CULTIVATION OF HUMAN LEUKEMIA FACTOR ON THE CHORIOALLANTOIC MEMBRANE OF CHICK EMBRYOS

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The majority of causative agents of virus diseases of man and animals possess a well-marked property of conservation and proliferation in the developing chick embryo.

The same results have been found in respect to a number of tumor viruses.

During the study of the nature of human leukemia factor,* the necessity for attempts at its cultivation on the membranes of fertilized chick embryos naturally arose.

In the present paper are described the results of investigations carried out in this field, under the direction of Active Member of the Academy of Medical Sciences of the USSR Prof. L. A. Zil'ber.

EXPERIMENTAL METHOD

The following were used for cultivation in chick embryos: fresh whole blood from 6 patients with acute leukemia; blood from 7 healthy persons (control); filtrate from the brain of a person dying from acute hemocytoblastosis; filtrate from the brain of a person dying from cardiovascular disease (control).

Cultivation was carried out by the usual method, as follows: 0.3 ml of blood (or brain filtrate) was placed in the intervascular region of the chorioallantoic membrane of a 7-day chick embryo of the Leghorn breed. The embryos were kept at 37°C. Five days later the allantoic fluid of the inoculated embryos was collected and injected (0.3 ml each) into fresh embryos.

Cultivation of the blood and brain filtrates from sick and healthy personswas carried out 7 times with different numbers of passages (4, 9, 10, 12, 15, 15, 40). The maximum number of passages was 40 and their general duration 6.5 months.

It has to be emphasized that the first inoculation of the embryos and all subsequent passages were made with the addition of 0.025 ml (0.625 mg) of cortisone to 0.3 ml of allantoic fluid. By adding cortisone we wished to suppress the organism-host reaction to the injected pathogenic agent.

For the mouse inoculations allantoic fluid was selected from the 3rd, 4th, 5th, 6th, 7th, 9th, 12th, 15th and 18th passages (for convenience in the table some experiments made with different numbers of passages from different patients are combined since no great variation was observed in their activity).

Animals were also injected with filtrates of the chorioallantoic membrane of the 7th and 9th passages of the blood from a patient with acute leukemia. To prepare the filtrate the membrane was finely chopped in cold sterile

* The term "human leukemia factor" conventionally describes an acellular agent isolated from human leukemia tissues [1].

Preparation	Age of mice used in the experiment	Mode of injection of preparation	Number of mice in the experiment	Time of appearance of 1st leukemia in mos.	No. of mice surviving until the appearance of the 1st leukemia	Mean latent period of development of leukemia in mos.	No. developing leukemia	No. of leukemoid reactions	Remarks
Allantoic fluid of the 3rd-4th passages of leukemic blood	15 days	Subcutaneously	23	2.0	13	4.0	2	3	Transplantation in one case negative (myeloid reaction)
Allantoic fluid of the 6th passage of leukemic blood	2—8 days	Subcutaneously	83	1.0	46	1.3	10	19	Transplantation in 2 cases successful (leukemia) and 1 case negative
	1 month	Into the spleen	21	2.0	4	4.0	2	4	
Allantoic fluid of the 12th and 15th passages of leukemic blood	1—1½ months	Into the spleen	82	1.0	55	3.9	12	23	Transplantation in 2 cases successful (leukemia) and in 3 others – negative (reaction)
Filtrate of the chorioallantoic membrane of the chick embryo of the 7th and 9th passages of leukemic blood	11—30 days	Subcutaneously	29	2.0	14	2.0	1	2	
	1 month	Into the spleen	14	8.0	4	8.0	1	2	
Allantoic fluid of the 6th and 12th passages of leukemic brain filtrate	1—5 days	Subcutaneously	40	2.5	28	3.3	2	4	Transplantation in one case successful (leukemia)
	1—2 months	Into the spleen	40	1.5	12	2.7	4	6	
Allantoic fluid of the 6th and 7th passages of normal blood	2—8 days	Subcutaneously	33	—	28	—	0	2	
	1 month	Into the spleen	41	3.0	28	3.0	1	16	
Allantoic fluid of the 18th passage of normal blood	7—11 days	Subcutaneously	55	1.0	16	1.0	1	6	
Allantoic fluid of the 5th passage of filtrate of normal brain	1 month	Into the spleen	21	—	11	—	0	6	Transplantation in 1 case negative (reaction)
	6 days	Suboutaneously	21	—	13	—	0	2	

physiological saline (in proportions of 1 : 5) at pH 7.3-7.5. The suspension was centrifuged at 2000, 4000 and 6500 rpm. The precipitate was removed. The supernatant fluid remaining after the last centrifugation was passed through a Seitz filter. Films of the filtrate were examined under the phase-contrast microscope and also were stained by Pappenheim's method. Whole cells were absent from the films. Penicillin was added to the filtrate. The filtrates were sterile.

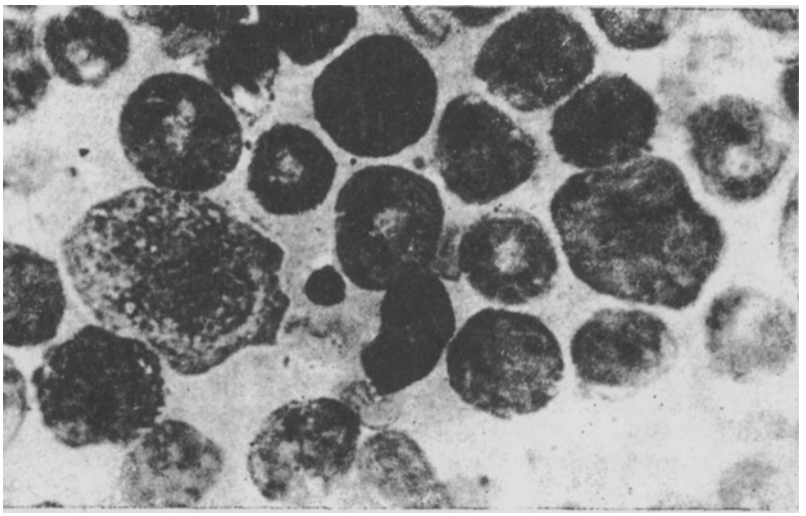

Fig. 1. Impression of the bone marrow. Myeloid leukemia. Magnification 720 X.

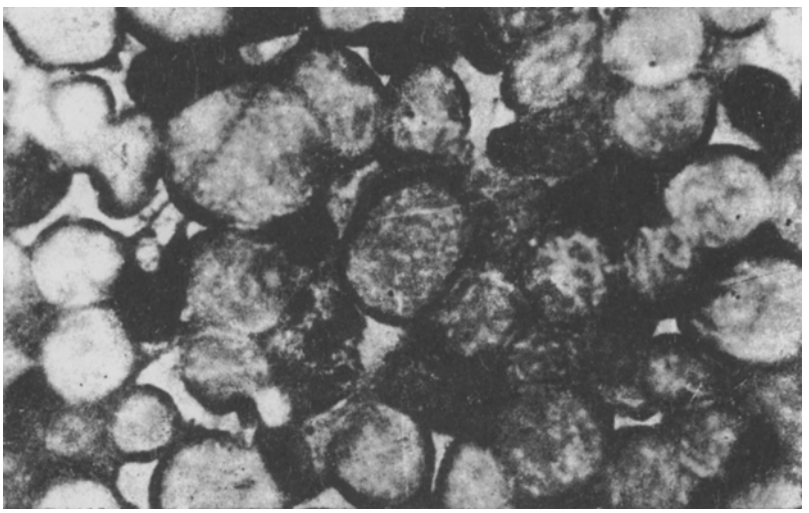

Fig. 2. Impression of the spleen. Leukemia. Magnification 720 X.

Filtrates of human brain tissue were prepared in the following way. The tissue was macerated in the cold for 4 minutes in a homogenizer in a proportion of 1 : 5 with sterile physiological saline to which was added a 10% solution of 96° ethyl alcohol. The supension was kept at 4° for 20 hours and then centrifuged at 2500 rpm for 10 minutes. The supernatant fluid was passed through a No. 3 Rublevskii filter.

We judged the presence of human leukemia factor in the inoculated embryos by the result of a test of the specific biological activity in animals of the allantoic fluid taken from the embryos (the results of its specific antigenic properties were given in another paper [2].

The allantoic fluid and filtrates of chorionic membrane of the chick embryos were injected in a dose of

0.05-0.1 ml subcutaneously in newborn mice or directly into the spleen of adult mice of low-leukemic strains (CC57, C3, HA, C57 and white of no particular strain). Experiments were performed on 576 mice.

216 animals were examined histologically and cytologically.

For confirmation of the diagnosis of true leukemia in ambiguous cases transplantations of the resulting lesions into other mice of the same strain were carried out. It must be pointed out that attempts to transplant material from animals showing severe myeloid reactions (distinguishable from leukemias morphologically with difficulty) did not lead to the development of true leukemias.

Cultures of the blood of the experimental animals revealed no paratyphoid infection which may give leukemoid reactions in mice.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the experiments are summarized in the table.

As can be seen from the table, the allantoic fluid of the 3rd-4th, 6th, 12th and 15th passages of blood from patients with leukemia causes the appearance in mice of true leukemias in 20.5% of cases (28 leukemias in 136 mice surviving until the time of appearance of the first leukemias), their average latent period of development being 3.8 months. No essential variation in the activity of the allantoic fluid from different passages could be detected.

The allantoic fluid of the 6th, 7th, and 18th passages of blood from healthy persons led to the development of true leukemias in only 2 mice (of 72), which amounts to 2.7% of mice surviving until the time of appearance of the first leukemias.

The allantoic fluid of the 6th and 12th passages of leukemia brain filtrate, when injected into animals, caused the appearance of leukemias in 15% of mice (6 leukemias in 40 mice surviving until the time of appearance of the first leukemia) with a mean latent period of development of 3 months.

The allantoic fluid of the 5th passage of filtrate of normal brain did not produce leukemias.

It should be mentioned that the activity of the 12th passage of leukemic brain filtrate was slightly lower than that of the 6th passage.

The leukemias produced in the mice by their morphological structure belonged to the myeloid leukemias and reticuloses (Figs. 1 and 2).

So far as the leukemoid reactions (as a rule, myeloid) are concerned, they were observed equally often in the experimental and control groups.

We did not intend to make a systematic study of the lesions produced in the embryos. Nevertheless we must point out the severe retardation in development (growth), the massive hemorrhages in the organs and membranes, and the appearance of white jelly-like formations on the body of the embryos inoculated with blood from leukemic patients.

Thus, the results of the experiments described show that human leukemia factor is capable of prolonged (at least over 3 months) cultivation on the membranes of the developing chick embryo. However, judging by these experiments, its biological activity does not increase with passage.

There are several reports in the literature of the possibility of cultivation of noncellular agents isolated from animal tumors in chick embryos. These include: the agent of fibroma of rabbits [13], myxoma of rabbits [11], fowl leukemia [8], fowl lymphomatosis [9], leukemia of mice [10] and cancer of the mammary glands in mice [3, 4].

In chick embryos inoculated with noncellular agents from lymphogranulomatosis [6] and from chorionepithelioma in man [12] changes are observed which, in the authors' opinion, indicate the presence of filterable etiological agents in these tumors.

The research of A. D. Timofeevskii and his co-workers [5], who used electron microscopy and immunological reactions to identify the etiological agents of several tumors of man and animals in chick embryos, is of great interest.

The prolonged survival of the agent of human leukemia on the membranes of chick embryos, which we established by biological experiments and also by immunological reactions [2], enable it to be grouped with these etiological agents enumerated above.

Although the findings described in this paper are in support of the virus nature of human leukemia factor, the experiments of Brachet [7] must not be forgotten, for he showed the possibility of cultivation of lipoprotein cell fractions on the membranes of chick embryos (these experiments, it is true, are still disputable).

SUMMARY

Allantoic fluid of chick embryos, infected by blood and filtrate of human brain tissue from patients with leukemia or persons dead from it, was injected to mice of low-leukemic breeds. It was demonstrated that the above substances cause the appearance of leukemia in 20.5 and 15% of animals. Allantoic fluid of chick embryos infected with blood of healthy persons caused leukemia only in 2.7% of mice, while allantoic fluid of embryos, infected by filtrate of normal brain tissue did not possess leukosogenic activity. Consequently, it is possible to cultivate the human factor for a long period of time on membranes of developing chick embryos (15 inoculations for over 3 months).

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* Original Russian pagination. See C. B. translation.
** In Russian.