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Original Articles

THE CONCENTRATION OF ADRENALINE IN HUMAN PLASMA AND ITS RELATION TO MENTAL ACTIVITY*

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IT is generally accepted that situations of danger, stress or emergency lead to an increased activity of the sympathetic system and to the discharge of adrenaline from the adrenal medulla. Until recently our knowledge was based, essentially, upon two lines of evidence; on the observation of effector organs and mechanisms, such as heart rate, blood pressure, width of skin capillaries, etc., and on the measurement of the adrenaline concentration in the suprarenals or the suprarenal veins, which contain comparatively large quantities. Lately, with the advent of more refined techniques, further methods have become available, which made it possible to estimate adrenaline in tissues other than the suprarenals, in urine and in peripheral blood. The last two methods are obviously of special importance for clinical studies.

In this paper work done in the writer's laboratory will be briefly reviewed. The first part deals with the development of a method for the estimation of adrenaline and noradrenaline in plasma and with experiments that were intended to establish the validity of the method. The concentration of adrenaline and noradrenaline, and the form of their occurrence, in plasma and CSF, will be discussed. In the second part results will be described, which suggest that there is a correlation of the blood-adrenaline level with mental activity.

THE ESTIMATION OF ADRENALINE IN BLOOD

The problem of estimating adrenaline and noradrenaline in blood has attracted many workers (see review by Pekkarinen, 1948), but the methods proposed were lacking in sensitivity or specificity. One of the greatest difficulties was the separation of adrenaline from the plasma proteins. When plasma is treated with any of the common deproteinizing agents, the recovery of added adrenaline in the protein-free filtrate is low, since a large part is adsorbed by the precipitated protein (Gaddum and Schild, 1934; D'Silva, 1937; Lehmann and Michaelis, 1942). Attempts were made to obtain a separation by dialysis or ultra-filtration, but these procedures were not very satisfactory.

* Awarded the Burlingame Prize for 1955.

The problem was elegantly solved by Lund (1949) who showed that adrenaline and noradrenaline were quantitatively retained when plasma, diluted with an equal volume of buffer, was filtered through a column of aluminium oxide at a slightly alkaline pH. The adsorbed amines are subsequently eluted by dilute acid and estimated in the eluate. The column of alumina can be replaced, with equal effect, by one of silica gel (Weil-Malherbe, 1954b).

Since the eluate contains only minute amounts of catechol amines (of the order of 0·01–0·05 μg.) the method of estimation had to be highly sensitive and in this respect a fluorimetric technique seemed most promising. Such a method has been developed by Lund (1949, 1950). Another method, which has been used for the investigations described in this report, is based on a condensation of adrenochrome with ethylenediamine, a reaction which results in the formation of a stable fluorescent compound (Weil-Malherbe and Bone, 1952a, 1953). Compared with the method of Lund it has the advantage of greater simplicity and of requiring less blood; moreover, since the derivative of adrenaline differs in fluorescence from that of noradrenaline, it is possible to estimate both substances side by side in the same solution (Weil-Malherbe and Bone, 1953).

The Validity of the Method

While there is no doubt that the sensitivity of this method is equal or superior to previous methods, it was of the utmost importance to establish its specificity.

To begin with, a large number of individual compounds was tested. Only some closely related amines reacted in a way similar to adrenaline and noradrenaline. Some substances yielded a fluorescent compound on treatment with ethylenediamine, but were not adsorbed on the alumina column; among these were catechol, adrenochrome and 5-hydroxytryptamine. Others, like dihydroxyphenylalanine, formed a fluorescent derivative that was not extracted from the reaction mixture under the conditions used (Weil-Malherbe and Bone, 1952a).

It was next shown that the unknown substance in the plasma behaved like adrenaline in two respects: it had the same rate of disappearance when exposed either to atmospheric oxidation in alkaline solution or to oxidation by a dialysed liver extract. The reactions of adrenaline and of the plasma substance with the liver enzyme were equally inhibited by ephedrine, a specific inhibitor of amine oxidase. Hence it was assumed that the reaction was due to the presence of amine oxidase in the liver extract. When the exposure of plasma to the action of the liver extract was sufficiently prolonged, the reacting material was completely removed (Fig. 1), the time curve being that of a first-order reaction. This indicated that the fluorescence reaction was either given by a single substance or by a mixture of substances with similar affinities for the enzyme.

In a further series of experiments (Weil-Malherbe and Bone, 1953, 1954a) plasma was extracted on a large scale and the purified extracts were studied by paper chromatography. After termination of the run, the paper strip was freed from adherent solvents and cut into sections. Each of these was extracted with dilute acid and the extracts were analysed by the ethylenediamine method. Solutions of authentic adrenaline and noradrenaline were applied to a guide strip; the position of the spots was here revealed with the aid of a spray reagent.

The analysis showed that, after correction for a slight blank, fluorescence was only obtained from those positions that corresponded with the adrenaline and noradrenaline spots on the guide strip. Moreover, the fluorescence obtained

from the adrenaline zone had the same spectral properties as that of the adrenaline derivative, while that given by the noradrenaline zone had the spectral properties of the noradrenaline derivative. The results were essentially the same in two different solvent systems. An example of these experiments is shown in Fig. 2.

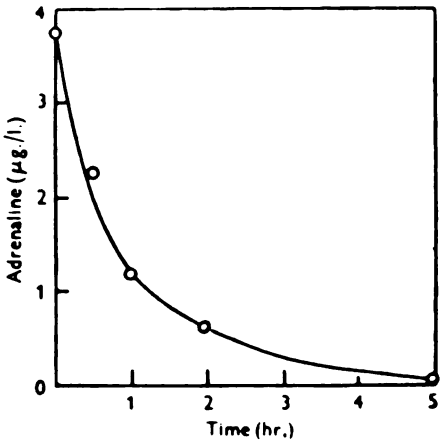

FIG. 1.—Action of amine oxidase on fluorogenic matter in human plasma. From Weil-Malherbe and Bone (1952a).

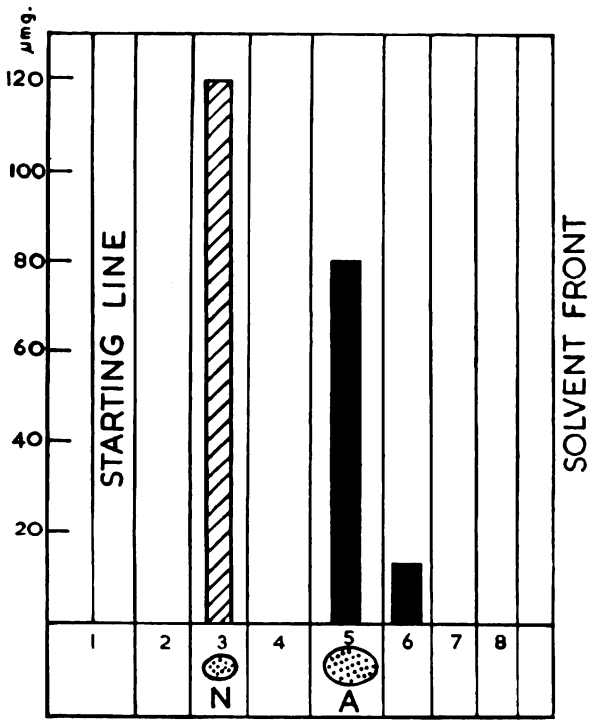

FIG. 2.—Paper chromatography of plasma extract. Solvent: phenol, 75 per cent.—water, 25 per cent. The oblong at the bottom of the Figure represents the paper strip with adrenaline (A) and noradrenaline (N) spots and with approximate position of cuts. The bars indicate quantities of adrenaline (black) and noradrenaline (hatched) calculated from fluorescence of extract of corresponding paper section. Data of Weil-Malherbe and Bone (1954a).

Particular emphasis was laid on the quantitative aspect of these experiments. It was found that the substances isolated by paper chromatography accounted for a proportion of the reacting material of plasma, which closely corresponded with the recovery of added catechol amines in model experiments.

A comparison of the ethylenediamine method with the method of Lund, on portions of the same sample of blood, showed that practically identical results were obtained in both (Table I). This agreement is regarded as highly significant since the two methods are based on different mechanisms, both with a high degree of specificity.

TABLE I

Simultaneous Estimation of Adrenaline and Noradrenaline in Plasma by Ethylenediamine Method (ED) and by Method of Lund (1950)

	ED		Lund	
	A	N	A	N
	(μg./l. of blood)			
1	1·59	5·12	1·56	5·40
2	0·86	2·45	1·15	3·26
3	1·46	3·70	2·37	2·96

A = adrenaline.
N = noradrenaline.

For final identification it will be necessary to demonstrate the biological activity of the catechol amines of plasma. This problem which is a particularly difficult one on account of the low concentrations and of the presence of interfering substances has not yet been studied systematically in the writer's laboratory. Hagen (1954) carried out parallel estimations on fractions prepared from adrenal medulla and found that the results obtained by the fluorimetric method agreed well with those obtained by biological assay. The concentration of catechol amines was of course so high that these extracts could be diluted to the point where interfering substances no longer mattered; the results, though they are reassuring, have, therefore, no immediate bearing on the validity of the method for plasma.

THE CONCENTRATION OF CATECHOL AMINES IN BLOOD

With the aid of the fluorimetric method the concentration of adrenaline in the plasma of peripheral venous blood is found to be of the order of 1–2 μg./l. of whole blood, while that of noradrenaline is of the order of 4–5 μg./l. In the plasma of arterial blood the concentration of adrenaline is about 0·5 μg./l. higher, while that of noradrenaline may be higher or lower than in venous blood. This finding is in harmony with the concept that noradrenaline is secreted not only by the cells of the adrenal medulla, but also at sympathetic nerve endings in peripheral tissues (Euler, 1950). On the other hand, a positive arteriovenous difference for adrenaline implies a continuous utilization of adrenaline by the peripheral tissues. Assuming a basal minute volume of 4 l., a mean body weight of 60 kg., and an identical arteriovenous difference in all parts of the vascular system, an adrenaline utilization rate of 0·033 μg./kg./min. is obtained. A continuous utilization, in turn, implies a continuous discharge of adrenaline. This concept differs from that advocated by Cannon (1929), among others, who maintained that adrenaline is only released in response to stress. According to more modern views, however, adrenaline is discharged continuously from the adrenal medulla, even under basal conditions, provided

the sympathetic innervation is intact. Several authors (Dunér, 1953; Folkow and Euler, 1954; Houssay and Rapela, 1953; Wada and Kanowoka, 1935) have estimated the basal discharge from both suprarenals in cats and dogs and found values of 0·03–0·06 μg./kg./min. These figures are in excellent agreement with the approximate rate of peripheral utilization deduced from the arterio-venous difference of adrenaline. The values for the adrenaline concentration of plasma obtained by the fluorimetric method are therefore compatible with the established adrenaline output of the suprarenals.

Nevertheless, some physiologists assume a much lower level of circulating adrenaline. Holzbauer and Vogt (1954), on the basis of a biological method of assay, came to the conclusion that the resting adrenaline level in the plasma of a normal human subject was below 0·06 μg./l. These authors used a different method of blood collection, designed to prevent the disintegration of the blood platelets. When plasma that had been collected with similar precautions was analysed by the ethylenediamine method it was indeed found that a considerable proportion of the total adrenaline and noradrenaline could be removed by intensive centrifugation, presumably because it was associated with the blood platelets (Weil-Malherbe and Bone, 1954b). Some results are shown in Table II.

TABLE II

Adrenaline and Noradrenaline in Plasma and in Blood Platelets

Exp.	Method of Blood Collection	Fraction	A (μg./l. of blood)	N (μg./l. of blood)
1	NaF-$Na_2S_2O_3$*	Plasma+platelets	1·46	3·70
	Heparin†	Plasma+platelets	1·47	4·25
		Plasma (platelet-poor)	0·34	1·20
		Platelets	0·72	2·11
2	NaF-$Na_2S_2O_3$*	Plasma+platelets	1·06	4·32
	Heparin†	Plasma (platelet-poor)	0·52	2·10
		Platelets	0·53	2·48

A=adrenaline.
N=noradrenaline.

* Three vol. of blood collected in 1 vol. of solution containing 2 per cent. NaF and 3 per cent. $Na_2S_2O_3$ (Weil-Malherbe and Bone, 1952a). Ordinary glassware.

† Blood collected in siliconized glass vessel containing heparin as anticoagulant (200 units/10 ml.). Centrifugations carried out in silicone-coated tubes.

This observation, however, does not completely account for the discrepancy, since even in platelet-free plasma the adrenaline concentration obtained by the fluorimetric method is 5–10 times higher than the upper limit admitted by Holzbauer and Vogt. The writer finds himself in disagreement with Holzbauer and Vogt on another issue: whereas our experiments indicated a reduction of the plasma-adrenaline level during insulin hypoglycaemia by 50 per cent. or more (Weil-Malherbe and Bone, 1952b, 1954c), Holzbauer and Vogt found increases of up to 6 μg./l. in dogs and up to 1·8 μg./l. in a human subject. While it is of course impossible to assert with complete confidence that the fluorimetric method estimates nothing but adrenaline, it is considered highly improbable that it would have failed to detect increases of this order.

The function of the blood platelets as carriers of adrenaline and noradrenaline is in line with the fact that they harbour other pharmacologically active amines, viz. 5-hydroxytryptamine (Rand and Reid, 1951) and histamine, at least in the rabbit (Code, 1952). The results reported in subsequent sections

of this paper were obtained by a method which did not differentiate between free and platelet-bound adrenaline, yet some of the changes observed were so large that they cannot be accounted for by the free fraction alone. It must be assumed therefore that the platelet-bound fraction is a readily available reserve that may undergo extensive changes. This assumption is borne out by preliminary results obtained in experiments on the effect of insulin hypoglycaemia. They show (Fig. 3) that the decrease of concentration elicited by insulin is

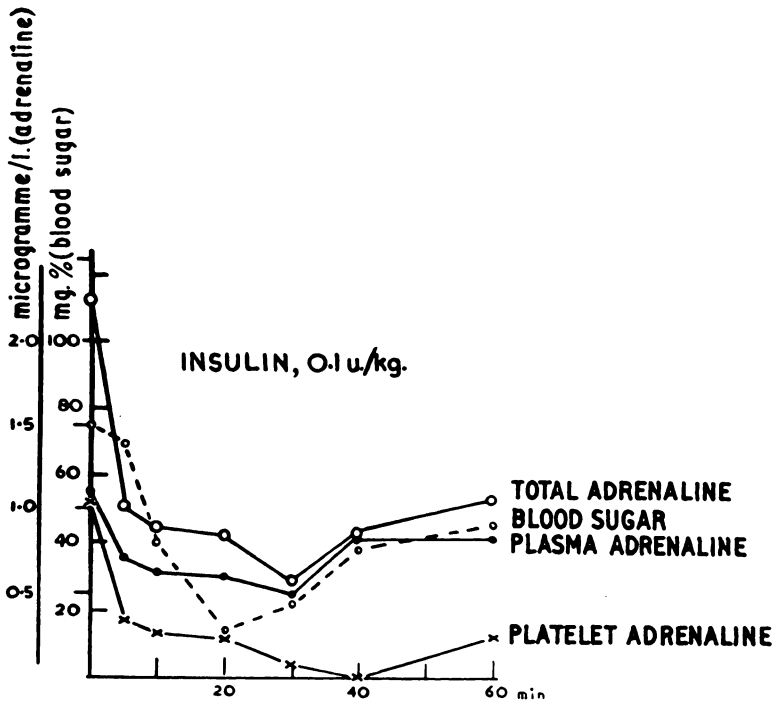

FIG. 3.—Effect of an intravenous injection of insulin (0·1 unit/kg.) on fractions of plasma-adrenaline. (Weil-Malherbe, 1954b.)

relatively much more marked in the platelet-bound, than in the free fraction of plasma adrenaline, and that at one point of the time curve no adrenaline whatever is found to be associated with the platelets. It seems probable, therefore, that, in order to be physiologically active, adrenaline must be released from its association with the platelets.

THE CONCENTRATION OF CATECHOL AMINES IN CEREBROSPINAL FLUID

The fluorimetric method has also been applied to determine adrenaline and noradrenaline in CSF, and mean concentrations of 0·9 μg./l. and 2·5–3 μg./l., respectively, were found (Weil-Malherbe and Liddell, 1954). The levels in CSF were only 40–60 per cent. of the concentration in plasma; the distribution ratio was statistically equal for adrenaline and noradrenaline and hence the ratio of adrenaline/noradrenaline was, on the average, the same in CSF as in plasma.

In some more recent experiments (Table III) the free and platelet-bound fractions of the catechol amines in plasma were determined separately. The corresponding fractions of CSF that had been collected simultaneously were

TABLE III

The Concentration of Adrenaline and Noradrenaline in Plasma and CSF and Their Occurrence in Free Solution and in Association with Particulate Matter

Exp.	Fraction	Concentration (μg./l. of plasma or CSF) A	Concentration (μg./l. of plasma or CSF) N	Ratio CSF × 100 / Plasma (platelet-free) A	Ratio CSF × 100 / Plasma (platelet-free) N
1	Plasma, platelet-poor	1·47	5·05		
	Plasma platelets ..	1·44	5·56		
	CSF, spun at 12,000 *g* ..	0·52	1·58	35·4	31·3
	CSF, precipitate ..	0	0		
2	Plasma, platelet-poor ..	1·00	3·88		
	Plasma platelets ..	1·50	5·35		
	CSF, spun at 12,000 *g* ..	0·52	2·82	52	72·6
	CSF, precipitate ..	(0·10)	(0·35)		

A = adrenaline.
N = noradrenaline.
Blood collected in heparin. Silicone-coated glassware used throughout.

processed by the same methods and with the same precautions as the plasma fractions. Intensive centrifugation had no significant effect on the concentration of the catechol amines in CSF and there is therefore no reason to assume the existence of a fraction of catechol amines associated with corpuscular matter.

A CORRELATION OF THE PLASMA ADRENALINE WITH THE AROUSAL FROM HYPOGLYCAEMIC COMA

The blood level of any substance is the resultant of two opposing processes, the rate of discharge and the rate of disappearance. One has become accustomed to associate a state of increased sympathetic activity with an increased level of blood adrenaline and *vice versa*. On the whole, our findings have shown this view to be justified, although there is one exception: whereas insulin hypoglycaemia is known to stimulate sympathetic activities, it has been found that the intravenous injection of insulin entails an immediate lowering of the plasma adrenaline concentration, which precedes the onset of hypoglycaemia (Weil-Malherbe and Bone, 1954c). The determination of the adrenaline arteriovenous difference revealed that the reduction of the blood level is linked with a transitory increase of the arteriovenous difference, presumably indicating an increased utilization of adrenaline. Further evidence that insulin enhances the utilization of adrenaline is provided by the work of Hökfelt (1951) who found an increased fixation of adrenaline in the liver and heart of insulinized rats, which could be reversed by the administration of glucose. It seems reasonable, therefore, to attribute the depression of the blood-adrenaline level by insulin to an increase of adrenaline utilization in the tissues rather than to a diminished discharge from the adrenal medulla.

It is tempting to connect this phenomenon with the effect of adrenaline on the phosphorylase reaction. Phosphorylase is the enzyme that is responsible for the reversible "phosphorolytic" splitting of glycogen, resulting in the formation of glucose-l-phosphate. The enzyme exists in an active and an inactive form in both liver and muscle. An increase of muscular activity accelerates the conversion of the active form of muscle phosphorylase into the inactive form, presumably because of an increased rate of the phosphorolytic reaction.

Adrenaline has the opposite effect: it accelerates the resynthesis of the active from the inactive form (Sutherland and Cori, 1951; Sutherland, 1951).

Insulin, by pushing increased amounts of glucose into the cell, activates the synthesis of glycogen while the blood sugar is high, particularly in muscle; during the later phases of insulin action, when the blood sugar is low, glycogenolysis is increased, particularly in the liver. Both phases of insulin action therefore lead to an increase of phosphorylase activity. This may be expected to have the same consequence as the increased phosphorylase activity resulting from muscular activity, viz. an increased conversion of the active into the inactive form.

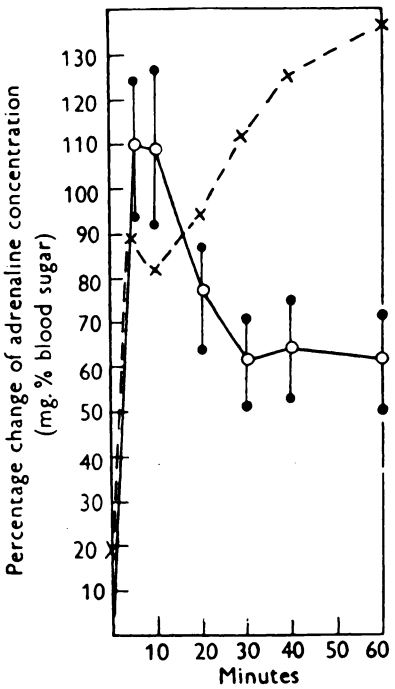

FIG. 4.—The effect of an intravenous injection of glucose (50 ml. 33·3 per cent. solution) on the plasma-adrenaline level during hypoglycaemic coma.
Full line: adrenaline, mean concentration change (per cent.) and its standard error.
Broken line: mean blood-sugar concentration (mg. per cent.).
From Weil-Malherbe and Bone (1954c).

It is suggested that an acceleration of the phosphorylase reaction is the cause of the increased utilization of adrenaline observed after insulin administration.

The spontaneous reversal of insulin hypoglycaemia is preceded by a gradual return of the blood-adrenaline level to its normal value. When, however, hypoglycaemic coma is terminated by the administration of glucose, the blood-adrenaline concentration rises dramatically to a peak well above the normal resting value and subsequently declines to the basal level (Fig. 4). This effect of glucose administration is not confined to insulin hypoglycaemia, but occurs also under the conditions of the simple glucose tolerance test (Fig. 5). The change in the adrenaline concentration is obviously not due to hyperglycaemia, since it precedes the change in the blood-sugar concentration. The effect is specific for glucose, since the ingestion of a fructose meal does not affect the blood-adrenaline level. The blood-noradrenaline level is unaffected by either glucose or fructose ingestion (Weil-Malherbe and Bone, 1954d).

It is difficult to imagine why the administration of glucose would induce an increased discharge of adrenaline from the adrenal medulla, especially in view of the results of Dunér (1953) who showed the existence of a reflex, probably mediated by a hypothalamic centre, by which a rise in blood sugar depresses the secretion of adrenaline by the adrenal medulla. On the other hand, it is likely that the administration of glucose, especially during hypoglycaemia or in the

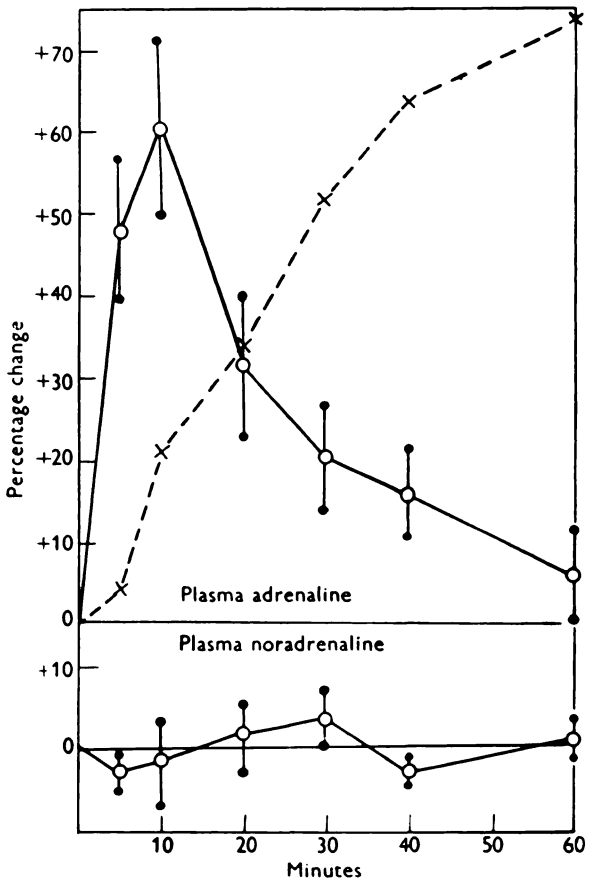

FIG. 5.—The effect of 50 g. glucose, taken by mouth, on the concentration of adrenaline and noradrenaline in plasma.
Broken line: mean concentration change of blood sugar.
From Weil-Malherbe and Bone (1954d).

post-absorptive state, reduces the activity of phosphorylase—and thereby the utilization of adrenaline—because exogenous glucose replaces the glucose mobilized from the glycogen reserves of the body. This mechanism is the logical counterpart of that proposed to explain the effect of insulin on the blood-adrenaline concentration.

When glucose is given intravenously during hypoglycaemic coma, consciousness usually returns within 5 minutes of the injection, coinciding with the peak of the blood-adrenaline curve. After the administration of glucose by nasal intubation arousal may be delayed for 10–30 minutes or more. It was observed regularly in a series of experiments that the time of arousal closely

corresponded with the peak of the blood-adrenaline curve. The simultaneity of these events suggested a causal relationship.

If we assume that adrenaline has other functions in addition to that concerning phosphorylase, then a decrease of adrenaline utilization in the phosphorylase reaction may, by raising the adrenaline concentration in blood, cause their stimulation. If the arousal from hypoglycaemic coma is connected with those other functions of adrenaline, it should be possible to obtain the same result that is observed after glucose administration by other means capable of elevating the blood-adrenaline concentration, such as the stimulation of adrenaline discharge from the adrenal medulla or the injection of exogenous adrenaline. This is indeed the case.

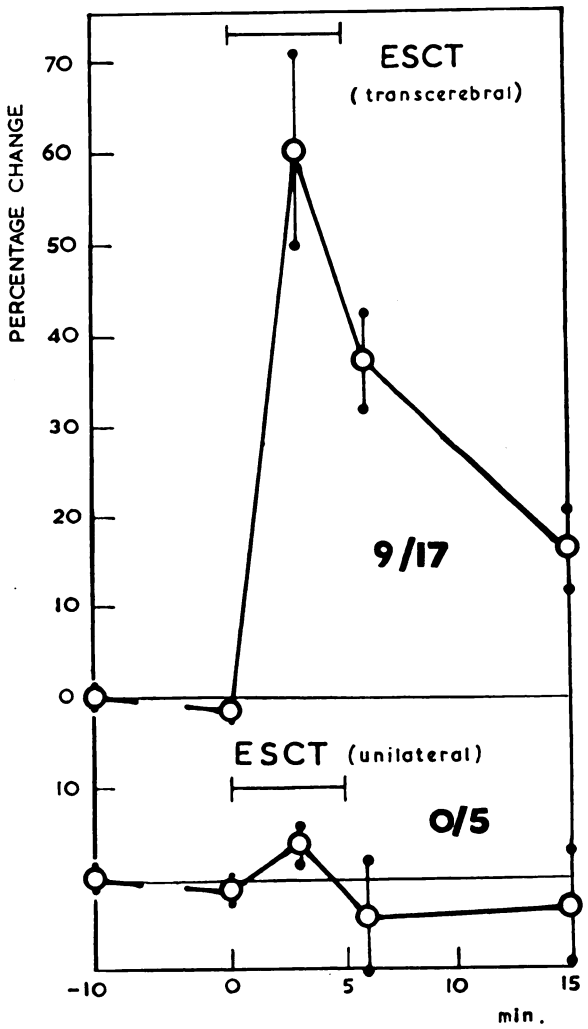

FIG. 6.—Effect of electric subconvulsive therapy (ESCT) on the concentration of catechol amines in plasma during hypoglycaemic coma.
The points indicate mean concentration changes and their standard errors.
The barred horizontal line indicates the duration of ESCT.
Top curve: electrodes in standard biparietal position.
Bottom curve: electrodes on one side of skull.
The ratios show the proportion of cases roused. Data of Weil-Malherbe and Bone (1952b).

Fig. 6 shows the mean percentage change of the concentration of catechol amines in blood resulting from the application of a subconvulsive electric current during hypoglycaemic coma.* The first two blood samples were taken before the treatment, with a 10-minute interval; their agreement shows the absence of spontaneous change during this period. The next blood sample was collected 3 minutes after switching on; it showed an increase of about 60 per cent. Treatment was discontinued after 5 minutes. One minute later the catechol amine concentration was only 37 per cent., and 10 minutes later 16 per cent., above the initial value. In spite of the fact that the elevation of the catechol amine level was poorly sustained, arousal was achieved in 9 cases out of 17 (Weil-Malherbe and Bone, 1952b). No effect either on the catechol amine level

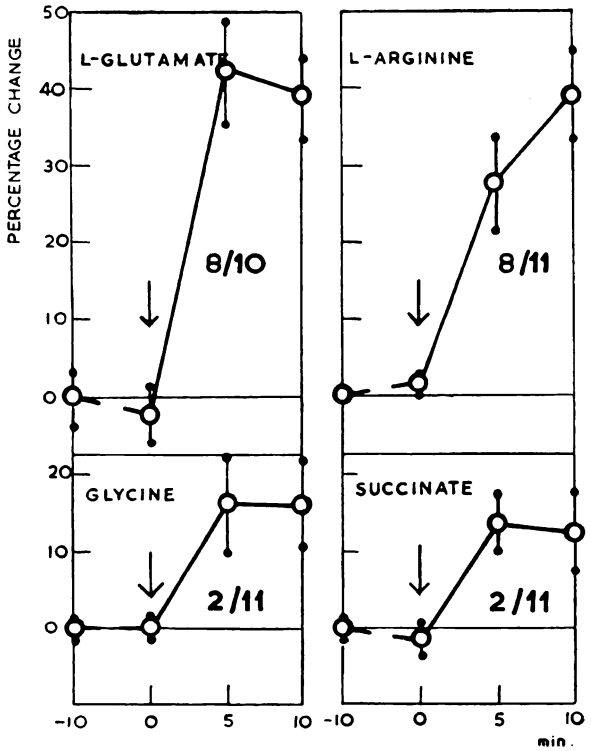

FIG. 7.—Effect of intravenous injection of various substances on the concentration of catechol amines in plasma during hypoglycaemic coma. The points indicate mean percentage concentration changes and their standard errors. The arrow indicates the moment of injection. The ratios show the proportion of cases roused. Data of Weil-Malherbe and Bone (1952b).

in blood or on the comatose state was observed when both electrodes were placed on the same side of the skull instead of in the usual biparietal position. The subjects chosen for these experiments had previously given a positive response to the same current when the standard electrode position was used. It may be concluded that the effect is only observed when the current is conducted across the brain and that it is presumably due to a stimulation of autonomic centres.

An increase of the catechol amine level in blood was also observed when certain amino acids were injected intravenously during hypoglycaemic coma. Fig. 7 shows the mean percentage changes produced by 3 amino acids, L-

* These experiments were carried out in collaboration with Dr. J. D. Montagu.

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glutamic acid, L-arginine and glycine, and by a related dicarboxylic acid, succinic acid. All substances were injected in amounts of 0·017 moles dissolved in 10 ml. and adjusted to pH 7·0. The first 2 blood samples, withdrawn before the injection, show the absence of spontaneous change within a period of 10 minutes. The ratios shown in Fig. 7 refer to the proportion of cases in whom consciousness was restored by the treatment. With glutamic acid and arginine 8 successes were achieved out of a total number of 10 or 11 experiments, respectively. With glycine and succinic acid, however, only 2 successes were scored out of 11 tries in each case. Correspondingly, the catechol amine level was markedly raised by the first 2 substances, much less by the second two. There is thus a clear-cut correlation between the effects on consciousness and on the blood-adrenaline level (Weil-Malherbe and Bone, 1952b).

Finally, arousal from hypoglycaemic coma is observed in a high proportion of cases (9/11) when adrenaline itself is administered by slow intravenous infusion (Weil-Malherbe, 1949).

It is concluded from these results that the coincidence of arousal with the rise of the plasma-adrenaline concentration is due not to a merely chronological, but to a causal relationship.

The Plasma-Adrenaline Level During Anaesthesia and Convulsive Activity

Further evidence for a connection between the blood-adrenaline level and the activity of the nervous system was obtained in studies dealing with the effects of various forms of electroconvulsive therapy (E.C.T.). In these experiments (Weil-Malherbe, 1955) electrical convulsions were produced (1) without modification, (2) after barbiturate administration and (3) after the combined injection of a barbiturate and a paralysing agent. The effect of leptazol convulsions, with and without modification by a paralysant, was also investigated. Four blood samples were collected during each treatment, 2 before and 2 after the seizure. In the experiments in which unmodified E.C.T. was used, and also in those in which leptazol, with or without modification, was used, the second sample was withdrawn 10 minutes after the first, but before the treatment was started; it thus served merely to show the absence of spontaneous variation (Figs. 8 and 9). In the experiments in which modified E.C.T. was studied the second blood sample was withdrawn within 1 minute after the injection, but before the application of the current. The injection of the barbiturate was followed by an immediate and dramatic reduction of the plasma-adrenaline level, which was unchanged by the addition of a paralysant (Brevedil M, succinylcholine). In the third blood sample which was collected as soon after the fit as twitchings or convulsions had ceased, a striking increase of the blood-adrenaline concentration was found. Ten minutes later, when the fourth blood sample was taken, the adrenaline level had returned to about the initial value; in those experiments in which a barbiturate was given it returned to a value below the initial level, indicating the persistence of some sedative action. But the rise of the adrenaline concentration in response to the convulsion was only slightly reduced when a barbiturate was injected. Measured by the difference of the adrenaline levels found in samples 3 and 4, the extent of the response is about equal in the 3 forms of E.C.T. After leptazol convulsions the rise of the adrenaline concentration is almost twice as great as after E.C.T. and this effect, too, is not significantly changed by the administration of a paralysant (Brevedil E).

The noradrenaline concentration of plasma is raised by about 40 per cent. after an unmodified convulsion, whether produced by electricity or leptazol. The effect decays more slowly than the adrenaline response and the level is still significantly elevated after 10 minutes. However, the noradrenaline response is completely suppressed when the convulsion is modified by a paralysant, and almost completely, when it is modified by a barbiturate. The rise of the noradrenaline concentration in plasma is thus clearly the result of secondary sequelae of the unmodified procedures, such as muscular contractions, cardiovascular changes and anoxia. The adrenaline response, on the other hand,

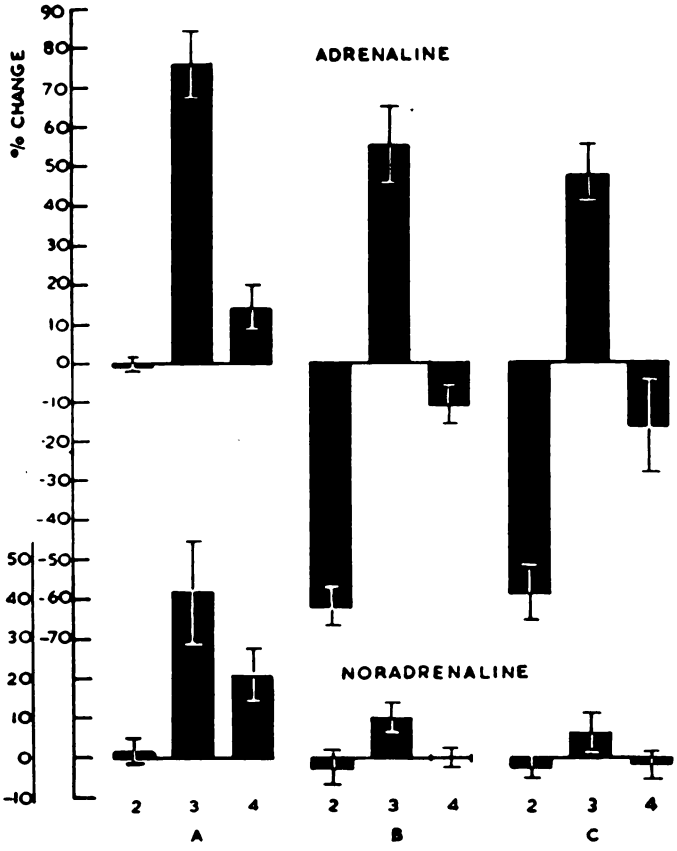

FIG. 8.—The effect of various forms of electroshock on the concentration of adrenaline and noradrenaline in plasma.
The bars indicate the mean percentage concentration changes in samples 2, 3 and 4 of each series. The barred lines indicate the standard error. A=unmodified E.C.T.; B=E.C.T. after injection of hexobarbitone; C=E.C.T. after combined injection of hexobarbitone and Brevedil M. From Weil-Malherbe (1955).

seems to be more directly connected with the increased neuronal activity of the convulsive discharge.

The inhibition of neuronal activity in anaesthesia has the opposite effect on the plasma-adrenaline level. The barbiturate used in these experiments was hexobarbitone, but other barbiturates act similarly, e.g. pentothal (Montagu, 1955) or sodium amytal.

What is particularly remarkable, is the rapidity with which the adrenaline level in plasma may change; thus, the change from the resting level to a value

60 per cent. below the resting level, and from there to a value 60 per cent. above the resting level is observed within the space of about 3 minutes, consequent upon the barbiturate injection and the electric shock, respectively.

Only a very superficial degree of anaesthesia was produced by the dose of hexobarbitone chosen. An effect on the plasma-adrenaline level is even found with purely sedative doses, such as are used as adjuvants to psychotherapy.

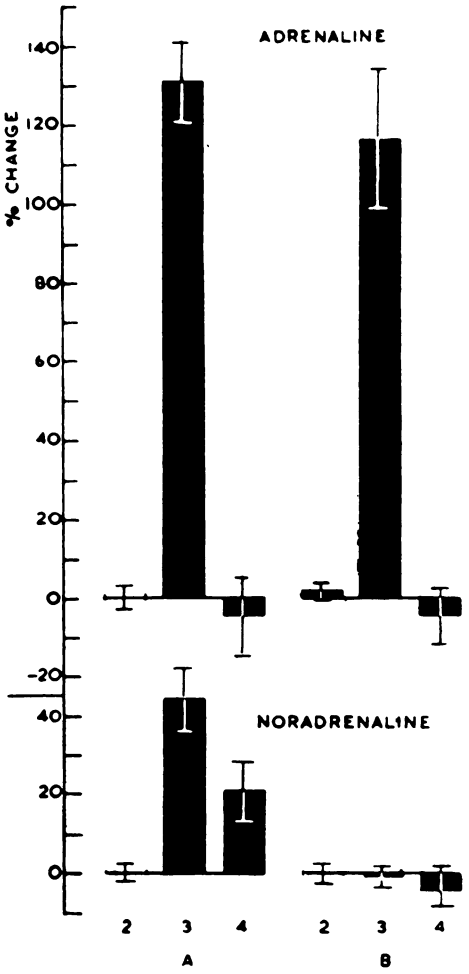

FIG. 9.—The effect of leptazol shock, with (B) and without (A) Brevedil E, on the concentration of adrenaline and noradrenaline in plasma. The bars indicate the mean percentage concentration changes, with the standard error shown on top. From Weil-Malherbe (1955).

The following case report may be of interest:

A woman of 35 was severely affected by ideas of depersonalization from which she experienced relief only under sodium amytal sedation. After a course of sodium amytal treatments it was suggested to her, under hypnosis, that she would receive her usual injection and that she would experience, post-hypnotically, her usual relief. After the suggestion had been made, the previously anxious and agitated patient manifested the symptoms of relaxation, sedation and somnolence and felt relief from her obsession, as she did previously after

sodium amytal injection. The change of the blood-adrenaline level was studied during a real and a suggested sodium amytal treatment and the results are shown in Table IV. It is evident that the suggestion had only a very slight effect on the blood-adrenaline level compared with the effect of the actual injection. Incidentally, this analysis was carried out before the method of differential estimation of adrenaline and noradrenaline was developed. The results are given in terms of catechol amines and the changes would probably have been more marked if it had been possible to estimate adrenaline separately.

TABLE IV

The Effect of an Actual and a Suggested Injection of Sodium Amytal (0·25 g., i.v.) on the Catechol Amines of Plasma. See Text

Exp. 1: Injection		Exp. 2: Hypnosis	
Time (minutes)	Catechol Amines (μg./l. of plasma)		Catechol Amines (μg./l. of plasma)
0	3·43	Before hypnosis	3·56
10	2·68	Under hypnosis	3·51
30	2·50	5 minutes after suggested "injection"	3·44
60	2·74	Half-hour post-hypnotic	3·39
90	2·92		
120	3·37		
240	3·46		

It is to be expected that the spontaneous seizures of epilepsy affect the blood-adrenaline level in the same way as seizures induced by electricity or leptazol. Unfortunately, it has not yet been possible to study a spontaneous epileptic fit, but observations were made on a patient whose EEG showed evidence of epileptic discharge in response to photic stimulation (Fig. 10). Two experiments are illustrated in Fig. 11. The height of the blacked-in columns indicates the plasma-adrenaline level, while the plasma-noradrenaline level is shown by the overall height of the column, including the black part. In the first experiment (Fig. 11, A) photic stimulation was carried out intermittently for 1–2 minutes, followed by several minutes' rest. Each stimulation produced an increase of the adrenaline concentration that was not very large, but probably significant, since the same resting level was reached in the intervals. There was a drop of the noradrenaline level during the experiment, but this could not in any way be related to the procedure. In the second experiment (Fig. 11, B) stimulation by a frequency that did not evoke an EEG response had no effect on the plasma-adrenaline level, but a considerable increase was found in three successive samples withdrawn immediately after periods of stimulation by the critical frequency. The noradrenaline level again showed a tendency towards a decrease without a clear correlation with the experimental procedure. The EEG response to photic stimulation and at the same time the effect on the plasma-adrenaline level was completely suppressed in this case by barbiturate medication.

THE PLASMA ADRENALINE LEVEL DURING NATURAL SLEEP

It may be argued that the reduction of the plasma-adrenaline level by anaesthetics is not connected with their action on the CNS, but due to a peripheral mechanism. The effect of natural sleep on the adrenaline concentration in plasma has therefore been investigated (Renton and Weil-Malherbe, 1955).

In these experiments a sphygmomanometer cuff was tied around the subject's arm above the elbow when he went to bed. The cuff was inflated while he was asleep, and though this usually woke him up, the arterial circulation of the forearm was arrested and a venous blood sample was taken immediately.

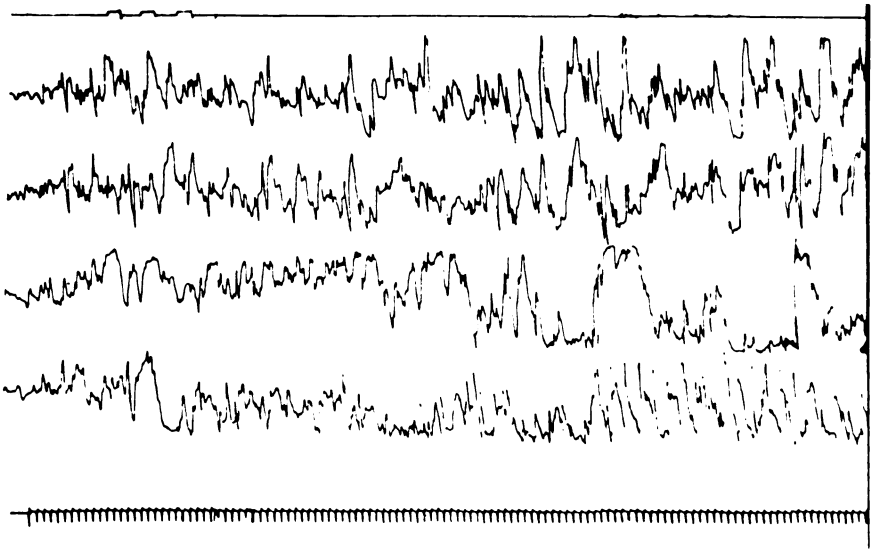

FIG. 10.—EEG response to photic stimulation. The bottom tracing is a photoelectric record of the stroboscope pulses.

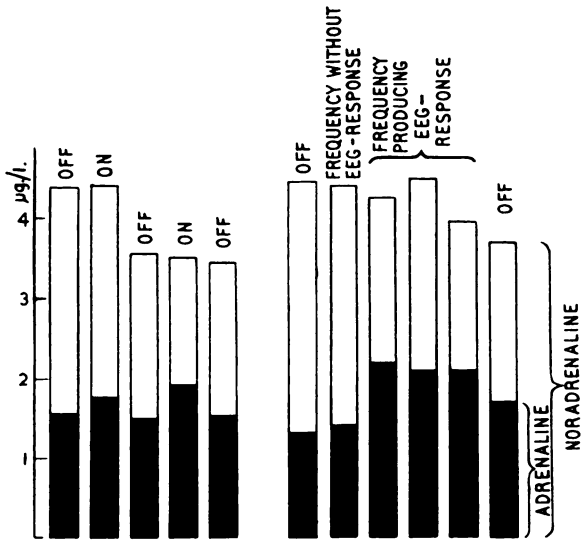

FIG. 11.—Effect of photic stimulation on the concentration of adrenaline and noradrenaline in plasma. Cf. text. (Weil-Malherbe, 1954b.)

Further blood samples were taken either 5–10 minutes after waking or on the following morning before breakfast, or at both these times. Table V shows the mean percentage increase of the plasma adrenaline level of the "waking" samples compared with the "sleeping" sample. The increases are significant

TABLE V

The Effect of Natural Sleep. Mean Percentage Concentration Changes of Adrenaline and Noradrenaline in Plasma after Waking, Compared with "Sleeping" Sample

	No. of Observations	Adrenaline	Noradrenaline
5–10 minutes after waking	10	+37·75%	+7·6%
Standard Error		6·19	4·88
P		<0·01	~0·15
Next morning (fasting)	13	+26·06%	−2·9%
Standard Error		7·08	3·06
P		<0·01	~0·35

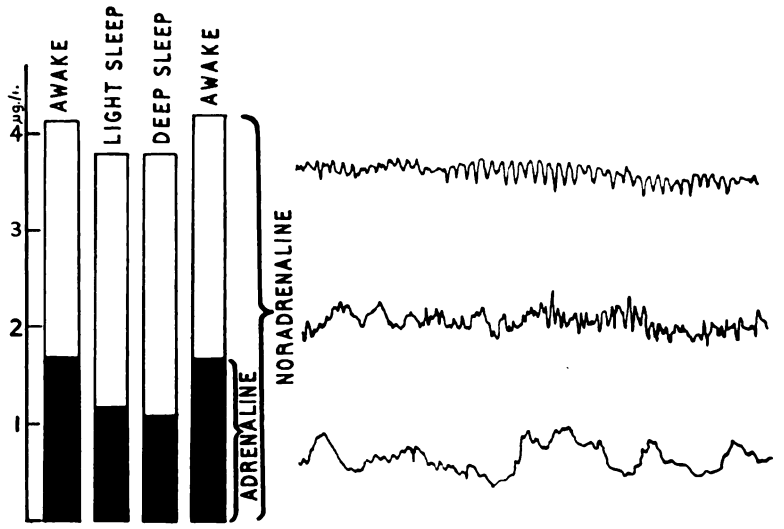

FIG. 12.—Concentration changes of adrenaline and noradrenaline in plasma during sleep. Cf. text. (Renton and Weil-Malherbe, 1955.)

whether the sample was collected immediately on waking or on the following morning. There was no significant effect on the plasma-noradrenaline level.

Another experiment, illustrated in Fig. 12, was performed on a healthy volunteer who stayed up all night and, on the following day, went to sleep while his EEG was recorded. Blood samples were taken before he went to sleep (normal alpha rhythm, top tracing of Fig. 12), when his EEG record indicated light sleep (middle tracing of Fig. 12), again when the record indicated deep sleep (bottom tracing of Fig. 12) and finally when he was awake at the end of the experiment. The plasma-adrenaline level, represented by the black columns, dropped during sleep and returned to the initial value after waking. There was also a slight reduction of the noradrenaline level during sleep.

The effect of sleep, though significant, is smaller than that of anaesthesia, which is not surprising in view of the ease with which natural sleep is reversible.

THE EFFECT OF SOME RELAXANT DRUGS ON THE PLASMA-ADRENALINE LEVEL

Not all reductions of the plasma-adrenaline level are of central origin. Preliminary experiments have shown that adrenergic or ganglionic blocking agents, such as dibenamine or hexamethonium, have this effect. In particular,

two drugs have been studied, which are now widely used in psychiatry for their relaxing and sedative effects: myanesin (Fig. 13) and largactil (Fig. 14). Myanesin, in doses of 2 g. by mouth, led to a fall of the adrenaline level in the 5 cases investigated. The effect was at its maximum after about ½ hour when the mean concentration was reduced by 50 per cent., a change that was statistically significant. There was also a tendency for the noradrenaline concentration to decrease, but this effect was less consistent. Largactil was injected intramuscularly in doses of 100 mg. In the 3 cases investigated, so far, a reduction of the adrenaline level was observed in two. The third case showed an initial rise, followed by a drop. Similar biphasic reaction curves were previously observed after the injection of methedrine and lysergic acid diethylamide (Liddell and Weil-Malherbe, 1953). The changes of the noradrenaline level observed after largactil medication again showed no definite trend or pattern and are difficult to interpret.

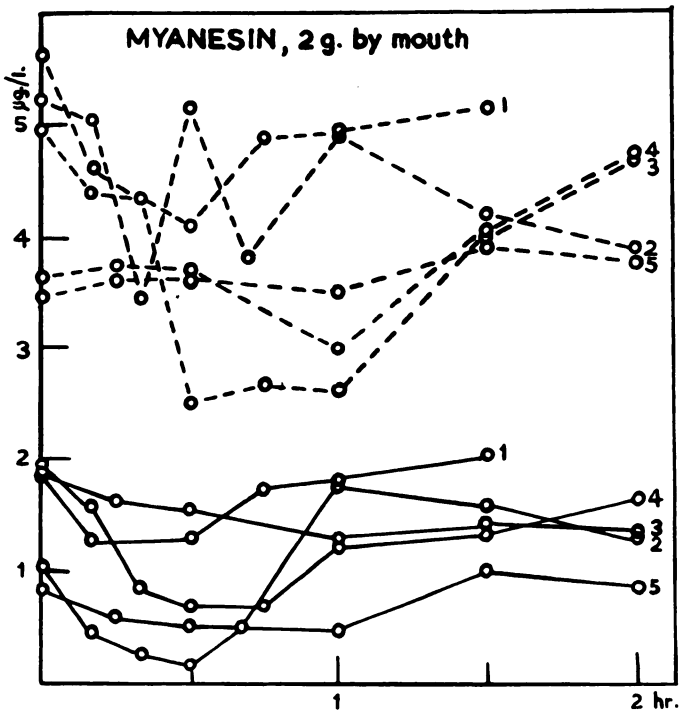

FIG. 13.—Effect of myanesin on the concentration of adrenaline (full lines) and noradrenaline (broken lines) in plasma. (Weil-Malherbe, 1954b.)

Clinically these drugs were found to alleviate symptoms of tension, anxiety and agitation, subjectively and objectively. Their mechanism of action is not yet fully understood and the writer does not wish to imply that they are peripheral blocking agents, but whatever the mechanism, it is relevant that a decrease of mental and physical tension is associated with a decrease of the plasma-adrenaline level.

THE PLASMA-ADRENALINE LEVEL IN MENTAL DEFICIENCY AND SENILE DEMENTIA

Although the investigations described in this section arose from an entirely different hypothesis, they turned out, in the end, to have some bearing on the

relationship between mental activity and the blood-adrenaline level. The original hypothesis was that the metabolic error of phenylpyruvic oligophrenia might entail an impairment of adrenaline synthesis; since the formation of tyrosine from phenylalanine is blocked in this condition and since tyrosine is the precursor of adrenaline, such a possibility was worth considering. When it was found that the plasma-adrenaline level was indeed abnormally low in these cases, the study was extended to other forms of congenital mental deficiency and to a group of patients suffering from senile dementia. For comparison, the results of adrenaline estimations obtained during one year in various groups of patients were collected (Weil-Malherbe, 1954a).

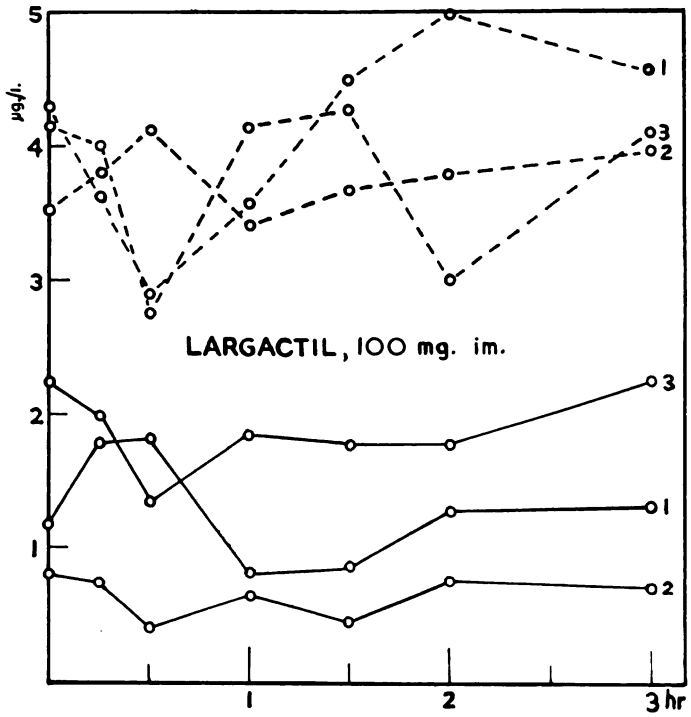

FIG. 14.—Effect of largactil on the concentration of adrenaline (full lines) and noradrenaline (broken lines) in plasma. (Weil-Malherbe, 1954b.)

In Fig. 15 the results are shown in the form of a histogram, with the bars arranged in the order of increasing adrenaline concentration. Each bar represents the mean value of the plasma-adrenaline concentration, with the limits of the standard error indicated by the barred line. The figures at the foot of each column indicate the number of observations and the number of cases. The two figures do not always tally, because some cases were examined repeatedly. Wherever multiple observations from the same individual were available, the mean value was calculated and entered as a single observation when the group average was computed. On the whole, the individual variation was similar to the variation within the group. Only blood samples that were collected under basal conditions were used for this survey.

On the left of Fig. 15 are 5 oligophrenic groups, on the right are groups of mental hospital patients and, in addition, a group of peptic ulcer patients, most of whom have had a gastrectomy; their blood samples were sent to us from

another hospital. These groups are very heterogeneous, including, as they do, cases of psychosis, both acute and chronic, psychopathy and neurosis, as well as organic diseases. What they had in common was the fact that their intellectual faculties were not grossly impaired.

Congenital mental defectives other than those belonging to the group of phenylpyruvic oligophrenia were classified as idiots, imbeciles and feeble-minded persons by conventional criteria. The group of idiots contained one case of hemiplegia and one of mongolism. Among the imbeciles were 8 cases of epilepsy, 2 of mongolism, 2 of spastic diplegia and 1 of microcephaly. The remainder of these groups was free from somatic abnormalities. Of the 13 cases of phenylpyruvic oligophrenia 7 were classified as idiots and 3 as imbeciles. No information was available on the remaining 3 cases.

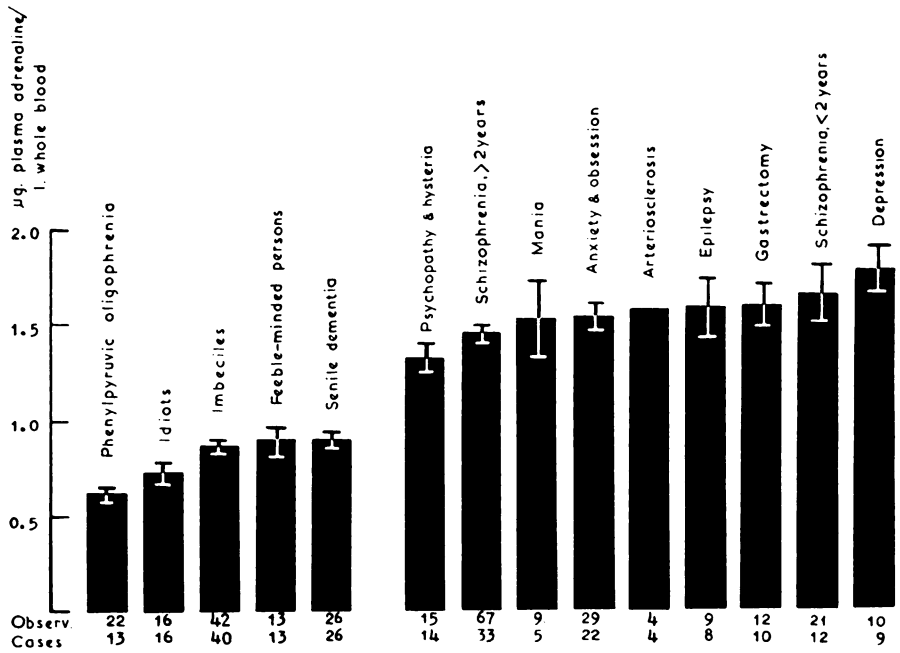

FIG. 15.—Mean plasma-adrenaline concentration in various groups of oligophrenic and non-oligophrenic patients. The barred lines indicate the standard error of the mean. (Data of Weil-Malherbe, 1954a.)

If the oligophrenic groups are considered first, it appears that the average plasma-adrenaline level is lowest in the group of phenylpyruvic oligophrenia, a little higher in the group of idiots, and higher still in the remaining three groups, which have almost identical mean values. This order suggests a correlation with the severity of the mental defect. The difference between the group average of the phenylpyruvic oligophrenia cases and the common mean of the other mental deficiency groups is statistically significant.

As far as the non-oligophrenic groups are concerned, it is remarkable how slight are the variations among the average plasma-adrenaline levels of the different sub-groups, in spite of their heterogeneity. The psychopathy-hysteria group deviates most from the common average in the negative direction, the depression group most in the positive direction. The difference between these two groups is significant, while those between most of the other groups are not (cf. Weil-Malherbe, 1954a).

Compared with the differences among the sub-groups, the difference between the oligophrenic groups on the one hand, and the non-oligophrenic groups on the other is quite striking. The common average of the latter exceeds that of the former by 83 per cent., a difference that is highly significant (Table VI).

TABLE VI

The Concentration of Adrenaline and Noradrenaline in the Plasma of Oligophrenic and Non-oligophrenic Hospital Patients

Groups	No. of Cases	Adrenaline			Noradrenaline		
		Mean (μg./l. of blood)	S.E.*	P†	Mean (μg./l. of blood)	S.E.*	P†
Non-oligophrenic, combined	117	1·52	0·035		4·10	0·062	
Oligophrenic, combined ..	108	0·83	0·024	<0·01	3·79	0·069	<0·01
Oligophrenic, omitting phenylpyruvic oligophrenia	95	0·86	0·025	<0·01	3·84	0·071	<0·01
Oligophrenic, omitting senile dementia and phenylpyruvic oligophrenia ..	69	0·84	0·030	<0·01	3·83	0·090	0·015

* S.E.=Standard Error of Mean.

† P=probability that difference from mean of combined non-oligophrenic groups is due to chance.

The groups are fairly well matched with regard to sex, but not so well with regard to age. The 3 mental deficiency groups (idiots, imbeciles and feeble-minded persons) consist of adults up to the age of 57 and are closest in age distribution to the bulk of the non-oligophrenic groups. The phenylpyruvic oligophrenia group contains only children up to the age of 13, while the group of senile dementia patients is composed of people in their seventies and over. Among the non-oligophrenic groups is a small group of 4 patients, aged 64–78, who suffered from arteriosclerosis without intellectual deterioration. The group was too small to calculate its standard error, but the agreement with the mean values of the other non-oligophrenic groups is sufficiently close to attribute the low values found in the senile dementia group to causes other than old age. We have also had an opportunity of analysing some blood samples of normal children; in most of them values were found that were normal by adult standards. The possibility must further be conceded that, in the case of phenylpyruvic oligophrenia, the depression of the plasma-adrenaline level is a consequence of the metabolic error rather than the mental defect. But even if the group of phenylpyruvic oligophrenia is omitted, the general conclusions remain unchanged (Table VI).

Fig. 16 shows the mean values of the plasma-noradrenaline levels in the same groups, arranged in the same order as in Fig. 15. The differences are smaller than those of the plasma-adrenaline levels. The average noradrenaline level of the oligophrenic groups is only about 7 per cent. below that of the non-oligophrenic groups, but, small though this difference is, it is statistically significant. The result remains the same even if the phenylpyruvic oligophrenia group —where the noradrenaline level is most strongly depressed—is omitted (Table VI).

These observations show that dementia and mental deficiency of different aetiology are associated with a reduced level of circulating adrenaline. It is tentatively suggested that this effect is a symptom of the reduced mentation processes in these patients.

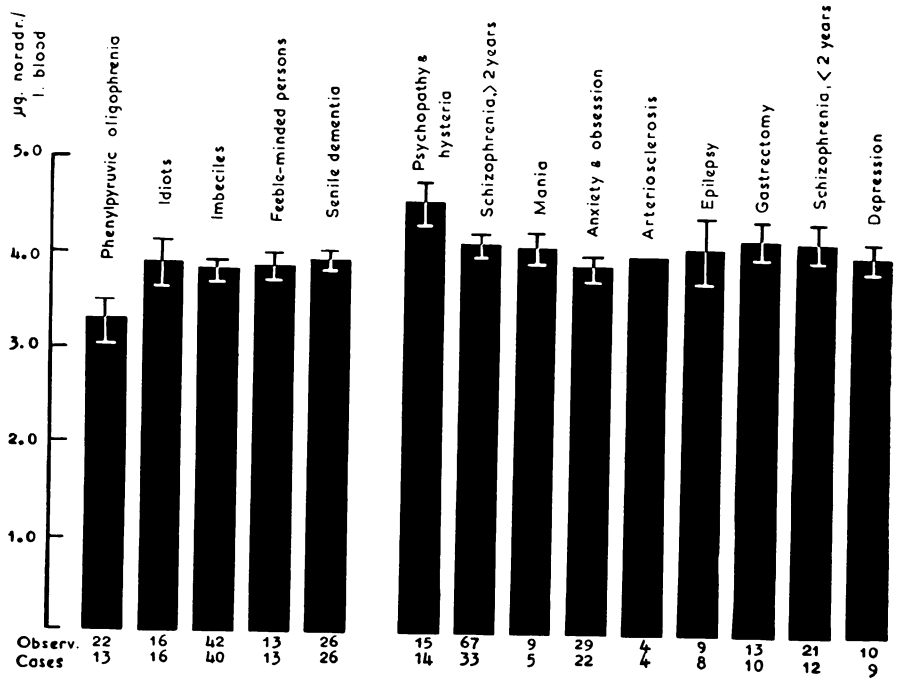

FIG. 16.—Mean plasma-noradrenaline concentration in various groups of oligophrenic and non-oligophrenic patients. The barred lines indicate the standard error of the mean. (Data of Weil-Malherbe, 1954a.)

SUMMARY AND CONCLUSIONS

The results described in this review seem to indicate a broad correlation between the plasma-adrenaline level and the level of consciousness or the extent of nervous activity. Anaesthetics and relaxing agents lead to a drop of the adrenaline level, sometimes within a minute of the injection. The plasma-adrenaline level is also depressed during natural sleep. On the other hand, the return of consciousness at the termination of hypoglycaemic coma coincides with a peak of the plasma-adrenaline concentration. Electrical stimulation of the brain, the injection of a convulsant drug, or photic stimulation in a susceptible subject, produce a great rise of the plasma-adrenaline level, even if convulsions are suppressed. Finally, the lowering of the adrenaline level in oligophrenia of different aetiology suggests that it is a symptom of the reduced mentation processes in these patients.

ACKNOWLEDGMENTS

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