THROMBOKINASE ACTIVITY OF TISSUES IN VARIOUS ENDOCRINE DISORDERS AND IN TUMOR GROWTHS

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There are several references [4, 5, 6, 8] to a possible association between tumor development and endocrine disorders; it seemed worthwhile, therefore, to study the effect of hormonal disturbances on the properties of tissues which are related to tumor growth.

One such property is evidently thrombokinase activity, because there is evidence that thrombokinase has a stimulating effect on the growth and division of cells in tissue culture [10, 11], as well as on the thromboplastic properties of the experimental carcinoma V2 in the rabbit.

We have not found any reference to the work on the relationship of thrombokinase activity and the condition of the endocrine system, thought there are isolated references [12, 13] to the fact that hormones may affect several components of the blood coagulatory system. There is no information about the thrombokinase activity of the tissues of many experimentally induced tumors or of the other tissues of the animal affected.

We therefore investigated thrombokinase activity in The tissues of rats with experimentally induced endocrine disorders and with sarcoma strains M-1, sarcoma 45, and with Biskind's ovarian tumors.

METHOD

Experiments were carried out on 68 white rats of an impure strain: Of these, 50 males weighed 250 - 300 g and the 18 females 200 - 250 g. The endocrine disturbance was induced by thyroidectomy, adrenalectomy, or castration. We also used castrated animals with ovarian tumors, as obtained in special researches on the transplantation of an ovary into the spleen using Biskind's method.

The sarcomas were induced by introducing a suspension of M-1 and 45 sarcoma tissue.

The animals were killed by decapitation after 1, 2, and 4 weeks, while those operated by Biskind's method and the control castrates were killed after 6 months. In a separate experimental group, blood was first taken from the jugular vein [1] to determine the thrombokinase (thromboplastic) activity of the blood using B. A. Kudryashov and P. D. Ulitina's method [3].

The thrombokinase activity of the tissue extracts was measured by Quick's method [14]. The relative concentration of thrombokinase in the extracts was determined by the minimum dilution of the extract necessary to destroy its activity [7].

The thrombokinase was tested on rabbit plasma containing a large amount of prothrombin [15] as well as of the thrombotropin [2] necessary for the activation of the inactive form of thrombokinase (prothrombokinase). The rabbit blood was taken from the heart by a syringe containing 0.1M oxalate solution in 1 ml per 10 ml of blood.

RESULTS

All the animals were divided into groups: 10 controls, 11 castrates six female castrates with one ovary transplanted into the spleen, 10 thyroidectomized, 10 from which two-thirds of the adrenal was removed, 10 totally adrenalectomized, six with sarcoma M-1, and five with sarcoma 45.

Study of the tissue extracts of the intact controls showed that each white rat tissue had a particular value of thrombokinase activity. The greatest was that of the lung (Fig. 1a, I) and the next in order were the tissues of the testes, brain, kidneys, and spleen (Fig. 1a – d, I). The liver had least. (Fig. 1f, I). Blood was intermediate in this respect between the kidneys and spleen.

Thrombokinase activity in corresponding tissues from different individuals varied by ± 2.5 sec for tissues with high and by ± 6 sec for those with low thrombokinase activity; blood showed variations from one individual to another of ± 10 sec.

Experiments on rats from whom different endocrine glands had been extirpated showed that the change in thrombokinase activity of their tissues was greater than the individual variations in the control group.

Castration caused an increase in activity chiefly in the lungs (Fig. 1a, K) and blood (Fig. 1g, K). Tissue extracts from castrated females with one ovary transplanted into the spleen did not show the increased thrombokinase activity of the other castrates (Fig. 1, B).

Thrombokinase Activity of Human Tumor Tissue

Tissue	No. of cases of the condition	Clotting time of control plasma	Thrombokinase activity (in sec)	
			tumor extracts	surrounding healthy tissue
Metastasis of omentum (cancer of the sigmoid colon)	1.109	67	16	–
Metastasis of omentum (cancer of the cecum)	1.069	100	25	–
Cancer of the stomach	1.097	90	18	–
Cancer of the breast	1,253	80	21	–
"	1.338	80	43	50
"	1.244	80	13	27
"	899	100	42	62
"	1.272	80	48	59
"	1.283	70	46	58
"	1.450	80	50	62
"	13.183	70	40	43
"	1.702	70	42	48
"	1.406	72	45	53
"	1.755	65	41	–
"	1.562	90	46	62

Thyroidectomy caused chiefly an increase in the thrombokinase activity of the blood (Fig. 1g, T), kidneys, and spleen (Fig. 1, T, g). There was little change in that of the lungs or brain in most of the thyroidectomized animals, and in the testes it was actually reduced (Fig. 1b, T).

The remarked changes in the activity of all tissues, and particularly that of the lungs, kidneys and brain, occurred in the adrenalectomized group (Fig. 1a, A, and d, c, A).

The experiments in which the extracts were diluted showed that the increased thrombokinase activity represented a disproportionate increase in the concentration of thrombokinase in the extracts (Fig. 2). For instance, whereas an extract of pulmonary tissue from the control (intact) group of animals reduced the clotting time of control plasma to 21 sec, an extract of castrate pulmonary tissue to 13 sec, and pulmonary tissue from an adrenalectomized animal to 10 sec (see Fig. 1), the correspondingly diluted extracts which failed to speed clotting were: for pulmonary tissue of the control (intact) animals – $10^{2.41}$, i.e. a 256-fold dilution, for castrate lung tissue – $10^{3.91}$, (8192 times dilution), and for pulmonary tissue of the adrenalectomized group – $10^{4.52}$, (32708 times dilution) (Fig. 2a, I, K, A).

The graph of Fig. 2 showing the relative concentration of thrombokinase in the extracts, where the concentration is shown as the logarithm of the dilution, illustrates the close relationship between the thrombokinase (more accurately prothrombokinase) content in the tissues and endocrine imbalance. Apparently individual variation in thrombokinase activity which may occur in control animals is also associated with endocrine balance.

In animals bearing tumors, the changes in thrombokinase activity were found to be much smaller than in those where there had been endocrine disorders (see Fig. 1, M-1, S-45). Only the blood of the sarcoma group, and particularly those with sarcoma M-1, showed a marked increase in thrombokinase activity (see Fig. 1, g, M-1, S-45).

Extracts of the tumors themselves of both kinds showed very high thrombokinase activity (see Fig. 1, 3, M-1). Extracts of sarcomatous tissue did not lose their activity until the dilution was $10^{4.21}$ and $10^{4.52}$ (see Fig. 2, 3, M-1, S-45), in other words the thrombokinase concentration in sarcomatous tissue approached the highest level i.e. that of pulmonary tissue in the adrenalectomized group.

Because of the results obtained on the thrombokinetic properties of the experimental sarcomas, we thought it

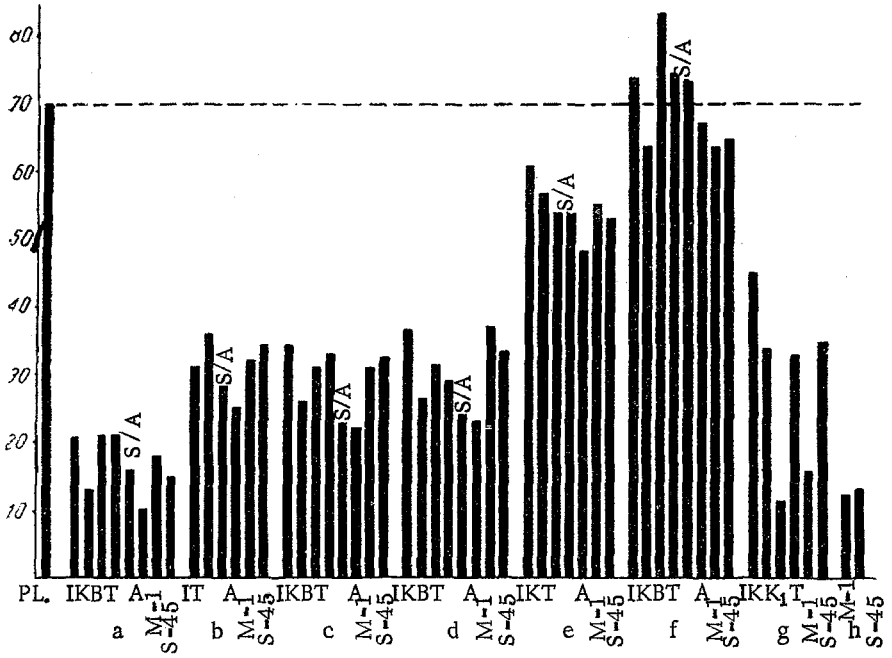

Fig. 1. Changes in the thrombokinase activity of different tissues as shown by the clotting time (in sec) of the control plasma (average values). a – Tissues of lung; b – of testes; c – of brain; d – of kidneys; e of spleen; f – of liver; g – of blood; h – of tumor. Pl – control oxalate rabbit plasma; clotting time after recalcifying shown by dotted line, I control (intact) animals, K – castrated males, K_1 castrated females, B – females operated by Biskind's method, T – thyroidectomized animals, S/A – subtotally adrenalectomized animals; A – completely adrenalectomized animals, M-1 – animals with 14-1 sarcoma; S-45 – animals with sarcoma 45.

essential to study those of the malignant human tumors removed at operation. The results are shown in the table.

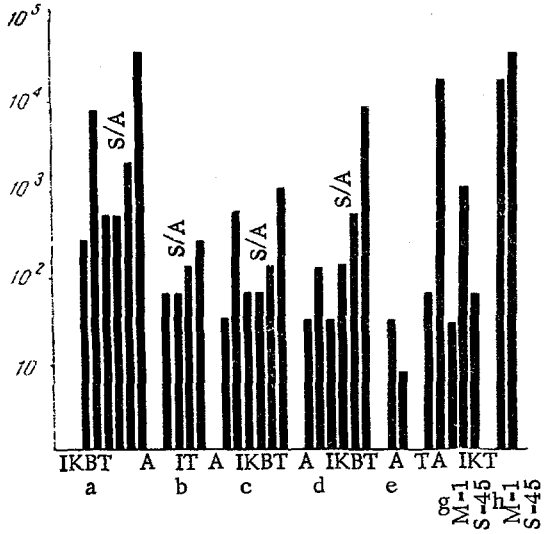

Fig. 2. Thrombokinase content of different tissues, expressed as the logarithm of the dilution of the tissue extracts. Indications as in Fig. 1.

As can be seen from the results, extracts of tumor tissue had the property of greatly reducing the clotting time of the plasma, and in most cases their thrombokinase activity was greater than that of the surrounding apparently normal tissue.

Our work has shown a definite similarity between changes in thrombokinase activity of tissues in endocrine disturbances and in tumors.

SUMMARY

Experiments were performed on white rats with various endocrine disturbances. Following adrenalectomy there was a considerable rise of thrombokinase activity of the lungs, kidneys, and brain. There was also an increased activity of the same organs after castration. In rats with tumor strains of sarcoma M-1 and sarcoma 45, there was a sharp rise in thrombokinase activity, mainly in the tumor tissue itself; the concentration of thrombokinase in the tumor tissue approached the highest concentration found elsewhere which was in the pulmonary tissue of castrated and adrenalectomized animals.

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