SOME PROPERTIES OF HUMAN "LEUKEMIC FACTOR" WHEN CULTIVATED ON THE EMBRYONIC MEMBRANES OF CHICKS

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Our previous investigations [1] showed that a noncellular factor (human "leukemic factor") is present in the tissues of patients with leukemia, and this may be cultivated on the membranes of developing chick embryos. It was also shown that after cultivation on the chorionallantoic membrane this factor possesses specific antigenic activity.

It appeared necessary (from the theoretical and, possibly, also from the practical point of view) to study some other important properties of the cultivated agent.

The present paper describes the results of experiments to investigate the effect of formalin, high temperatures, freezing and drying on the activity of "leukemia factor" after cultivation on the embryonic membranes of chicks. We also studied the electron microscopic picture of the cultivated factor. We tried, further, to discover whether it was possible for the "leukemia factor" to be adsorbed on red cells. The answers to these questions might be of considerable importance to the elucidation of the nature of human "leukemia factor."

EXPERIMENTAL METHOD

Blood and brain tissue filtrate from patients with leukemia were cultivated on the chorionallantoic membrane of fertilized chick embryos. The allantoic fluid of the inoculated embryos was subjected to various treatments (formalinization, heat, freezing and drying, and adsorption on red cells).

At the 6th passage of blood from patients with acute leukemia, the allantoic fluid of the inoculated embryos was heated on a water bath to 80°C for 30 and 60 min; the same fluid was treated with 1% formalin. Allantoic fluid at the 15th passage of leukemic blood was heated to 80°C for 40 min and to 100°C for 30 min, and at the 12th passage of filtrate of leukemic brain tissue it was heated to 100°C for 30 min. Allantoic fluid at the 20th passage of leukemic blood was autoclaved for 40 min at 1.5 atmos. Chick embryos were inoculated with this fluid and 4 "blind" passages were carried out, after which the fluid was injected into mice. At the 23rd passage of leukemic blood the allantoic fluid was frozen to -70°C and dried. This preparation was injected into animals 9 days and 2½ months after lyophilization. Allantoic fluid at the 5th and 12th passages of leukemic blood and also at the 10th passage of leukemic brain filtrate and the 3rd passage of filtrate of normal brain were added, in proportions of 1:1 or 3:1, to a 5% suspension of human red cells (from a donor of Group I (0), Rh negative) in physiological saline. The mixture was kept at a temperature of 4°C for 3 hours. The red cells were then precipitated by centrifugation two or three times and washed with cold physiological saline. The precipitate of red cells was made up to a 5% suspension in physiological saline.

To find out whether adsorption of "leukemic factor" on red cells could take place, we also studied its specific antigenic properties, using L. A. Zil'ber's test of anaphylaxis with desensitization.

TABLE 1

The Properties of "Leukemia Factor" After Passage through Chick Embryos

Preparation injected	Age of mice	Site of injection of preparation	Number of experimental mice	No. of mice surviving until first sign of leukemia	Average latent period of leukemia development in	Number of leukemias developing
Allantoic fluid at the 6th passage of leukemic blood (crude)	2—8 days	Subcutaneously	79	45	1,5	9
	1 month	Into the spleen	21	4	4,0	2
The same fluid heated to 80°C	2—10 days	Subcutaneously	61	32	—	0
	1 month	Into the spleen	21	16	—	0
The same fluid treated with 1% formalin	3 days	Subcutaneously	21	19	—	0
	3 months	Into the spleen	20	16	—	0
Allantoic fuid at the 20th passage of leukemic blood, autoclaved	1 month	The same	62	41	5,8	7
The same fluid heated to 80°C	1 »	» »	41	6	10,0	1
The same fluid heated to 80°C	1 »	» »	32	7	—	0
Allantoic fluid at the 20th passage of leukemic blood, autoclaved	25 days	Subcutaneously	22	5	—	0
Allantoic fluid at the 23rd passage of leukemic spleen	1 month	The same	33	6	—	0
	1 »	Into the spleen	20	2	2,0	1
	1 »	Into the thymus gland	22	11	1,0	4

TABLE 2

Adsorption of "Leukemic Factor" on Red Blood Cells

Preparation injected	Age of mice	Mode of injection of preparation	No. of expt. mice	No. of mice surviving to develop leukemia	Av. latent per. of leukemia development in mo.	No. of leukemias developing
Allantoic fluid at the 5th passage of leukemic blood; adsorption on red cells	1— 10 days	Subcutaneously	32	6	4.5	2
The same, heatd to 100°C	10 days	» »	10	3	—	0
Allantoic fluid at the 5th passage of leukemic blood; adsorption on red cells	1 month	Intraperitoneally	20	11	1.7	2
Allantoic fluid at the 10th passage of a filtrate of leukemia brain; adsorption on red cells.	1 »	The same	20	18	1.7	2
Allantoic fluid at the 12th passage of normal blood; adsorption on red cells	1 »	» »	20	12	—	0
Allantoic fluid at the 3rd passage of filtrate of normal brain; adsorption on red cells	1 »	» »	20	12	—	0

All the preparations described above were injected into mice of CC_{57}, C_{57}, C_3HA and unidentified strains, to test their leukemia-producing activity. They were injected into the animals subcutaneously (usually in the

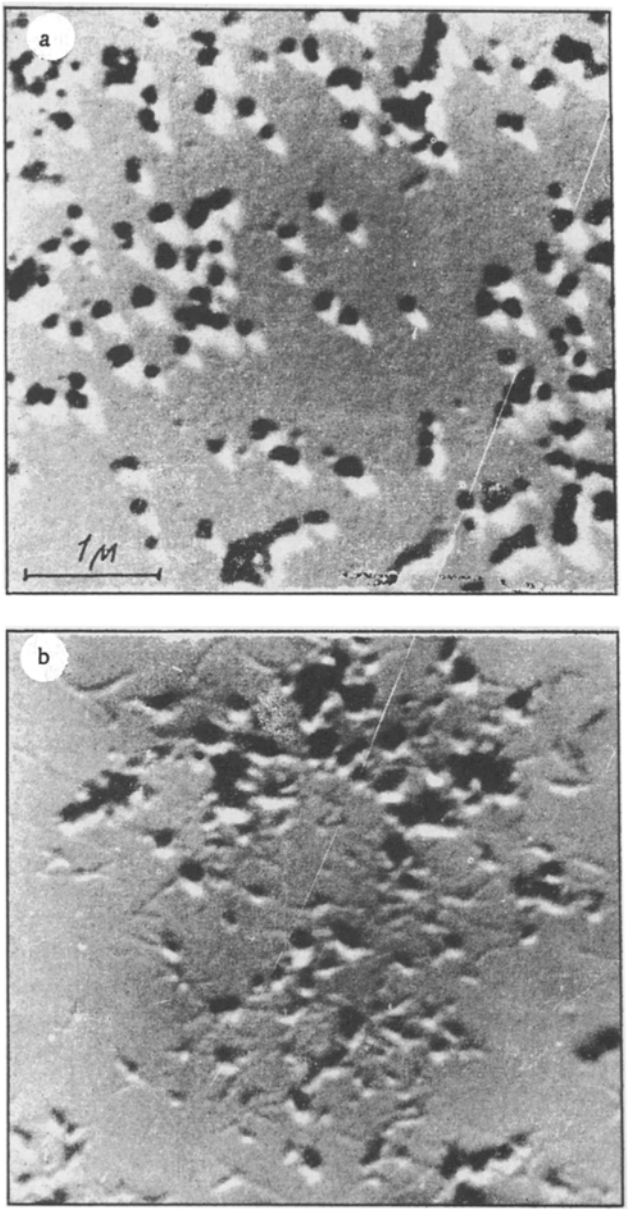

Fig. 1. Allantoic fluid under the electron microscope. a) From a chick embryo inoculated with leukemia; b) from a healthy chick embryo.

case of newborn mice), directly into the tissue of the spleen and directly into the tissue of the thymus gland of mice aged 25-30 days.

Blood cultures were taken from the experimental mice in order to detect any paratyphoid infection which could give a leukemoid reaction. In only one mouse (which had received autoclaved allantoic fluid) was Salmonella Gärtneri found; no paratyphoid bacteria were discovered in the remaining cases.

The animals which died or were killed were examined histologically and cytologically with great care, in order to make a diagnosis of true leukemia. In case of necessity (7 mice) the systemic diseases produced in the mice were transplanted into other mice of the same strain to differentiate true leukemias from leukemoid reactions.

EXPERIMENTAL RESULTS

As seen from Table 1, crude allantoic fluid at the 6th passage of leukemic blood on embryonic membranes of chicks produced leukemia in 22.4% of mice. The same allantoic fluid, when heated to 80°C or treated with 1% formalin, completely lost its ability to produce hemocytoblastoses or myeloses in mice.

When injected into the spleen of mice at the 15th passage of leukemic blood, crude allantoic fluid led to the appearance of leukemia in 17% of the mice, after an average latent period of development of 5.8 months. The same allantoic fluid, if heated to 80°C, caused leukemia in only one mouse of every 6 which survived for 10 months from the beginning of the experiment (the period of development of leukemia); heating the allantoic fluid to 100°C inactivated it. Heating to 100°C also destroyed the leukemia-producing activity of allantoic fluid at the 12th passage of a filtrate of leukemic brain.

"Leukemic factor" is also inactivated by autoclaving. Autoclaved leukemic allantoic fluid was subcultured 4 times by blind passage on embryonic membranes of chicks. Inoculation of mice with allantoic fluid at the 4th passage of autoclaved "leukemic factor" did not cause the appearance of hemocytoblastoses nor myeloses.

Human "leukemic factor" is thus inactivated by high temperatures and by 1% formalin.

As we know, viruses possess considerable resistance to cold. In a frozen state, suspensions of viruses remain active for a long time: at a temperature of -70°C viruses survive for a year and more; and in the dried form (after freezing) preparations of viruses do not lose their powers of infection for several years.

We also investigated the leukemia-producing activity of frozen and dried preparations of leukemic allantoic fluid.

It was shown that frozen and dried allantoic fluid at the 23rd passage of leukemic blood kept its activity for at least 2½ months. Of 19 mice which survived to show the first signs of leukemia, leukemia was observed in five, with an average latent period of development of 1½ months. Leukemia was found in mice receiving injections of the preparation directly into the tissue of the spleen or thymus gland (when the latter method of injection of the preparation was used, very malignant hemocytoblastoses developed).

Many viruses have the ability to undergo adsorption on red blood cells. We studied the ability of human "leukemic factor" to undergo such adsorption by means of a biological test of the specific leukemia-producing activity of the preparations obtained and by the anaphylaxis with desensitization reaction.

As seen from Table 2, allantoic fluid at the 5th and 12th passages of leukemic blood, adsorbed on red cells, possessed leukemia-producing activity and caused leukemia in 23% of mice with an average latent period of development of 3.1 months. Allantoic fluid at the 10th passage of a filtrate of leukemic brain, adsorbed on red cells, was also active (leukemia was observed in 2 of the 18 mice which survived the time for the first case of leukemia to develop).

In control experiments in which human red cells were added to allantoic fluid at the 12th passage of normal blood or at the 3rd passage of a filtrate of normal brain, no leukemia was observed.

The results of these experiments indirectly confirm the results of the study of the specific antigenic properties of the "leukemic factor" adsorbed on the red cells. In 3 guinea pigs of six sensitized to leukemic allantoic fluid at the 14th passage and adsorbed on human red cells, an anaphylactic reaction (accessed +) developed in response to an assaulting injection of leukemic allantoic fluid at the 22nd passage.

Allantoic fluid at different numbers of passages of leukemic and normal blood was studied under the ÉM-3 electron microscope by G. I. Abdeev. The specimens were prepared as follows: one drop of allantoic fluid, centrifuged at 6000 rpm, was placed on a collodion film; excess fluid was removed; after drying, the film was washed 5-6 times with distilled water; the preparations were shadowed with nichrome at an angle of 15°. On examination of the "leukemic" films (Fig. 1 a), at some passages round bodies were found, usually 100-125 mμ in diameter, and sometimes grouped in clusters. Similar particles were also encountered in control films, but they were few in number and rarely seen (Fig. 1 b).

To conclude this account of the concrete results of the experiments we must emphasize particularly that all the leukemias produced in these experiments were, by their morphological structure, hemocytoblastoses or myeloses. Besides these, we often noted the appearance of extensive systemic lesions of reticular tissue, from time to time indistinguishable under the microscope from true reticuloses (hemohistioblastoses). Under these circumstances areas of systemic hyperplasia of reticular tissue were found not only after injection of the "leukemic factor" but also in the control experiments. However no hemocytoblastoses not myeloses were present in the latter.

The facts described suggest that reticuloses may develop in mice as a result of various nonspecific agents. It has still to be explained whether these "reticuloses" are true malignant neoplasms (hemohistioblastoses) or whether they are severe systemic reactions of the reticular tissue. This interesting and formidable problem will be the subject of a special study. So far as human "leukemia factor" is concerned, it possesses the specific property of causing hemocytoblastoses and myeloses in experimental animals.

Judging by the results of the investigations which we have described, the human "leukemic factor", like the majority of the known viruses (including the tumor-producing viruses), is inactivated by the action of high temperatures and of 1% formalin.

At the same time it evidently is adsorbed on red blood cells and is preserved for at least 2½ months by freezing to -70% and subsequent drying.

SUMMARY

The author presents results of examination of the properties of human "leukemic factor" cultured on the chorion allantoic membrane of developing chick's embryos. It was established that "leukemic" allantoic fluid loses its leukosogenic activity in action of high temperatures and its treatment with 1% formalin solution. Lyophilized preparations of "leukemic" allantoic fluid (which was kept in dried condition up to 2½ months) caused

the appearance of myeloid leukemias and of hemocytoblasts in mice (especially when administered into the thymus gland). Leukemic allantoic fluid adsorbed on human erythrocytes possessed leukosogenic activity. The preparations of "leukemic" allantoic fluid when examined microscopically were found to contain globular bodies 100-125 mμ in size (such particles were rarely revealed in control preparations and were few in number).

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*Original Russian pagination. See C. B. Translation.