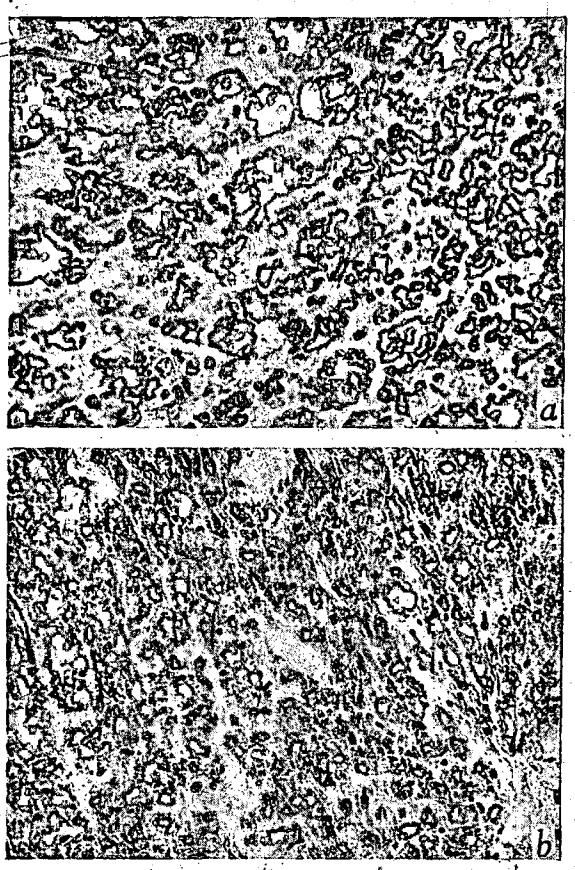

Fig. 2. Livers of leukaemic *AKR* mice (*a*) before, and (*b*) after treatment with DNAse. Haematoxylin-eosin. (× 225.)

Gradually the cellular detritus was digested. This was probably the reason for the diminution of the lymph nodes, liver, spleen and thymus, and the return towards normal structure. These various changes do not occur in normal mice or in untreated leukaemic mice.

The mitotic index in the nodes of leukaemic animals was six times greater than normal; treatment with DNAse decreased the index to the normal level (Table 3).

Examination of the blood cells revealed that the number of leucocytes in the blood of leukaemic mice injected with DNAse was almost half that of the control group. The number of lymphoblasts and Gumprecht cells decreased concurrently.

It is well known that leukaemia can be induced in normal mice by the inoculation of suspensions of lymphoid cells from leukaemic animals[21]. DNAse treatment deprived lymphoid tissue cells of leukaemic animals of the power to transfer leukaemia. For example, after subcutaneous injection into normal 3 week old *AKR* mice of 0·2 ml. of cell suspension from the lymphoid tissue of leukaemic mice, subcutaneous tumours at the site of inoculation and general lymphatic leukaemia developed within 2–3 weeks in twenty-six of the thirty-seven inoculated animals. Not a single tumour developed after the inoculation of forty-five normal mice with cell suspensions obtained from lymphoid tissue of leukaemic animals treated with DNAse. Our preliminary data suggest that the injection of ribonuclease does not produce a similar effect on the course of lymphatic leukaemia in mice of the *AKR* strain.

At present there is not enough evidence to explain the mechanism of the action of DNAse on leukaemia. It may be that lymphoblasts, in contrast to normal cells, have an increased sensitivity to DNAse. The loss of the ability of leukaemic tissue of DNAse-treated *AKR* mice to induce leukaemia after transfer to normal mice may suggest a direct action of DNAse on filtrable leukaemic factor.

The Gross virus, like other leucosis viruses, probably contains RNA. An intermediate DNA template may, however, be involved, analogous to that which arises during the reproduction of Rous virus[22]. This assumption is susceptible to experimental verification.

The results of the present work served as the basis of a test of the therapeutic effect of DNAse in lymphoid leukaemia in man. Preliminary data point to a positive effect of DNAse: injection of enzyme leads to rapid decrease of the size of hypertrophic lymph nodes and of the spleen and liver; the general condition of patients also improves[23].

R. I. Salganik
R. P. Martynova
N. A. Matienko
G. M. Ronichevskaya

Institute of Cytology and Genetics,
Academy of Sciences,
Novosibirsk, U.S.S.R.

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by Stodolnik-Baranska

~~RADIOBIOLOGY~~

Lymphoblastoid Transformation of Lymphocytes *in vitro* after Microwave Irradiation

It is well established that lymphocytes grown with various added substances *in vitro* can transform into cells resembling blastoid forms (lymphoblastoid transformation)[1,2] or macrophages (lymphoid macrophages)[3,4]. The added compounds can be classed as unspecific, including phytohaemagglutinin (PHA) and streptolysin *A*, and specific such as tuberculin and diphtheria toxoids[5]; lymphocytes can also transform when incubated with allogenic cells[6].

During experiments on the influence of microwaves on mitosis, it was found by chance that this type of phenomenon could also be induced by a physical factor, namely microwave irradiation. Human lymphocytes were separated in plastic containers[8] and then placed in 2 per cent calf serum in medium 199 containing antibiotics. The cultures were irradiated in an anechoic chamber with pulsed 10 cm microwaves from a box horn antenna. The test-tubes containing the cultures were placed in

Table 1. PERCENTAGES OF TRANSFORMED LYMPHOCYTES IN IRRADIATED AND CONTROL CULTURES

Power density	Day of incubation	Transformation following irradiation (%)* Lymphoblastoid	Macrophages	Mitoses	Control non-irradiated (spontaneous transformation) (%)* Lymphoblastoid	Macrophages	Mitoses
7 mW/cm²	3	20	1·2	0	10·8	0	0
	5	60	12	2	12·5	0	0
20 mW/cm²	3	20	9	1	11	0	0
	5	61	14	3	17	2	0

* Lymphocytes to 100 per cent.

'Plexiglass' thermostat maintained at 37° C. 'Plexiglass' has a similar impedance to that of air.

A series of control experiments was carried out to measure the eventual heating effect. Thermocouples were immersed in the culture media, which were then subjected to power densities of 7 mW/cm² over 4 h and 20 mW/cm² over 15 min. There was no rise in temperature under these conditions. Two series of cultures were then irradiated for a period of 3–5 days. The first series was irradiated for 4 h daily at a power density of 7 mW/cm² and the second for 15 min daily at a power density of 20 mW/cm².

The results of the control experiments show that the observed effects can be attributed to the effect of microwave irradiation and not to any effect of heating. Selective overheating of cells suspended in medium is theoretically possible under these conditions. In view of the ratio of cell volume to the volume of the medium, however, and given the heat exchange characteristics of the medium and the cells, such "pin point" heating seems improbable. Moreover, our experience with cell cultures, mixed cultures of human lymphocytes, and phytohaemagglutinin cultures has been that increases in temperature of the order of 2°–3° C cause degeneration and cell death.

Non-irradiated cultures obtained from the same individuals served as controls. The cultures were collected in the usual manner after 3 and 5 days[7], and smears prepared and stained by the Giemsa method.

The percentage of transformed lymphocytes (lymphoblastoid forms and macrophages), lymphocytes and mitoses was determined by counts of 1,000 cells each. The results are shown in Table 1. After incubation for 3 days in cultures irradiated at a power density of 7 mW/cm² the percentage of transformed cells was twice as high as in control cultures.

Similar results were obtained in cultures irradiated at a power density of 20 mW/cm².

After incubation for 3 days a slight increase in the percentage of macrophages was noted, and in some cases sporadic mitoses were observed.

After incubation for 5 days similar changes were observed at both power densities. The changes were more distinct at the higher power density. The percentage of transformed lymphocytes was five times as high as that in control cultures; the percentage of macrophages was also high, and there were 2–3 per cent of mitoses. Moreover, fragmented nuclei could be seen among the transformed cells. Bridges joining separated parts of nuclear material, complete breakdown of nucleus into small fragments and vacuolization of cell nuclei were also found. The macrophages ingested fragments of damaged nuclei, cytoplasm and chromosomes.

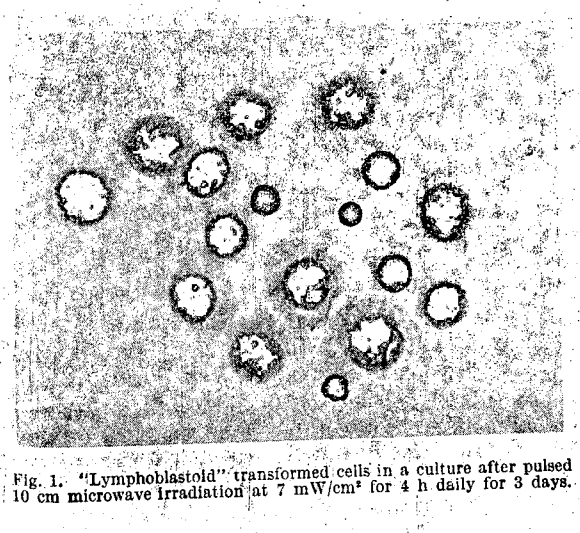

Fig. 1. "Lymphoblastoid" transformed cells in a culture after pulsed 10 cm microwave irradiation at 7 mW/cm² for 4 h daily for 3 days.

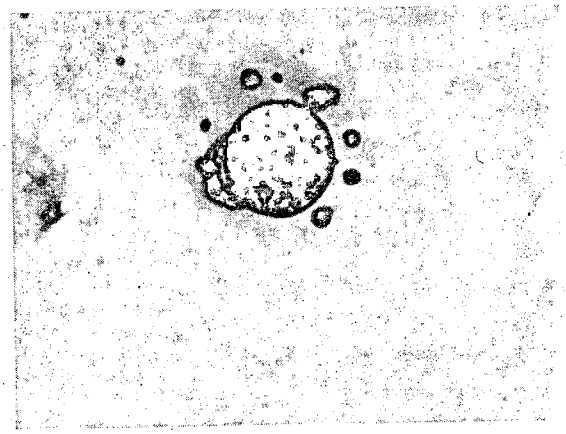

Fig. 2. Same preparation as Fig. 1. Lymphoid macrophage.

These observations indicate that lymphoblastoid transformation can be induced by the action not only of specific or unspecific antigenic substances but also of a purely physical factor. I have no explanation of this phenomenon at present, but further investigations are under way.

W. STODOLNIK-BARAŃSKA

Department of Histology and Embryology,
Warsaw University Medical School.

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PHYSIOLOGY

Intrinsic Innervation of the Human Placenta

THE placenta and foetal membranes are usually described as being devoid of neural elements[1,2]. In other descriptions, the possibility of an innervation is not considered[3,4]. Nevertheless, reports of the presence of nerve elements in these foetal tissues continue to appear in the European literature[5–7]. Improvements in techniques for the examination of neural elements in fresh tissue have allowed us to re-investigate this apparent contradiction. Samples of fresh placentas and their attached foetal membranes were removed by sharp dissection for staining in methylene blue within 30 min of delivery. The excised pieces (about 2 mm thick) were stained and prepared for examination as whole mounts with the use of an immersion technique[8]. In this method, fresh tissues are immersed in methylene blue solution, fixed in chilled ammonium molybdate solution, washed with water, rinsed in 95 per cent alcohol, and dehydrated in absolute alcohol. The dehydrated tissues are cleared in xylene and benzyl benzoate and stored and examined in the latter fluid.

The most striking finding in the placenta was the presence of numerous large nerve trunks coursing among the placental villi. These trunks were readily identified by their organization and the relative acellularity of the