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THE CATECHOLAMINE CONTENT OF THE PERIPHERAL PLASMA IN HUMAN SUBJECTS WITH COMPLETE TRANSVERSE LESIONS OF THE SPINAL CORD

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It is now believed that noradrenaline is the main and possibly the only catecholamine liberated at post-ganglionic adrenergic nerve ending (Peart, 1949; von Euler, 1951). There is still doubt however whether, in the intact animal, any of this noradrenaline finds its way into the circulation (Celander, 1954). Using sensitive chemical methods various workers (Weil-Malherbe & Bone, 1953; Valk & Price, 1956; Cohen & Goldenberg, 1957; Robinson & Stott, 1958; Munro & Robinson 1958*a*; Keenan, Kleitsch & Humoller, 1959), have consistently found noradrenaline, as well as adrenaline, in the normal human plasma although the levels observed have varied widely. The origin may be, of course, the adrenal medulla rather than the sympathetic nerve endings, but the information available on this point from animal experiments is difficult to interpret. If, as appears likely, adrenaline and noradrenaline are liberated into the peripheral blood stream, the question arises whether the concentration present at a particular time reflects the prevailing level of activity of the sympathetic and adrenal-medullary systems.

Our particular interest was the sympathetic nervous system in human spinal subjects; and with this object measurements of plasma adrenaline and noradrenaline were made on a group with complete transverse lesions at different levels of the spinal cord. If noradrenaline is liberated into the blood in proportion to the degree of sympathetic nervous activity, it might be expected that the circulating level in resting spinal subjects would be below the normal and possibly related to the level of the cord lesion. Furthermore, some information might be gained regarding the character of the human adrenal-medullary secretion by noting the change, if any, in the proportion of plasma adrenaline to noradrenaline occurring within the range of lesions at the spinal levels giving origin to the nerves

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supplying the gland. For comparison, data have been included for a group of normal persons and another group before and after bilateral adrenalectomy.

Eosinophil counts were made on blood samples from the spinal group. Since normal individuals develop an eosinopenia in response to injected adrenaline, it appeared likely that a significant eosinophilia in the spinal group could be taken as additional, although admittedly indirect, evidence of subnormal levels of adrenaline in the plasma.

Chemical methods of assay for adrenaline and noradrenaline in peripheral plasma have been criticized on the grounds of specificity. Biological methods have been regarded as more acceptable in this respect, but they lack the required sensitivity. We have used in the present investigation the chemical method originally developed by von Euler & Floding (1955*a*, *b*) for analysis of adrenaline and noradrenaline. The method has been modified by the incorporation of extra blanks and by using a highly sensitive fluorimeter (Robinson & Stott, 1958).

METHODS

The blood samples were collected from the median cubital vein into 20 ml. all-glass syringes containing the anticoagulant. Unless otherwise stated the blood was collected after the subjects had been seated for 2–3 min but not specially rested.

Blood samples were obtained from sixteen healthy medical students and laboratory workers. Since posture affects the catecholamine values (Munro & Robinson, 1958*b*) the collections were made from these subjects in both the supine and the upright sitting positions so that the results could be more strictly compared with those of other workers.

Blood was also collected from forty patients with complete transverse lesions of the spinal cord, the result of fracture-dislocations of the spine. The level of the cord lesions varied from the 4th cervical to the 3rd lumbar segments. These patients were in the upright sitting position during blood sampling and were well recovered from their initial injury.

Blood samples were also obtained from eight female patients before and after bilateral adrenalectomy for carcinoma. The first samples were collected 2–4 days before the first operation and the second 6–8 days after the second operation. The collections in these cases were made with the patients lying in bed. A ninth patient was examined 9 months after operation for bilateral adrenalectomy but not before operation.

Five millilitres of a solution containing sodium fluoride 2 g and sodium thiosulphate 3 g/100 ml. were drawn into a 20 ml. syringe, and the blood sample then drawn from the medial cubital vein up to the 20 ml. mark. With the minimum of delay the blood was centrifuged at approximately 2000 rev/min and the plasma separated.

In a few of the normal subjects blood was collected with special precautions to maintain the integrity of the plasma. The collection was made into silliconed syringes containing approximately 500 i.u. heparin in a volume of 0·05 ml. Immediately after collection it was put into siliconed centrifuge tubes packed in ice and centrifuged as above, and the plasma separated (Holzbauer & Vogt, 1954).

Eosinophil counts were carried out by the method of Pilot (1950) on forty-five fit subjects with spinal cord lesions of long standing. These subjects were in the upright sitting position during sampling.

The catecholamines were isolated on micro-columns of alumina by the method of

Weil-Malherbe & Bone (1952). The adrenaline and noradrenaline content of the eluate was determined fluorimetrically by a modification (Robinson & Stott, 1958) of the method of von Euler & Floding (1955*a*, *b*).

RESULTS

Adrenaline and noradrenaline levels in peripheral plasma

Normal subjects. In normal healthy persons who had been sitting upright for 2–3 min after being engaged in the usual laboratory and ward duties the plasma adrenaline, as shown on Table 1, was $2{\cdot}3 \pm 0{\cdot}36\,\mu g/l.$ (mean of sixteen subjects $\pm$ S.D.) and the noradrenaline $4{\cdot}28 \pm 0{\cdot}64\,\mu g/l.$ (mean of fourteen subjects).

After the subjects had been supine for 40–50 min the corresponding values were: adrenaline $1{\cdot}72 \pm 0{\cdot}51\,\mu g/l.$ (mean of seventeen subjects); and noradrenaline $2{\cdot}2 \pm 0{\cdot}26\,\mu g/l.$ (mean of seventeen subjects). The differences between the adrenaline values of the subjects in the supine and upright sitting positions is not statistically significant but the difference between the noradrenaline figures is significant ($P < 0{\cdot}05$).

Samples taken as described by Holzbauer & Vogt (1954), i.e. into heparin in siliconed syringes from four normal seated subjects, gave adrenaline and noradrenaline values very much lower than those quoted above. The mean adrenaline value as shown in Table 1 was below the threshold of measurement by the method used, i.e. $< 0{\cdot}3\,\mu g/l.$ plasma, whilst the mean for noradrenaline was $0{\cdot}9\,\mu g/l.$ (range $0{\cdot}6$–$1{\cdot}6\,\mu g/l.$) The difference between the results when the blood is taken into the sodium fluoride and sodium thiosulphate solution and when taken into heparin is altogether outside the range of experimental error or of individual variation.

Patients with complete transverse lesions of the spinal cord. Figure 1 shows the mean values for plasma adrenaline and noradrenaline, plotted in the form of histograms, in relation to the segmental level of the cord lesion. Plasma values for adrenaline and noradrenaline of normal subjects are also shown for comparison. There are considerable differences between the plasma adrenaline and noradrenaline values of the normal and of the spinal subjects with lesions high in the cord.

Table 2 shows that the concentration of plasma adrenaline in patients with complete cervical lesions is just above the limit of measurability with the methods used ($0{\cdot}3\,\mu g/l.$). These low values persist in cases with lesions down to a level below T3. A distinct increase occurs with lesions below T4 and T5 but the values are still below those found in normal controls. The plasma adrenaline attains its maximum in subjects with lesions below T6 and from this point is not significantly different from the normal value.

The changes in plasma noradrenaline are comparable to those of

TABLE 1. Comparison of reported values for adrenaline and noradrenaline in the peripheral plasma of normal human subjects

Condition of subjects	Method of assay*	Adrenaline (μg./l. $\pm$ S.D.)	Noradrenaline (μg./l. $\pm$ S.D.)	Method of collecting blood*	Authors
Seated for 3 min after normal activity	THI	2·3 $\pm$0·36	4·28 $\pm$0·64	Fluoride-thiosulphate	Munro & Robinson (1958*a*)
After lying supine for 50 min	THI	1·72 $\pm$0·51	2·2 $\pm$0·26	Fluoride-thiosulphate	Munro & Robinson (1958*b*)
After lying down for 30 min	EDA	1·3	4·1	Fluoride-thiosulphate	Weil-Malherbe & Bone (1953)
Seated for 3 min after normal activity	THI	< 0·3	0·9	Siliconed heparinized syringe	Munro & Robinson (1958*b*)
	THI	0·00 $\pm$0·01	0·2 $\pm$0·19	Heparin	Valk & Price (1956)
	EDA	0·097 $\pm$0·14	—	Heparin	Millar (1956)
	THI	0·06 $\pm$0·05	0·3 $\pm$0·07	Heparin	Cohen & Goldenberg (1957)
	Rat uterus	0·06	< 1·0	Siliconed heparinized syringe	Holzbauer & Vogt (1954)

* THI indicates a trihydroxyindole method; EDA indicates an ethylene diamine condensation method. The blood samples were collected into solutions of the substances indicated.

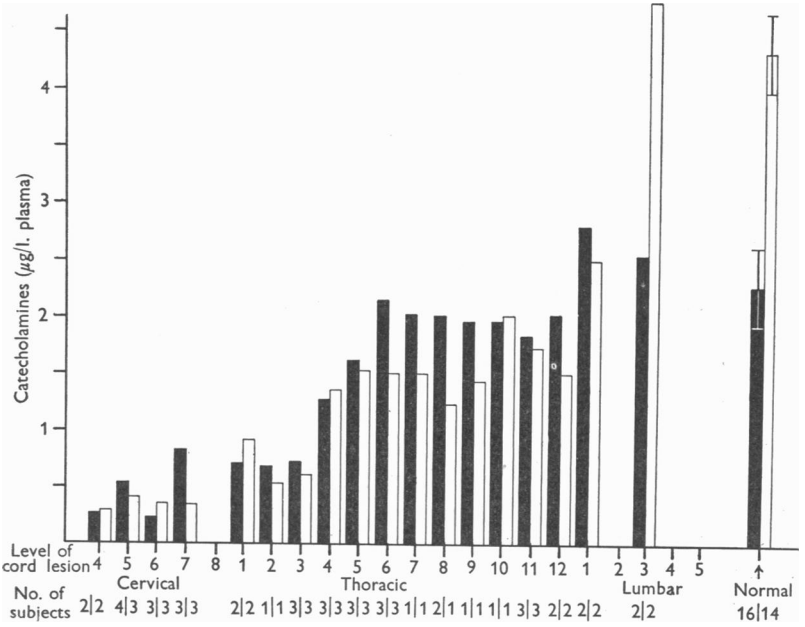

Fig. 1. Plasma adrenaline (■) and noradrenaline (□) in patients with spinal lesions at different levels, and in normal man.

adrenaline in so far as very low values are found with cervical and high thoracic cord lesions. A relatively abrupt rise in concentration of plasma noradrenaline also occurs with a level of lesion just below T3, but again the values obtained with lesions below T4 and T5 are definitely lagging behind those of the normal controls.

The noradrenaline values rise gradually with lowering of the level of lesion down to L1 and then rise abruptly between L1 and L3 to the normal value. Table 2 also shows the statistical significance to be attached to the above data.

TABLE 2. Plasma adrenaline and noradrenaline concentrations in subjects with complete transverse lesions of the spinal cord at different levels

Level of cord lesion	Adrenaline (μg/l. $\pm$ S.D.)	Noradrenaline (μg/l. $\pm$ S.D.)	
C4–T3	0·4 $\pm$ 0·13	0·25 $\pm$ 0·24	15
T4–T8	1·65 $\pm$ 0·48	1·15 $\pm$ 0·49	9
T9–L1	1·92 $\pm$ 0·62	2·0 $\pm$ 0·47	9
L3	2·0 $\pm$ 0·70	4·6 $\pm$ 1·2	2
Normal	2·33 $\pm$ 0·47	4·1 $\pm$ 0·46	16

Significance of differences (t test)

Between	Catecholamine determined	P
C4–T3 and T4–T8	Adrenaline	$< 0·05$
C4–T3 and T4–T8	Noradrenaline	$< 0·01$
T9–L1 and normal	Noradrenaline	$< 0·01$
L3 and normal	Noradrenaline	Not significant

The ratio of noradrenaline to adrenaline also varies. In normal subjects the plasma noradrenaline is always the higher, but with few exceptions the reverse occurs in the spinal subjects at all segmental levels between T4 and L1. On the other hand, in those with lesions at L3 the proportion is the same as in normal subjects. It would appear that some change of physiological significance, probably related to sympathetic nerve activity, occurs in subjects with lesions between L1 and L3.

Subjects before and after bilateral adrenalectomy. In this group blood samples were taken with the patients lying in the supine position. Before operation the plasma adrenaline did not differ significantly in value from that of normal subjects in the supine position. It was $1·75 \pm 0·51$ μg/l. (mean of eight subjects). The second blood samples taken 6–8 days after the second stage of adrenalectomy showed, in eight out of the nine patients, plasma adrenaline values too low to be measured by the method used, i.e. less than 0·3 μg/l. The ninth patient had a plasma adrenaline of only 0·6 μg/l.

The plasma noradrenaline before the operation was $1·94 \pm 0·39$ μg/l.

(mean of eight subjects). After bilateral adrenalectomy the noradrenaline value rose to $2{\cdot}98 \pm 0{\cdot}76\,\mu g/l.$ (mean of nine subjects). Of the eight patients who were studied both before and after adrenalectomy six showed a post-adrenalectomy increase in plasma noradrenaline, one a slight fall, and one no change. The differences between pre- and post-adrenalectomy values, however, are not statistically significant.

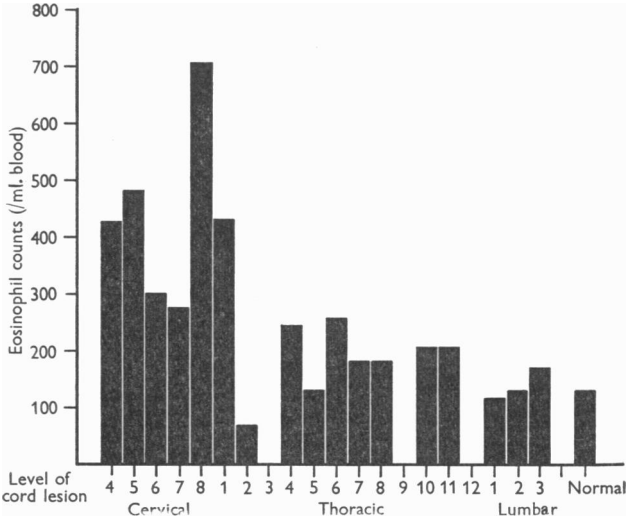

Fig. 2. Eosinophil counts in 45 patients with spinal lesions at different levels, and in normal man.

Eosinophil counts in spinal subjects

Figure 2 shows in histogram form the eosinophil counts of forty-five patients with complete and long-standing transverse lesions of the cord. Thirteen out of twenty-one patients with lesions above T5 showed some degree of eosinophilia, i.e. counts above 300/mm^3. Twenty-three patients with lesions complete at T5 and below all showed normal counts. The impression obtained is that eosinophil counts are about normal with lesions up to T2 level. With lesions immediately higher in the cord than this the eosonophil count rises significantly to an abnormal value and remains elevated about the same amount with higher levels of lesion. It is interesting to note the complementary character of the histograms for plasma adrenaline and eosinophil count.

DISCUSSION

Much of the previous work on the catecholamine content of the peripheral plasma has been concerned with the circulating levels of adrenaline and noradrenaline in the resting normal subject (Table 1), and the present

observations in this respect compare most closely with those of Weil-Malherbe & Bone (1953). These have been criticized, and their relatively high values explained, on the basis of a poor specificity of the method employed, but this cannot account for the present values, which were obtained by a different and more specific method of assay. The differences shown in the table appear to be due less to the method of assay than to the way in which the blood samples were taken. Weil-Malherbe & Bone (1958) found that human platelets contain adrenaline and noradrenaline and that they would take up both adrenaline and noradrenaline against a concentration gradient. More recently Born & Hornykiewicz (1957) and Born, Hornykiewicz & Stafford (1958) have made a similar finding with pig platelets. It is possible that the platelets, by their capacity to absorb catecholamines, form a 'trap' taking up any transmitter which escapes into the blood from the nerve endings and receptor tissue: this could serve to help to localize a sympathetic response. Inspection of Table 1 suggests that where great care is taken to maintain the platelets and the integrity of the plasma generally (Valk & Price, 1956), the adrenaline and noradrenaline values are at their lowest. Where maximum disruption of platelets is likely to occur, as in the fluoride solution, maximum catecholamine values are found. If it were possible to sample the completely undisturbed plasma then zero values might conceivably be found. On the other hand, in acute stress with maximum stimulation of the sympathetic and adrenal medullary systems the platelet trap might not be completely effective and measurable amounts of the catecholamines might be found 'free in the plasma'.

The present investigation shows that by measuring the 'total extractable' adrenaline and noradrenaline rather than the 'free' and biologically active, a reasonable estimate may be made of the prevailing level of sympathetic and adrenal medullary activity. For instance, the increase in plasma noradrenaline with an unchanged level of adrenaline when normal subjects rise from the supine to the sitting position suggests increased activity of the vasoconstrictor nerves associated with an escape of noradrenaline from the endings into the peripheral blood. Celander (1954), on the other hand, using a biological method of assay, was unable to demonstrate the presence of circulating sympathin even after maximal stimulation of the splanchnic nerves, that is, at a rate much higher than 6–8/sec, which Folkow & Uvnäs (1948) estimated as the upper limit of the physiological rate of discharge of sympathetic vasomotor nerves. Obviously the fall in the catecholamine content of the plasma with progressively higher levels of cord lesions reflects the increasing degree of isolation of the sympathetic chain from the normal tonic control by centres in the brain. Small amounts of adrenaline and noradrenaline nevertheless persist

in the peripheral plasma of patients with long-standing cervical lesions. Sympathetic reflex activity at the spinal level, the presence of extra-medullary chromaffine tissue, or direct stimulation of the adrenal medulla by circulating metabolites, may account for this. We have found for instance that the occurrence of active infection in spinal patients leads to higher than normal plasma adrenaline values.

Our observations confirm the view that the adrenal medulla is normally maintained in an active state by higher nervous centres. The plasma adrenaline values are significantly lower in the case of lesions above than below T4, but not different from the normal in the patients with complete lesions below T6. The main outflow of secretory fibres to the adrenal medulla thus seems to occur between T4 and T6. Teitelbaum (1942) on the basis of anatomical studies describes a much wider secretory outflow from T5 to T11. Vogt (1952) found that the denervated adrenal medulla of the cat continued to secrete both adrenaline and noradrenaline, but much less actively than the innervated gland. An abnormally high circulation of metabolites due to the operative procedures might at least partly account for the observed levels in the denervated animals.

The existing information with regard to the catecholamine composition of the adrenal medullary secretion has been mainly obtained by analysing the adrenal vein effluent in anaesthetized animals, i.e. under some degree of stress (Folkow & Uvnäs 1948; Vogt, 1952; Dunér, 1953). This indicates a significantly greater proportion of noradrenaline than adrenaline, which becomes more pronounced when the nerves to the gland are stimulated. The present investigation affords some information on this point in the unstressed human subject. Since spinal subjects with lesions below T4 have a predominance of adrenaline in the plasma, it may be assumed that a normal level of adrenal medullary activity which is apparent only in subjects with lesions below this level in the cord provokes a secretion composed predominantly of adrenaline.

The disappearance of adrenaline from the peripheral plasma after bilateral adrenalectomy suggests that all of it originates from the adrenal medulla and that the plasma adrenaline level in the intact subject is an index of the prevailing activity of this organ. The noradrenaline content of the plasma rose in most cases after bilateral adrenalectomy; it is unlikely therefore that this substance forms any considerable part of the medullary secretion in man. Von Euler, Franksson & Hellström (1954) observed a similar increase in urinary noradrenaline associated with a reduction of urinary adrenaline to 1/5 of the pre-operative level after bilateral adrenalectomy in human subjects. Since adrenaline has an important role in metabolism it may be that noradrenaline acting similarly but less strongly is increased in the circumstances to offset the loss of

adrenaline. The noradrenaline increase may also signify an enhanced stimulus to vasoconstriction in order to compensate for the loss of adrenocortical function.

It is difficult to provide any explanation other than a change in plasma catecholamine concentration which would fit the fact that eosinophilia occurs only in those patients with lesions sufficiently high in the spinal cord to isolate completely the splanchnic outflow from the higher nervous centres. There is so far no evidence that other substances capable of changing the eosinophil levels, e.g. histamine, undergo a change in concentration about this level of lesion. The possibility remains that the effect is only indirectly mediated by adrenaline, since it has been shown that the ketogenic steroid excretion of spinal patients follows approximately the same pattern when related to the level of the cord lesion as does plasma adrenaline (Robinson & Munro, 1958). That the adrenal cortical hormones are not necessary for an eosinopenic response to adrenaline is shown by the fact that it can be produced by adrenaline after removal of both adrenals. The present findings therefore suggest that subnormal levels of circulating adrenaline are directly responsible for the observed eosinophilia.

SUMMARY

1. The peripheral plasma levels of adrenaline and noradrenaline have been measured in a group of normal human subjects, in a group with complete transverse lesions of the spinal cord, and in another group before and after bilateral adrenalectomy. Eosinophil counts were made in a corresponding spinal group.

2. The plasma noradrenaline of the normal subjects was significantly higher in the sitting than in the supine position.

3. Both the adrenaline and the noradrenaline of the plasma were significantly below normal in spinal subjects with cervical and high thoracic lesions. The data provide evidence for the presence, normally, of tonic activity in the sympathetic nervous system dependent upon its functional continuity with higher nervous centres. Adrenal medullary activity is similarly affected.

4. The eosinophil counts in the spinal group were inversely related to the plasma adrenaline levels.

5. After bilateral adrenalectomy there was a significant reduction in plasma adrenaline but no corresponding fall in plasma noradrenaline.

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