[6] M. K. Petrova, The Role of Functional Impairment of the Cerebral Cortex in the Production of Various Pathological Processes in the Body* (Leningrad, 1946).

[7] E. E. Pogosiants, Trudy Akad. Med. Nauk, Voprosy Onkologii 26, 6, 19-30, 291-292 (1953).

[8] N. M. Turkevich, Voprosy Onkol. 1, 6, 64-70 (1955).

[9] V. K. Fedorov, Zhur. Vysshei Nerv. Deiatel. 1, 5, 744-752 (1951).

[10] F. M. Khaletskaia, Zhur. Vysshei Nerv. Deiatel. 4, 6, 869-876 (1954).

THE LEUKEMIA-PRODUCING ACTIVITY OF CELL-FREE FILTRATES OF HUMAN LEUKEMIC TISSUE

V. M. Bergol'ts

The Virological Laboratory of the A. I. Gertsen State Oncological Institute (Head – Prof. A. N. Novikov), Moscow

(Received November 29, 1957. Presented by Active Member Acad. Med. Sci. USSR, A. D. Timofeevskii)

The ability to pass through a semipermeable membrane is a characteristic property of the so-called filtrable viruses. By the use of special filters it is possible to isolate from a tissue extract a virus component, leaving undamaged cells and bacterial microorganisms on the filter [2]. If injection of the filtrate into an animal causes the appearance of a disease, this implies that the filtrate contains a virus.

The possibility of transmission of a tumor from an affected animal to a healthy one by means of a filtrate is the main factor in support of the virus etiology of these tumors. Tumors of this sort include carcinoma of the breast and leukemia in mice, papilloma and fibroma in rabbits, fibroma in deer, sarcoma and leukemia in fowl and so on.

There are no indications in the literature of the possibility of transmission of leukemia in man by means of cell-free filtrates. We have produced leukemia in mice by injecting them with centrifuged extracts of human leukemic tissue in which the presence of whole cells was practically impossible. However, the objection that isolated undamaged cells may still be left in the extract persists.

For this reason it was essential to attempt to produce leukemia in mice by means of cell-free filtrates of human leukemic tissue. In the present paper we give the results of experiments undertaken in this direction.

EXPERIMENTAL METHOD

We prepared cell-free filtrates from tissue from lymphatic glands, blood, brain and tumor-like leukemia infiltrations from 4 human patients suffering or dying from acute leukemias (hemocytoblastoses). In control experiments we used extracts of donated blood and of brain tissue from a patient dying from vascular disease. We had studied previously the biological activity of extracts of "normal" lymphatic glands: the extracts possessed no leukemia-producing activity [1]. In the same report the findings are given of the leukemia-producing activity of extracts of human sarcoma (as a control of tumor-like leukemia infiltration).

* In Russian.

The filtrates were prepared as follows: the tissue was ground up in a blender in the cold with physiological saline in a proportion of 1 : 5; the suspension was strained through gauze and then centrifuged at 2500-3000 rpm for 10-15 min. The supernatant fluid was filtered through a Seitz apparatus at a negative pressure (oil pump) with a two-layer asbestos filter. The filtrates were tested for sterility.

In one case, after preparation of filtrates from brain tissue and a tumor-like leukemic infiltration from a patient dying from acute hemocytoblastosis (chloroma) we changed the method of obtaining the filtrate; the suspension contained 10% of 96% alcohol and before centrifuging it was allowed to stand at 4° for 20 hrs. It was then filtered through a No. 3 Rublevskii filter (diameter of pores 700 mμ).

The filtrates were injected into mice of the low leukemic strains C_{57} and C_3HA and impure strains, directly into the tissue of the spleen (adult mice) or subcutaneously (newborn). Altogether 309 mice were used in the experiments. Animals injected with filtrates of leukemic brain tissue and tumor-like infiltrates were given 5 mg of cortisone one month later (in 4 doses at intervals of 2-3 days). Blood from these mice was inoculated in nutrient media in order to detect paratyphoid infection which may give severe leukemoid reactions. No paratyphoid bacteria were discovered.

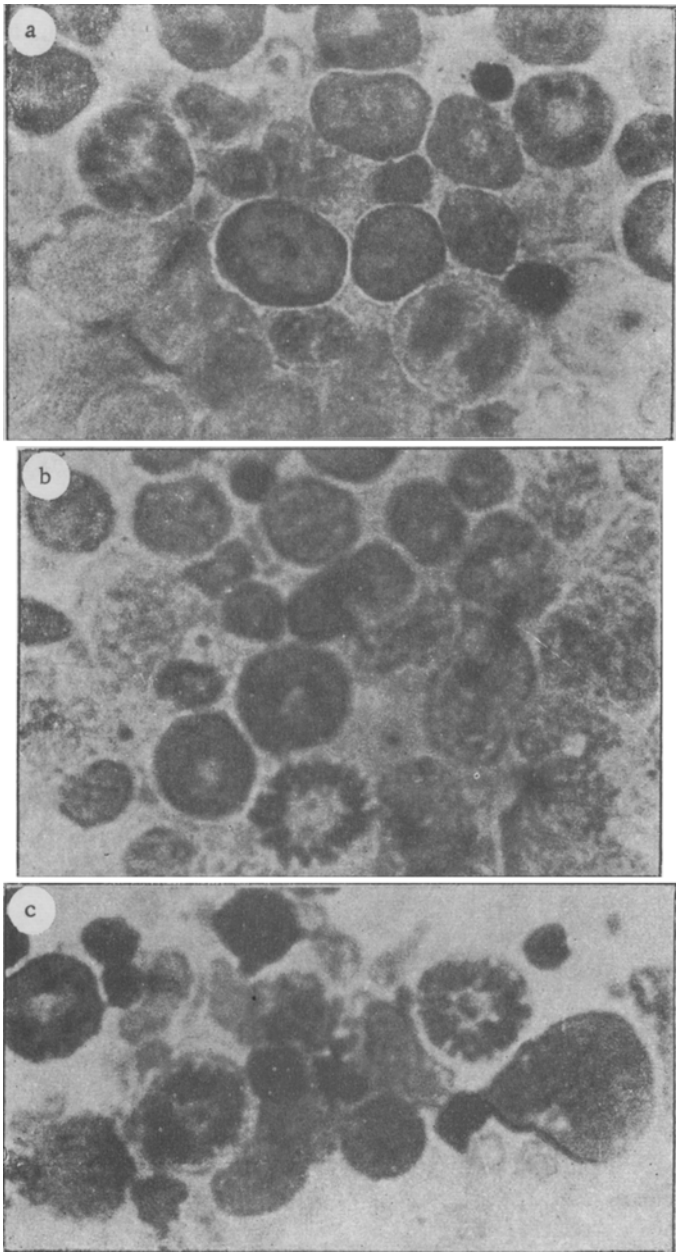
The diagnosis of true leukemia was made on the basis of the careful histological and cytological study of organs of the experimental animals. Where necessary the true malignant nature of the lesion was confirmed by transplantation into other mice of the same strain.

EXPERIMENTAL RESULTS

Essential details of the experimental results are shown in the summarized table.

Summarized Table of the Experiments

Extract	Age of mice	Method of injection of extract	No. of mice used in the experiment	Time of first appearance of leukemia	No. of mice surviving to time of 1st appearance of leukemia	Average latent period of development of leukemia in months	No. of leukemic mice	No. of leukemoid reactions
Filtrate of leukemic brain	1.5 month	Intraperitoneal	21	3	19	4.7	5	3
	7 days	The same	8	5	4	5	1	0
The same, in 10% aqueous alcohol	1.5 month	Into spleen	41	1.5	22	2.3	5	6
	1-6 days	Subcutaneous	28	5	3	5	1	4
Filtrate of normal brain in 10% aqueous solution	1 month	Into spleen	40	2.5	25	2.5	1	9
	3-14 days	Subcutaneous	21	—	4	—	0	1
Filtrate of leukemic tumor	1.5 month	Into spleen	19	2.5	8	3	2	3
Filtrate of leukemic blood	5-14 days	Subcutaneous	23	1	14	1	1	2
	5 days	"	33	6	4	6	1	5
Whole donated blood	1 month	Into spleen	26	—	20	—	0	4
Filtrate of leukemic lymphatic glands	1 month	Into spleen	9	1	8	1.5	2	1



Cellular composition of the bone marrow and spleen of a mouse after injection with a cell-free filtrate of human leukemic brain tissue. a, b) Bone marrow films; c) spleen film. Pappenheim's stain.

It will be seen from the table that cell-free filtrates of human leukemic tissues are undoubtedly able to produce leukemia in mice of a low leukemic strain. Leukemia developed in 25% of the animals surviving for the period of appearance of primary leukemia, their average latent period of development being 3.7 months. In a rather smaller percentage (11) leukemia blood.

The first cases of leukemia were observed from 1-1.5 months after the beginning of the experiment (they were confirmed by positive transplantation results). In their morphological structure the leukemias were of the myeloid type (see figure a,b,c).

In the control experiments only one case of true leukemia (3.4%) was found after the use of a filtrate (in 10% aqueous alcohol) of "normal" brain tissue. A leukemoid reaction was observed equally often in the experimental and control series. It must be mentioned that attempts to transplant organs of mice with severe myeloid reactions did not lead to the development of true leukemias.

On injection of newborn mice with filtrates of leukemic blood, we observed in those mice which died 2 weeks after the beginning of the experiment an increased content of immature cells in the bone marrow, with a normal composition of the spleen and liver. A similar phenomenon has been observed by Czech workers in young rats injected with leukemic cells. This is the so-called transitory "phase of blasts" [4].

The high leukemia-producing activity of filtrates of human brain tissue must be emphasized. This fact has also been pointed out by other workers [3, 5], but in relation to extracts of the brain of leukemic mice.

At present it is still difficult to account for such a high specific activity of extracts of leukemic brain; we think that it is concerned with the high lipoid content of this tissue, for lipoids play an essential part in the realization of the biological action of the "leukemia factor."

The results of the experiments just described, it seems to us, make it possible to include leukemia in man among the malignant neoplasms which are transmissible by means of cell-free filtrates. This illustrates the virus-like properties of the etiological factor of leukemia in man.

SUMMARY

Cell-free filtrates of leukemic human tissues were administered to mice of low leukemic lines. True leukoses developed on the average in 18% of mice, which survived the period of appearance of the first case of leukosis (with the average latent period of development of leukosis equal to 3.7 months). Extracts of "normal" human tissues do not possess such activity.

LITERATURE CITED

[1] V. M. Bergol'ts, Problemy Gematol. No. 1, 11-17 (1957).

[2] V. I. Tovarnitskii and G. P. Glukharev, Ultrafilters and Ultrafiltration * (Moscow, 1951).

[3] W. Krischke, A. Graffi and E. Krischke, Naturwissenschaften 44, 400 (1957).

[4] J. Riman and J. Vesely, Neoplasma 4, 100-113 (1957).

[5] S. O. Schwartz, H. Schoolman et al., Cancer Res. 17, 218-222 (1957).

* In Russian.