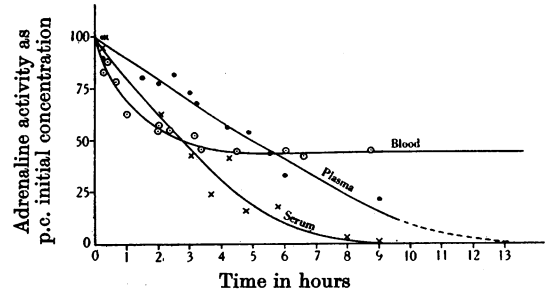

Fig. 1. To show rate and extent of inactivation of adrenaline at body temperature in (i) oxalated or defibrinated whole blood, (ii) blood plasma, and (iii) blood serum. Initial concentration of adrenaline 40γ/ml. in all cases. Data from Exps. A 47, 50, 58, 63 and 64.

linear manner during the greater part of its course and then tails off; it is more rapid in serum than in plasma. In oxalated or defibrinated blood inactivation of adrenaline does not proceed to completion: it proceeds in an exponential manner and ceases when an amount, which depends on the initial concentration of adrenaline, has disappeared. These facts are illustrated in Fig. 1, which shows the combined results from five typical experiments.

The difference between the behaviour of adrenaline in plasma and in serum seems to be chiefly one of rate. It is not accounted for by the absence of oxalate from the serum—since oxalated serum behaves in exactly the same way as normal serum—and it may be due simply to the difference in protein content of the two fluids. Comparison of the behaviour of adrenaline in whole shed blood, and in plasma or serum, shows that the difference here is of another order: it is a difference in kind. In shed blood, whether oxalated or defibrinated, it seems that inactivation

proceeds to what it is convenient to term an "equilibrium concentration" of adrenaline beyond which no further inactivation can be demonstrated.

It thus appears at the outset, from the marked contrast between the action of whole blood on adrenaline and its action on acetylcholine, that the hypothetical "oxidase" for adrenaline, corresponding to the known esterase for acetylcholine, either does not exist in the blood or, if it does, is incapable of exerting its full action under the conditions of our experiments. That we still get this equilibrium concentration even with full oxygenation of the blood and adrenaline shows that, apart altogether from the question of oxidizing enzymes, and apart, too, from the question of what has happened to the inactivated portion of the original adrenaline, full auto-oxidation itself is in some way prevented in whole blood.

Having failed to show complete inactivation of adrenaline by blood, we turned our attention to the possible effects of tissues added to blood and adrenaline *in vitro*.

Effects of tissues on adrenaline activation in blood. The tissues were removed from the animal used to supply blood for the experiment, cut into thin slices with a razor, rapidly washed to free them from blood and the excess moisture removed by filter papers, weighed, and added to the blood in the proportion of 1 g. of tissue/ml. blood. Adrenaline was then added to the mixture and to the control blood to give the same initial concentration per ml. of blood in each. Often the reverse procedure was adopted, i.e. 1 ml. of blood and adrenaline per g. tissue, mixed immediately beforehand, was added to the weighed liver slices. Control mixtures of tissue and blood were also set up and the different mixtures incubated in the usual way.

Of the tissues studied liver showed by far the greatest activity, and so attention was concentrated on it. Of the others, skeletal muscle was slightly active and kidney tissue intermediate between skeletal muscle and liver. Experiments on lung tissue proved fruitless because of the large amounts of an atropine-resistant histamine-like substance liberated during incubation.

That addition of liver slices to blood and adrenaline leads to the complete disappearance of adrenaline activity from the blood is shown in Fig. 2. This activity of liver is diminished by dipping the tissue in boiling water, and abolished by boiling for 2 min.

The activity of liver appears to be diminished by acid. This statement is based on two experiments (A 16 and 17) in which the activity of liver was so little in evidence as to lead us to suspect some error. It was found that the acid used in making up the stock adrenaline was *N* instead of

$N/10$. The activity of liver does not seem to be dependent on a simple catalysis of auto-oxidation, consequent upon contact of the tissues with metal in the course of their preparation. Even a bright copper strip or copper filings added to blood and adrenaline, or to blood, liver and adrenaline, does not affect appreciably the disappearance of adrenaline activity under conditions where neither the blood nor the liver has hitherto come into contact with metal. This is in direct contrast to what happens when metallic copper is added to a solution of adrenaline in Ringer's fluid, as first pointed out by Schild [1933]. The fact that the inactivating power of liver is abolished by boiling is also against the view that such an effect could be responsible.

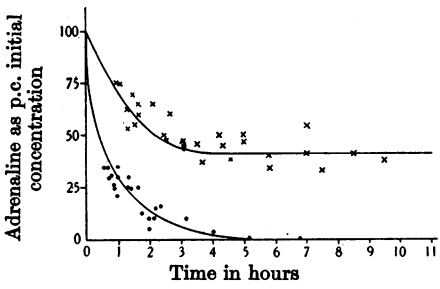

Fig. 2.

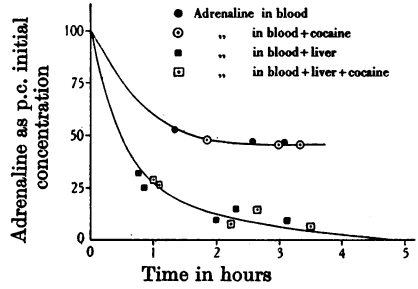

Fig. 3.

Fig. 2. To show contrast between inactivation of adrenaline in oxalated or defibrinated blood with and without addition of liver slices. Upper curve shows inactivation in blood alone: lower curve inactivation in blood to which liver (1 g./ml.) has been added. Initial concentration of adrenaline 20γ/ml. Data from Exps. A 30–34, 36, 37, 41–43, 51, 52, 56, 62, 67 and 68.

Fig. 3. To show that cocaine does not inhibit the inactivation of adrenaline in blood or in blood to which liver has been added. Initial concentration of adrenaline 20γ/ml. and of cocaine 10γ/ml. Data from Exps. A 61 and 62.

Liver ground with sand is active; but, when ground with sand in saline or Locke, filtrates from such concoctions, as free as possible from cells and from cell fragments, have proved inactive in our hands. Similarly, cell free extracts with $N/100$ HCl, with 25 p.c. glycerol and with acetone were inactive. Our results with extracts were thus disappointing [cf. Schütz, 1933; Blaschko *et al.* 1937] and we could only be sure of results when we used liver slices or the unfiltered concoctions got by grinding the material with sand. We preferred the slices.

Attempts to abolish the activity of liver by means other than heating were not satisfactory. We could diminish the activity considerably by withholding oxygen but we never succeeded in abolishing it completely

even by bubbling N_2 through the blood. Addition of KCN had no effect in the doses which we investigated (10–20γ/ml.) and formol, which we also tried, proved useless because of its own inactivating effect on adrenaline [Cramer, 1911], an effect quite evident with as little as 5γ/ml.

Effect of cocaine on adrenaline inactivation. Though the point was perhaps not yet proven, liver appeared to produce an effect on adrenaline which might provisionally, at least, be attributed to an enzyme (oxidase) and the last part of our first object, as explained in the introduction, was to determine whether cocaine had an inhibitory effect on this action and on the action of blood itself so far as this went. (For a review of the early literature relating to the action of cocaine on the effects of adrenaline and of sympathetic nerve stimulation see Trendelenburg [1924].)

In making up the experimental solutions cocaine hydrochloride was first added to a portion of the blood; liver slices were then added to a portion of the blood and cocaine, and to a portion of control blood; the adrenaline was added to control and to experimental fluids last of all. In some experiments the liver was added last of all. The particular procedure used made no difference to the results. The concentrations of cocaine used were from 5 to 20γ/ml., i.e. a total per ml. many times greater than that sufficient to produce a demonstrable effect on adrenaline sensitivity when injected intravenously into a cat.

In determining the adrenaline values of blood, etc., in which cocaine was present, it was found convenient to inure the test animal to the effects upon adrenaline sensitivity of further additions of cocaine by administering from 1 to 10 mg. of cocaine hydrochloride subcutaneously before the experiment started. Failure to do this resulted in highly inconsistent results due to the fleeting and/or step-like changes in adrenaline sensitivity induced by the minute amounts of cocaine in the experimental injections.

Fig. 3 illustrates the results of two such experiments. Cocaine, added to blood or to blood and liver, has no inhibiting effect on the inactivation of adrenaline under the conditions of our experiments, and thus, presumably, does not produce its well-known potentiating action on the effects of adrenaline, and on certain actions mediated by sympathetic nerves, by inhibiting adrenaline inactivation.

From the experiments so far described we see that the hypothetical analogy between the mechanisms responsible for the inactivation of adrenaline and of acetylcholine breaks down in one important respect, for while whole blood is extremely active in destroying acetylcholine this is not so with adrenaline. The hypothetical analogy between the actions

of eserine and cocaine, in so far as these substances affect the actions of acetylcholine and adrenaline and the actions of autonomic nerves, breaks down completely. Thus while eserine inactivates choline-esterase, cocaine fails to inactivate the inactivating principle (or principles) of tissues and so must produce its potentiating or "sensitizing" action to adrenaline and to adrenergic nerve stimulation in some other way.

Part II. Further observations on adrenaline inactivation in blood

That an "equilibrium", with respect to inactivation, is reached in blood was confirmed by many experiments. For any given initial concentration the equilibrium concentration varies somewhat from blood

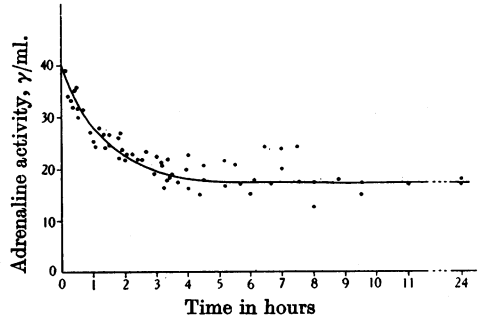

Fig. 4. To show variations of equilibrium value in different experiments with the same initial concentration of adrenaline (40γ/ml.). Data from Exps. A 47, 49, 51, 52, 53, 55, 57, 58, 63, 64, 67, 74 and 85.

to blood. This fact is evident from Fig. 4, which gives the graphed results of a number of experiments in which the initial concentration of adrenaline was 40γ/ml. In any individual experiment the equilibrium concentration is quite definitely established. The average value of the equilibrium concentrations for an initial concentration of 40γ/ml. was 17·8γ (13 experiments) and the individual values ranged from 14γ to 24γ/ml. (see Fig. 5*a*).

It is obvious that these individual variations may partly be due to errors and thus be insignificant; for if the initial concentration is more than that estimated by, say, 2γ/ml. and the standard solution used for assay less by 2γ/ml. than estimated, the assay (assuming there are no inherent errors in this) will give an equilibrium value 4γ/ml. higher than the actual; but the greatest care in measuring the blood and the adrenaline solutions has not diminished the variation, and we therefore consider it to be probably significant. As we shall see later, it may be related to differences in the corpuscle : plasma ratio in different samples of blood.

In addition to these individual variations for a given initial concentration there is a variation in the equilibrium concentration related to differences in the initial concentration of adrenaline. This relationship for different initial concentrations from 60γ downwards is shown in Fig. 5*a*. The points through which the graph is drawn represent the average values for that number of observations indicated for each initial concentration by the bracketed numerals on the right-hand side of the chart; while the small vertical lines, lateral to the main points, represent the range of the equilibrium values for each initial concentration. From

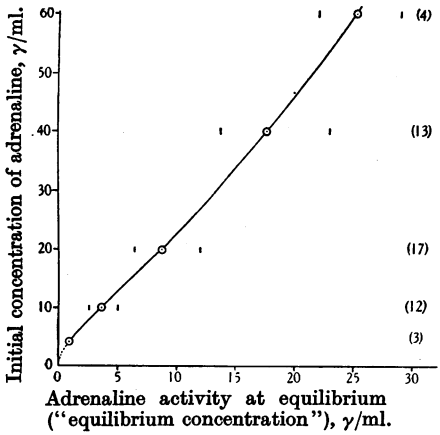

Fig. 5*a*.

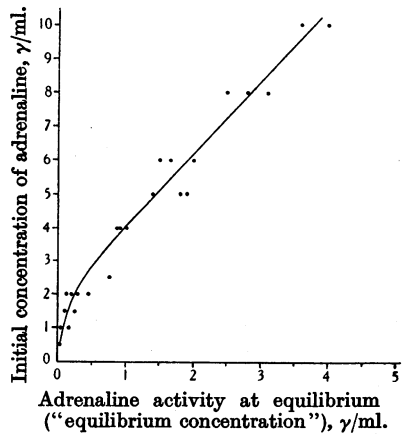

Fig. 5*b*.

Figs. 5*a* and 5*b*. To show variation in equilibrium value of blood and adrenaline with different initial concentrations. Fig. 5*a* shows the variation for initial concentrations from 60γ/ml. downwards. For description see text. Fig. 5*b* shows in greater detail the variations of the equilibrium value for initial concentrations from 10γ/ml. to 0·5γ/ml. Each point for a particular initial concentration on this chart represents a separate experiment. Data for Fig. 5*b* from Exps. A 8, 44, 64, 65, 66, 89 and 92.

a physiological point of view the most interesting part of this curve is that which relates to initial concentrations between, say, 10 and 0·5γ, and so that part of the curve is reproduced in greater detail as Fig. 5*b*. Unfortunately, however, it is with these small initial concentrations that our method tends to break down, for the simple reason that accurate estimates of the adrenaline activity at equilibrium involve the injection of relatively enormous quantities of blood (2–3 ml.). Nevertheless, the results, carefully controlled, are concordant so far as they go. But the experiments will have to be repeated using other techniques before we could feel justified in laying much stress on them. In the meantime

we can confine our attention to the results got with the relatively unphysiological initial concentrations of 20–40γ/ml. without prejudice as to what happens with very small initial concentrations.

The part played by the corpuscles in the inactivation of adrenaline by blood. The fact that adrenaline becomes completely inactivated in plasma and in serum but only partially in oxalated or defibrinated blood led us to consider the possible significance of the corpuscles in the phenomena exhibited by blood. We therefore made experiments in which oxalated blood, with added adrenaline, was allowed to come to equilibrium, the corpuscles then being spun off and the adrenaline value of the plasma determined as soon as possible after separation and at intervals thereafter. We soon saw that the adrenaline value of the plasma under these circumstances diminishes in the same way as it does when adrenaline is added to plasma in the first place. Thus, whatever the property of the corpuscles which prevents complete disappearance of adrenaline from blood, the corpuscles do not confer on the plasma any "protective" action which persists after they have been removed.

Extrapolation of the equilibrium plasma inactivation curve, after separation of the cells, indicates that at the time of separation of the corpuscles the adrenaline value of the plasma represents the whole adrenaline activity of the equilibrium mixture. Exp. A 57 may be given as an example: A sample of blood and adrenaline, 40γ/ml., came to equilibrium at 15γ/ml. Separation of cells from the plasma from 40 ml. of this equilibrium mixture gave 18 ml. of cells and 22 ml. of plasma. The adrenaline value of the plasma 30 min. after separation was 25γ/ml. and 2 hr. 50 min. after separation 18γ/ml. (average values for triplicate readings at 5 min. intervals). Extrapolation from these points, which lie in the linear part of the plasma inactivation time curve, gives the plasma adrenaline concentration at the time of separation as 26·5γ/ml. Now, if all the adrenaline of the equilibrium mixture (i.e. 15γ/ml.) is in fact present in the plasma, the adrenaline content of the separated plasma at the time of separation should be $(15/1 \times 40/22)\gamma$/ml., i.e. 27$\gamma$/ml. approximately, which is not significantly different from that found. Direct determination of the plasma or serum adrenaline immediately after separation confirms this type of result, and so we have the further important fact that all the adrenaline activity of an equilibrium mixture, as determined by bio-assay of the intact mixture, resides in the plasma or serum of the mixture.

It immediately appears, therefore, that the blood corpuscles play an essential part both in the establishment and in the maintenance of the equilibrium phenomena which we have described.

Adrenaline associated with the cells of an equilibrium mixture. Having shown that serum or plasma, freshly separated from an equilibrium mixture, appeared to contain an amount of adrenaline which accounted for the whole activity of the equilibrium mixture, it remained to confirm this by showing that the corpuscles from an equilibrium mixture showed by themselves no adrenaline activity. This was done. Whole corpuscles from an equilibrium mixture show no adrenaline activity when taken up in Locke solution and immediately injected into the test animal. If, however, the cells are left in Locke solution, or in fresh plasma or serum, the mixture soon acquires an adrenaline activity which gradually increases to a constant value, a fact which immediately suggests the survival of some of the missing adrenaline in reversible association with the corpuscles. This is dramatically demonstrated when corpuscles separated from an equilibrium mixture are laked either by the action of acid or by freezing: they then exhibit a marked adrenaline activity not shown by control laked corpuscles. From such observations it became apparent that the adrenaline equivalent of an equilibrium mixture, i.e. the equilibrium concentration, does not in fact represent the total amount of adrenaline in such a mixture, but that a further amount is present in or on the corpuscles, an amount incapable under ordinary circumstances of exhibiting its presence by an action on the test animal.

The next step was to determine the amount of adrenaline associated with the corpuscles of an equilibrium mixture. Two methods of doing this were open to us: One was to separate the cells from the mixture, determine the relative cell/plasma or cell/serum volumes, lake the cells and find their adrenaline value per unit volume; the other was to lake the equilibrium mixture with dilute hydrochloric acid and find the adrenaline value of the laked mixture. Both methods were used and some results are summarized in Tables I*a* and I*b*.

TABLE I*a*. Recovery of adrenaline from equilibrium mixtures. Plasma adrenaline, from separated equilibrium plasma, is calculated as adrenaline equivalent per ml. original whole blood. Corpuscle adrenaline, from separated equilibrium cells, is calculated as adrenaline equivalent per ml. original whole blood

Initial conc. (γ/ml.)	Adrenaline value plasma (γ/ml. whole blood)	Adrenaline value cells (γ/ml. whole blood)	Adrenaline value cells + plasma (γ/ml.)	Total adrenaline recovered (p.c. of initial adrenaline)
20	9·4	5·9	15·3	76·5
40	14·8	14	28·8	72
40	15·8	12·4	28·2	70·5
60	26·2	19·1	45·3	75
			Average	73·5

TABLE I*b*. Recovery of adrenaline from equilibrium mixtures. Corpuscle adrenaline by laking equilibrium mixture

Initial conc. (γ/ml.)	Equilibrium conc. (γ/ml.)	Adrenaline equivalent laked equilibrium mixture (γ/ml.)	Total adrenaline laked equilibrium mixture (p.c. original)
20	6·7	17·0	85
20	7·7	14·5	72
40	13·3	30·9	77
40	15·5	33·5	84
60	29·0	43·3	72
60	26·0	52·0	87
		Average	79·5

It will be seen that a somewhat greater "recovery" of adrenaline was got by laking the equilibrium mixture than by separating the cells from the serum or plasma. This difference may be accounted for by loss of cell-adrenaline in the experimental procedures necessary with the separation method. The average "recovery" or "total equilibrium" adrenaline from 10 equilibrium mixtures selected at random (initial concentration 40 and 20γ/ml.) represents approximately 80 p.c. of the original amount added. Thus while an average of 56 p.c. of the initial adrenaline (40 or 20γ/ml.) becomes physiologically inactivated by the time equilibrium is reached, all of this amount is not destroyed; a further 36 p.c. of the original amount can be recovered from the corpuscles in which situation it is ordinarily incapable of manifesting a pressor action on the test animal. We conclude therefore that the total irrecoverable adrenaline of an equilibrium mixture is actually only about 20 p.c. of the original, as against the apparent 56 p.c. of the original as given by the equilibrium adrenaline value.

If the procedure for obtaining an inactivation curve for blood and adrenaline is followed out and alternate samples of blood are laked immediately before assay two separate curves result, the normal ("apparent") inactivation curve, and what one might call for convenience the "virtual" inactivation curve. The uppermost graph in Fig. 6, constructed from the data of two experiments (A 63 and 64), is an example of such a "virtual" inactivation curve. It would seem from such results that the "irrecoverable" adrenaline is lost before equilibrium is reached, since the assay curve for the laked samples becomes horizontal before the normal inactivation curve does so.

Whether the irrecoverable adrenaline of an equilibrium mixture is destroyed by oxidation or otherwise, or is inactivated in the same way as the recoverable cell adrenaline but in a less easily reversible manner, or is inactivated by combination with some constituent of the blood,

irreversibly or otherwise, we are not at present in a position to say. Nor are we able to say whether the adrenaline recovered by laking an equilibrium mixture, or separated equilibrium corpuscles, is inactive because it is inside the corpuscles in a free state, or is adsorbed on the corpuscles, or both. Whatever be the truth it is certain that, so far as the recoverable adrenaline associated with the corpuscles is concerned, the mechanisms involved can be partially reversed under circumstances not involving destruction of the corpuscles. If, for example, cells from an equilibrium mixture are added to fresh serum or plasma this mixture acquires and shows a progressively increasing adrenaline value which ultimately becomes constant—that is, comes to equilibrium: the adrenaline value of the separated cells diminishes accordingly. Similarly, if fresh blood is added to an equilibrium mixture the equilibrium changes and in 3–5 hr. has a value near to that which it would have had if the initial adrenaline had been added to the final volume of blood in the first place, and the adrenaline value of the cells is diminished in consequence.

What is perhaps a more important point, and one which may be considered as giving evidence that the recoverable adrenaline associated with the cells is normally inactive simply because it is within them, or is adsorbed by them, and that the irrecoverable adrenaline may be lost in another way, is the fact that the *integrity* of the corpuscles does not appear to be necessary either for the loss of the irrecoverable adrenaline or for the persistence of that amount normally associated with the cells and normally recoverable after laking either the equilibrium mixture or the separated equilibrium cells. This is illustrated by the following experiment (A 85):

Oxalated blood, collected aseptically, was divided into two portions. One of these was laked.

In this experiment, and also in most of those in which we studied inactivation of low initial concentrations, the blood was laked by freezing with liquid air. 10 ml. or so of blood at a time were placed in a hard glass test-tube the bottom of which was dipped in liquid air until the blood froze solid. It was then rapidly thawed by running warm water over the outside of the tube. Often the whole process was repeated to ensure thorough laking.

Samples of each portion having been retained as controls adrenaline was added to each of the remaining portions to give an initial concentration of 40γ/ml. Experimental and control samples were then incubated with continuous mechanical agitation in stoppered sterile vessels. Immediate assay gave initial values of 39 and 41γ/ml. for the normal and the laked portions respectively. 24 hr. later the adrenaline value of the normal sample was 16·7γ/ml. and of the laked sample 29γ/ml. A portion

of the normal 24 hr. sample laked with liquid air had an adrenaline equivalent of 30γ/ml. The 24 hr. equilibrium value is thus well within the normal limits, as is the total recoverable adrenaline from this equilibrium mixture. The adrenaline value of the sample laked before the adrenaline was added to it 24 hr. previously is, however, not significantly different from the adrenaline value of the laked equilibrium mixture. It thus appears (1) that the irrecoverable adrenaline does not depend for its loss upon the integrity of the corpuscles, and (2) that the total recoverable adrenaline is the same whether the corpuscles are intact or not, that is, whether part of it is in a state in which it is incapable of exercising an effect on the test animal by reason of association with intact corpuscles, or is free in the plasma along with destroyed corpuscles, in which situation it is free to exercise its action on the test animal from the beginning.

Relative amounts of adrenaline associated with cells and with plasma or serum of equilibrium mixtures. A further interesting point relating to the corpuscle adrenaline is that at equilibrium the adrenaline equivalent of the corpuscles appears to be greater, volume for volume, than that of the plasma. If, for example, making use of the 4 : 6 corpuscle/plasma ratio determined by the hæmatocrite and the equilibrium and laked equilibrium values given in Table I*b*, we calculate the corpuscle adrenaline and plasma adrenaline concentrations respectively for each of the six blood samples of that table, we find the average values for each of the three sets of initial concentrations to be 21·4 and 12γ/ml. for the original 20γ/ml. samples, 44·5 and 24γ/ml. for the original 40γ/ml. samples, and 50·4 and 45·8γ/ml. for the original 60γ/ml. samples.

If we calculate the plasma and corpuscle adrenaline concentrations from the average equilibrium values for different initial concentrations (see Fig. 5*a*), assuming an 80 p.c. recovery and a 4 : 6 corpuscle/plasma ratio, the differences are not so striking as those just quoted; for an initial concentration of 10γ/ml. they are for corpuscles and plasma, in that order, 10·25 and 6·5γ/ml.; for 20γ, 18 and 14·7γ/ml.; for 40γ, 35·7 and 29·5γ/ml.; and for 60γ, 56·2 and 42·5γ/ml. respectively. The differences are, of course, reduced still further if the total recovery is reduced or the corpuscle/plasma ratio approaches nearer to unity. But that the corpuscle adrenaline concentration at equilibrium probably is significantly greater than the plasma adrenaline is confirmed by experiments in which the total recovery was accurately estimated and the corpuscle/plasma ratio determined at the outset by the addition of known volumes of spun cells to known and widely different volumes of separated plasma. In the

experiment which we quote, spun cells from oxalated blood were added to separated plasma in four different ratios; adrenaline was added to give an initial concentration of 20γ/ml. of the mixtures, and allowed to come to equilibrium. In addition to the equilibrium values, the total adrenaline recovery was determined in each instance; from these figures, together with the known corpuscle/plasma ratios, the plasma and corpuscle adrenaline concentrations were calculated. These results are set forth in Table II. (It must be clearly understood, however, that in speaking of

TABLE II. Equilibrium values, total recovery, and corpuscle and plasma adrenaline values in blood with altered corpuscle/plasma ratios. Initial concentration of adrenaline 20γ/ml. in all cases. Cell (or plasma) conc. $= \left(c\ [\text{or } p] \text{ amount} \times \frac{\text{total volume}}{c\ [\text{or } p] \text{ vol.}}\right)$

Ratio cells : plasma	Equilibrium value (i.e. plasma adrenaline) (γ/ml.)	Adrenaline equivalent laked equilibrium mixture (i.e. plasma and cell adrenaline) (γ/ml.)	Total adrenaline recovered (p.c. initial conc.)	Plasma adrenaline conc. (γ/ml. plasma)	Cell adrenaline conc. (γ/ml. cells)
4 : 2	3·9	15·3	76·5	11·7	17·1
4 : 6 (normal)	7·5	14·3	72·0	12·5	17·0
4 : 10	9·7	16·0	80·0	13·6	22·0
4 : 16	11·0	16·7	84·0	13·7	28·5

the "cell concentration" of adrenaline we do not infer that the adrenaline is in fact in simple solution within the cells at equilibrium: adsorption may well be the main, or indeed the exclusive factor at work. At the present time we cannot commit ourselves either one way or the other.)

In an earlier part of this paper it was pointed out that the individual variations in the equilibrium concentrations for different initial concentrations of adrenaline might be partly accounted for by differences in the corpuscle/plasma or corpuscle/serum ratios in the different samples of blood. The effects upon the equilibrium value of alterations in the corpuscle/plasma ratio, as in the experiment just quoted, show that this view is perhaps justified.

Experiments with low initial concentrations of adrenaline. We have hitherto dealt for the most part with results arrived at in the study of initial concentrations of 20 and 40γ of adrenaline per ml.; we revert finally to the question of much lower initial concentrations. We have already alluded to these (p. 243), and to the difficulties of investigating them by our method. The chief difficulty arises from the relatively enormous quantities of blood that have to be injected in the later stages of such experiments in order to get an estimate of the adrenaline present.

The number of injections is thus limited and the difficulties both of making an assay and of making adequate controls are further increased by this restriction. More serious, however, than the difficulties introduced by the simple volume factor is the fact that the controls often cause an atropine-resistant depressor effect which is greatly exaggerated when the blood is laked. Sometimes these presumably histamine effects are absent even with 5 ml. injections of blood: there is considerable variation either in the content of the depressor substance in different samples of blood and/or in the sensitivity of the test animals to it. In view of these difficulties, however, we regard our results with these low initial concentrations with caution until such time as the experiments can be repeated using other techniques.

Keeping this proviso in mind we present in Table III the results of an experiment (A 92) conducted under the best conditions, i.e. where the

TABLE III. To show equilibrium values, total recovery and relative corpuscle and plasma adrenaline concentrations with low initial concentrations. Figures in brackets are the percentages of the original concentration represented by the figures which precede them

Initial conc. (γ/ml.)	Equilibrium conc. (γ/ml.)	Adrenaline conc. laked equilibrium mixture (γ/ml.)	Cell conc. (γ/ml. cells)	Plasma conc. (γ/ml. plasma)
5·0	1·8 (36)	3·7 (74)	4·75	3·0
2·0	0·45 (22·5)	1·24 (62)	1·97	0·75
1·5	0·25 (16·6)	0·70 (50)	1·12	0·42
1·0	0·075 (7·5)	0·25 (25)	0·44	0·12
0·5	0·025 (5)	0·10 (20)	0·19	0·04

sensitivity of the test animal to adrenaline was extraordinarily high and the controls had no apparent histamine activity. The chief points of interest appear to be: (1) that as the initial concentration of adrenaline is lowered from 5 to 0·5γ/ml. the percentage of the original adrenaline represented by the equilibrium value falls from 36 to 5, and the total recovery from 74 to 20; (2) at equilibrium the "cell concentration", expressed as a multiple of the plasma concentration, rises from about 1·6 for an initial concentration of 5γ/ml. to about 4·7 at 0·5γ and this despite the fact that if histamine is interfering with the assays it would tend to diminish the observed cell concentration of adrenaline to a greater extent than the plasma concentration. If adsorption is the main factor at work in determining the phenomena which we have described in blood it may be that the comparatively low equilibrium values with the very small initial concentrations are due to more complete adsorption, and the low recoverable adrenaline to very incomplete reversal of the process, under these circumstances.

Concluding remarks

Detailed discussion of the results reported in this paper would be premature at this stage and must wait until some further facts are forthcoming. One or two observations of a general nature may, however, be permissible now. In the first place, we would point out the advantage of using blood as a medium in experiments on adrenaline inactivation. Despite the fact that Oliver & Schäfer in 1895 pointed out the feeble adrenaline inactivating power of blood, most studies of adrenaline inactivation since that time have been carried out using Ringer fluids as the medium. The rapidity of free oxidation in this medium, taken together with the rapid inactivation of adrenaline *in vivo*, appears to have led many, at least implicitly, to identify the processes in Ringer's fluid and *in vivo*, or if not going so far, at least to regard the process in Ringer's fluid as the standard against which inactivation by tissues or other factors is to be measured. This is seen, for example, in Miss Wiltshire's [1931] work. By taking blood as a medium and the inactivation of adrenaline in blood as a physiological standard the fallacious arguments and the contradictory interpretations which arise when other media are used would to a large extent be obviated.

The only general conclusion which we can safely draw from the work recorded here is that blood itself is probably but of small significance in determining the inactivation of adrenaline in the body and that it is in the tissues almost exclusively that the conditions for this inactivation are fulfilled. It is true that under the conditions of our experiments complete inactivation of 40γ adrenaline by 1 g. of liver—the most active of all the tissues we examined—takes some 4–5 hr. But this length of time does not in our view make it unlikely, as Bacq [1936] rather seems to suggest, that the same mechanism is in fact the one responsible for the rapid inactivation of adrenaline *in vivo*. In the first place, the conditions under which our experiments are carried out are manifestly much less favourable to the inactivating property of the tissues than are the conditions *in vivo*. In the second place, even if 1 g. of liver does take 4 hr. to inactivate 40γ of adrenaline, surely it is not too much to expect the 80 g. or so of cat liver *in vivo*, taken together with the lesser but very significant inactivating effect of many if not most of the other tissues of the body, to inactivate the same amount in a few minutes. Apart from such considerations, however, an examination of the inactivation-time curves for the different media we have investigated will immediately show how different are the inactivation *rates* in these media. Thus the

approximate times for 25 p.c. inactivation are in blood plasma 180 min., in serum 80 min., in blood 40 min., and in blood + liver 6 min. These facts are illustrated in Fig. 6 which summarizes most of our results. The relatively high initial rate of inactivation by liver is obvious from the graph, and the tailing-off may well be due partly to accumulation of oxidation products, a factor which might not operate to anything like

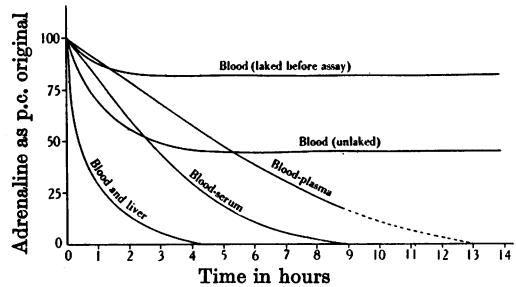

Fig. 6. To summarize the principal data given in Figs. 1, 2 and 3, and to show in addition the type of inactivation curve obtained by laking, immediately before assay, blood to which adrenaline has been added at zero time (see p. 246).

the same extent *in vivo*. But in a later paper one of us will revert to this topic and will show, among other things, that the activity of cat's liver in respect of the adrenaline inactivating principle is not nearly so great as is that of the liver of some other mammals, including man.

Summary

1. The methods used in studying the inactivation of adrenaline are described; all the experiments were made with cat's blood and cat's tissues and the adrenaline determinations made by blood pressure assay on spinal cats.

2. In oxalated blood plasma and in blood serum inactivation of adrenaline proceeds slowly to completion; it is more rapid in serum than in plasma.

3. In oxalated or defibrinated whole blood inactivation is never complete; it proceeds to what it is convenient to call an equilibrium value beyond which no further inactivation can be demonstrated.

4. Addition of tissue slices, or of concoctions of tissues, to blood and adrenaline leads to the complete disappearance of adrenaline activity from the blood. Many tissues show this activity to some extent but the most active, weight for weight, is liver.

5. The adrenaline inactivating power of liver is not diminished by cyanide or cocaine: it is diminished by increased acidity of the medium and by the withholding of oxygen; it is absent after the tissue has been boiled.

6. Further study of the inactivation of adrenaline by blood showed that the equilibrium concentration varies with the initial concentration and that there is also some variation, for a given initial concentration, from sample to sample of blood. These last variations are probably due to variations in the cell/plasma or cell/serum ratios.

7. The adrenaline value of an equilibrium mixture is represented exclusively by adrenaline in the plasma or serum, but a further amount of adrenaline is present in or on the cells of such a mixture, an amount which ordinarily is incapable of manifesting its presence by an action on the test animal.

8. This additional adrenaline is in reversible association with the cells and can be recovered in maximum amount by laking the separated cells or the equilibrium mixture, or, in lesser amount, by placing separated equilibrium cells in fresh plasma or serum or Locke solution. Over 80 p.c. of the original adrenaline (20–40γ) added to blood is recoverable by laking an equilibrium mixture.

9. The total adrenaline recoverable from blood seems to be independent of the integrity of the cells.

10. Whether the association of adrenaline with the cells is one of simple adsorption, or of passage of adrenaline within the cells, or both, is not clear. Nor is it clear whether the 20 p.c. of original adrenaline irrecoverable from such a laked equilibrium mixture is irrecoverable because it is destroyed, or is combined in some way with some constituent of the blood, or is held inactive in the same way as the recoverable cell adrenaline but in a manner which renders its association irreversible under the conditions of our experiments.

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REFERENCES

Bacq, Z. M. (1936). *J. Physiol.* **87**, 87 *P.*
Bain, W. A. & Suffolk, S. F. (1936). *Ibid.* **86**, 34 *P.*
Bain, W. A., Gaunt, W. E. & Suffolk, S. F. (1936). *Ibid.* **87**, 8 *P.*
Blaschko, H., Richter, D. & Schlossmann, H. (1937). *Ibid.* **90**, 1.
Cramer, W. (1911). *Ibid.* **42**, 36 *P.*
Elliott, T. R. (1905). *Ibid.* **32**, 401.
Oliver, G. & Schäfer, E. A. (1895). *Ibid.* **18**, 230.
Schild, H. (1933). *Ibid.* **79**, 455.
Schütz, F. (1933). *Biochem. Z.* **265**, 282.
Trendelenburg, P. (1924). *Heffter's Handbuch der experimentellen Pharmakologie*, **2**, 2, p. 1167.
Wiltshire, M. O. P. (1931). *J. Physiol.* **72**, 88.