ADRENALIN-LIKE SUBSTANCES AND THE BIOLOGICAL ACTIVITY OF THE BLOOD IN DIENCEPHALIC DISORDERS

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The pathology of the diencephalon, and particularly of its hypothalamic area, which contains the so-called autonomic centers, has been extensively investigated in the Clinic for Nervous Diseases of the I-Moscow Medical Institute. The appearance at an early stage in a disease of clinical signs of diencephalic involvement is of great practical importance; frequently these disturbances constitute the principal symptoms and may determine the subsequent course of the disease.

Neurophysiological and biochemical methods make it possible to reveal small changes due to disturbance of the subcortical autonomic centers which would not be revealed by the usual clinical tests. A detailed clinical and physiological examination may indicate diencephalic disorder in organic cerebral disease, and in various infectious or toxic conditions, and in disturbances of either temporary or permanent disorders of the relationship between cortex and subcortical structures.

We have previously shown that a single determination of certain physiological or biochemical constants is not sufficient to reveal the condition of the fundamental physiological systems. We have applied various tests and loads [1, 2, 3] which have enabled the limits of homeostasis to be determined, and the adaptive reactions to be measured; this process facilitates diagnosis, and distinguishes the primary pathological condition from its secondary manifestations. In this connection a study of neurohumoral regulation is of great importance as an index of the condition of the sympathetic and parasympathetic divisions of the autonomic system.

For many years we have studied patients with various abnormalities affecting the adrenalin-like substances (ALS), acetylcholine concentration, or sympathetic and parasympathetic substances of the bloodstream [4, 5, 6].

In the present paper we present the results obtained during brief spells of cooling or heating the body designed to induce a condition of "stress" (the term used by Selye).

METHOD

The ALS concentration in the blood was determined by Shaw's method, as modified by A. M. Utevskii and M. G. But [8] for the detection of dehydroforms (reoxidized forms of ALS) in the presence of ascorbic acid. A detailed description of the method has been given previously [4, 5, 6]. The fraction of unoxidized adrenalin in the complex was described by the coefficient of specificity (KSp). We have somewhat arbitrarily assumed that when the KSp is equal to or greater than 2, the ALS fraction contains only adrenalin. When the KSp is less than 1, no adrenalin is present, and the whole complex consists of unidentified ALS, which have been described by A. M. Utevskii by the term "chromogens". If the KSp lies between 1 and 2, the substrate investigated contains both adrenalin and chromogens. Their ratio is indicated by the value of the KSp. We also calculated the total ALS (total adrenalin) and the ratio of the dehydroforms to total adrenalin (dehydroadrenalin coefficient).

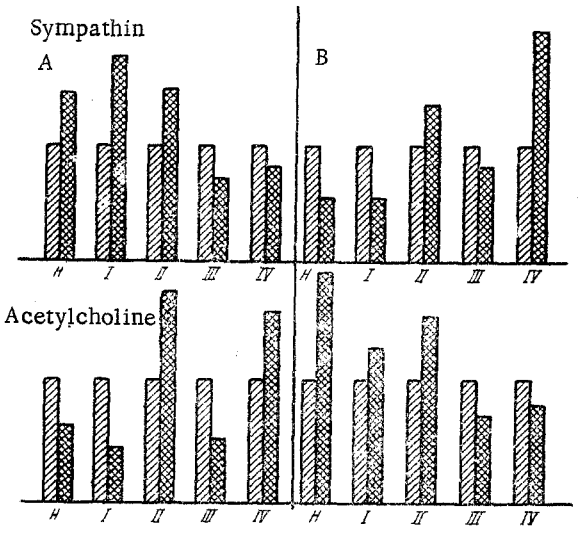

Fig. 1. Diagram showing the effect of (A) cold and (B) hot tests on the sympathin and acetylcholine content of the blood in healthy subjects (H) and in patients with diencephalic disorders (I-IV: 4 types of reaction) (diagonal shading – before testing, cross shading – after test)

The biological activity of the blood (or blood serum) was determined by applying it to the isolated frog heart or to leech muscle. We used the method developed at the Institute of Physiology of the USSR Academy [5, 7] which is based on observing changes in the amplitude and frequency of the contractions.

Tests were also made on 22 healthy human subjects, and on 112 patients with various diencephalic disorders; they were tested on an empty stomach, and immediately after a period of immersing the hands up to the wrist in cold water at 4° (the cold test), and immediately after a three-minute period with the hands immersed to the wrist in hot water at 44° (the hot test).

In considering the results obtained, it must be remembered that: 1) Increase in sympathetic activity of the blood may be due to an increase in concentration of sympathomimetic substances (sympathins) as well as to a reduced concentration of parasympathomimetic substances (parasympathins), particularly acetylcholine; 2) sympathetic activity of the blood may be reduced either by a reduction in the concentration of sympathins, or by an increase in parasympathins, particularly acetylcholine.

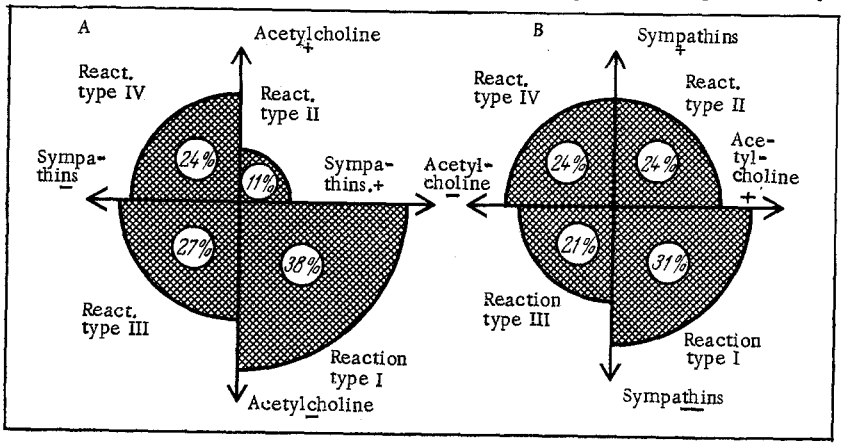

Fig. 2. Classification of patients with diencephalic disorders into 4 groups; reactions to (A) cold and (B) hot tests according to degree of increase (+) or decrease (-) in the sympathin and acetylcholine blood levels.

As we have shown previously [4, 5, 6], increase in sympathetic activity may depend firstly on an increase in the KSp, i.e., on the presence of the most active unoxidized forms of adrenalin, and secondly on an increase in the total ALS in the form of chromogens (noradrenalin ?). Similarly, a reduction in sympathetic activity may be caused either by a reduction in KSp, i.e., by a disappearance or a reduction in the amount of unoxidized forms of adrenalin, or by a reduction in the total ALS through fall in the content of chromogens (noradrenalin ?).

Because blood always contains different amounts os sympathins and parasympathins, its action on the isolated frog heart was tested repeatedly before and after adding atropine. The difference in the effect produced enables the true sympathin level of the blood to be determined.

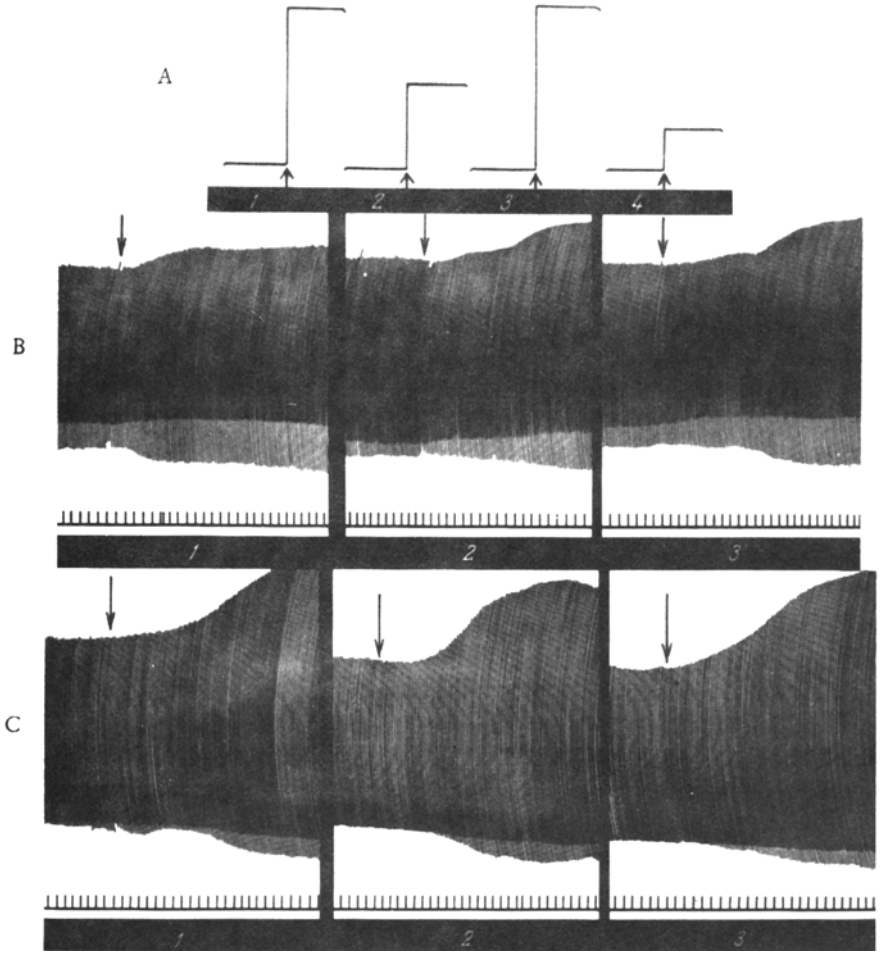

Fig. 3. Biological activity of the blood as shown by heat test (reaction type IV). A) Acetylcholine level in blood (1, 3 — effect of standard solution of acetylcholine [1 : 10^{-8}]; 2) level of acetylcholine before test; 4) level of acetylcholine after tests); B) effect of patient's serum, taken before test, on the isolated frog heart; C) effect of serum from patient taken after test on isolated frog heart; (B and C: 1) dilution 1 : 1500; 2) dilution 1 : 150; 3) dilution 1 : 15).

RESULTS

In healthy human subjects, the cold test causes a marked sympathetic effect on the frog heart (the positive inotropic effect of the plasma on the heart increases by 40-50%) while the acetylcholine level is reduced, on average by 40%. In some cases, after the cold test there is a reduction in the concentration of sympathins, and at the same time a marked fall in the amount of acetylcholine. Thus the relation of the sympathins to the parasympathins alters so as to result in an increased sympathetic activity.

In 65% of the cases, the ALS content increased, but the KSp either remained unchanged or was reduced. Some increase in the level of dehydroforms (reoxidized forms) of ALS was also found. It may be concluded, therefore, that in healthy subjects the cold test causes an increased sympathetic activity of the blood, chiefly on account of an increase in the chromogen (noradrenalin?) fraction, and a reduction in acetylcholine.

In healthy subjects the hot test produces a well marked parasympathetic effect (the positive inotropic effect of the plasma on the frog heart is reduced or fails entirely), and the acetylcholine level increases on average by 175%. There are no characteristic changes in the ALS, KSp, or dehydroform ALS; their values increase, decrease, or remain unchanged in approximately the same numbers. However, as a rule the variation was small and had no effect on the sympathetic activity of the blood, which in most cases was reduced by the hot test.

TABLE

Adrenalin-like Substances and the Biological Activity of the Blood in Patient A. (Diencephalic Disorder Affecting Endocrine Balance)

Test	ALS (in γ%)	DALS (in γ%)	TA (in γ%)	KSp	DAC	Inotropic effect in dilution 1:1500	Acetylcholine (in γ%)	Group assigned as result of tests
Cold	8.8	0	8.8	0.8	0	197	0.92	IV
	8.7	0	8.7	0.7	0	78	1.48	
Heat	6.7	0	6.7	0.4	0	129	0.94	IV
	7.8	1.2	9.0	0.8	0.15	184	0.36	
After course of x-ray therapy (400 r applied to diencephalic region)								
Cold	11.3	0	11.3	0.9	0	38	0.62	I
	12.2	0	12.2	0.8	0	113	0.24	

Note. 1. ALS) adrenalin-like substances, DALS) dehydro-adrenalin-like substances, TA) total adrenalin, KSp) coefficient of specificity, DAC) dehydro-adrenalin coefficient. 2. Line one – before test, line two – after test. 3. The graph shows a 7% increase in the positive inotropic effect as compared with that due to a standard solution of adrenalin diluted 1 : 10^{-7}.

Different diencephalic disorders may cause the most varied changes in ALS content and in the biological activity of the blood, whose limits are determined by the process of homeostasis. In each separate case, they may be caused by a preponderance either of sympathetic or of parasympathetic tone, by the time course of the reactions, through a replacement of the primary by secondary pathological symptoms, and by the lability or inertia of the nervous processes.

From the results of the cold and hot tests, patients with diencephalic disorders may be referred to one of the following groups (see also Figs. 1 and 2).

Cold test. First group – increased sympathetic activity and reduced acetylcholine. Qualitatively the results were the same as in the control group. In diencephalic disorders, considerable changes in the amount of the substances investigated occur, and are often several times greater than the normal physiological variation. The KSp often has a much higher value than in contols, thus indicating the presence of unoxidized forms of adrenalin.

Group II – increase in both sympathetic activity and acetylcholine level. N. I. Grashchenkov and G. N. Kassil' [1] have suggested that the primary sympathetic reaction is rapidly compensated by an increased parasympathetic response which may even overcompensate, and the acetylcholine level may increase 3-4 times.

Group III – simultaneous reduction in sympathetic activity and acetylcholine level. The ALS are reduced; the KSp either falls or remains unchanged. The sympathoadrenal apparatus is less reactive, and there is a compensatory reduction in parasympathetic tone.

Group IV – reduced sympthetic activity and increased acetylcholine. The ALS content falls, the KSp falls or remains unchanged. This reversed cold test result is frequently observed in cases of severe disturbance of the regulatory and compensatory mechanisms.

The results obtained show that diencephalic patients of all four groups show differences which are significantly greater than the fluctuations in healthy subjects.

Hot test. Group I – increase in acetylcholine and decreased sympathetic activity of blood. Qualitatively the tests were not different from normal; however, the acetylcholine level increases on average 33% less than in the control group. This indicates a reduced parasympathetic nervous response.

Group II – simultaneous increase in acetylcholine and in sympathetic activity. In a considerable number of cases there was an increase in ALS. Probably the primary parasympathetic reaction was rapidly compensated by increased sympathetic activity. The KSp was more frequently increased than in normal subjects, indicating the presence of unoxidized forms of adrenalin.

Group III – simultaneous fall in acetylcholine and sympathetic activity. In some cases the ALS level was reduced, as was also the KSp. This result indicates parasympathetic failure compensated by a relative fall in sympathetic tone.

Group IV – the acetylcholine was reduced by 34% on average, and the sympathetic activity was increased up to 5-6 times (Fig. 3). In this group the ALS was usually increased and the KSp raised. These results were usually obtained in cases of severe disorders of the regulatory and compensatory mechanisms.

In healthy subjects, when the hot test was applied, the ALS sometimes increased without there being any increase in sympathetic activity of the blood, because of the considerable increase in the amount of acetylcholine. Evidently, increase in ALS in the blood in the hot test is associated with the "stress" nature of the test.

In diencephalic patients, there is more frequently an increase in ALS and a raised KSp than in healthy subjects. In these patients there is also an increased acetylcholine level, both in the heat and cold tests, which is compensated by an increased ALS due chiefly to unoxidized forms of adrenalin (the KSp is raised).

Patients on which both the hot and cold tests were applied were very interesting. As a rule the results coincided, and both indicated that the patient should be referred to a particular group. Application of various therapeutic measures (x-ray therapy, nasal therapy, or hormone therapy) in many cases enabled the normal autonomic balance of the body to be restored (see Table).

Thus in routine clinical examination a single (hot or cold) test is sufficient. We carried out both tests only when there was difficulty in distinguishing the primary pathological signs from secondary compensatory processes.

From the results obtained we have drawn the following conclusion:

From studies of changes in the biological activity of the blood and of its content of adrenalin-like substances, it is possible to obtain evidence as to the condition of the autonomic nervous system and of its adaptive (homeostatic) mechanisms in healthy subjects and in patients with diencephalic disorders.

In healthy subjects the cold test causes an increase in sympathetic activity and a fall in the acetylcholine level of the blood. The hot test increases acetylcholine and decreases sympathetic activity.

The change in the biological activity of the blood depends on the content both of adrenalin-like substances and of acetylcholine.

In different forms of diencephalic disorder, the cold and hot tests cause characteristically different changes in the amount of sympathetic and of parasympathetic substances. We have found four different reactions corresponding to different regulatory mechanisms and different limits of homeostasis in various diencephalic disorders.

SUMMARY

Measurements have been made of the physicochemical and biological properties of the blood, based on tests designed to find the subjects' reactions to stimulation. From these it was possible to determine the limits of homeostasis and the nature of the adaptive reactions, and the results were used diagnostically; it was also possible to distinguish between primary pathological processes and secondary compensatory phenomena. Measurements were made of the concentration of adrenalin-like substances, acetylcholine, sympathins, and parasympathins in normal subjects and in those with diencephalic disorders. The two tests were based on the reaction to heat and to cold. Four types of disturbance of control and compensatory mechanisms were found.

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