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Resistance to the growth of cancer induced in rats by injection of autolyzed rat tissue.[1]

By **ISAAC LEVIN.**

[*From the Department of Pathology of Columbia University, at the College of Physicians and Surgeons.*]

It is a fairly well established fact that an immunity, or rather a resistance to the growth of a transplantable tumor may be induced in white mice and rats by artificial means. This acquired resistance is of a peculiar type and is not similar to the usual form of the anti-bacterial immunity. Clowes and Baeslack's assertion, that the serum of recovered mice cures cancer in other mice, has received no confirmation. Nor has any other known method of detecting the existence of immunity in an organism met with success in the animals refractory to growth of implanted cancer.

While the best manner of immunizing an animal against tumors consists in a previous unsuccessful inoculation of a tumor, this resistance does not appear to be specific. Ehrlich observed that an animal made resistant to a certain class of tumors, carcinoma for instance, is also resistant to the growth of an implantable sarcoma. Furthermore, a resistance may be artifically induced by previous inoculation of normal tissue of the same species of animals. Michaelis produced such an immunity by injection of normal mouse liver; Bashford by injection of blood or washed blood cells, but not by blood-serum; Schoene with different embryonic tissue; Bridré with liver and spleen.

All these methods of immunization seemed to be successful only when uninjured cells constitute a part of the substances used for the injections. Blood-serum, deprived of the cells, or normal or tumor tissue heated or crushed and frozen, does not induce any resistance—as was shown by the investigations of Michaelis and Haaland.

In view of all these facts, the opinion seems to prevail that an artificial immunity or resistance can only be induced by the inoc-

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ulation of living tumor cells or of living normal cells. As Ehrlich puts it, we are dealing here with a cellular immunity, which consequently can only be caused by the live activities of the injected cells.

It has been shown by the investigations of Buchner, Salkowsky, M. Jacoby, P. A. Levene and others, that the majority of the so-called vital functions of the cell are produced by its endocellular enzymes. These enzymes, while constituting an integral component part of the cell, may remain under certain conditions uninjured after the death of the cell. The best method of liberating these endocellular enzymes consists in the autolysis of tissues.

It seemed feasible a priori that the resistance induced by normal mouse or rat tissue injected subcutaneously may also be due, not to the function of a live cell, but to some peculiar type of an endocellular ferment. With the aim in view to investigate this possibility, the present research was undertaken. The work was done with Ehrlich's sarcoma of a white rat, for a transplant of which the author is indebted to Dr. S. Flexner. This tumor is of a very malignant type and takes in from 100 per cent. to 80 per cent., grows to very large size, frequently gaining the size of two inches by one inch in about three weeks after inoculation. The tissue used for immunization was autolyzed liver of a "Nuller" — *i. e.*, a rat naturally resistant to sarcoma. Though resistance is induced by tissue of a normal animal, it was deemed advisable to enhance the chances for success by taking the tissue from a resistant animal. The liver was removed and kept under aseptic precautions at body temperature for two weeks; then the autolyzed liver tissue was mixed with about double the quantity of normal salt solution, ground thoroughly with sand, filtered, and 1 c.c. of the solution injected subcutaneously at different periods before or after the inoculation of the tumor. The rats used for the experiments were approximately of the same size and consequently of about the same age.

A diagram of the experiment follows (page 66).

The final examination and measurement of the tumors was done in about twenty-five days after inoculation, when the tumors reached already the highest point of growth. These results compare quite

	1	2	3	4	5	6	Total.	Control Rats.
Number of rats inoculated with tumor.	10	10	10	10	10	10	60	60
Number of rats survived at the final examination 25 days after inoculation.	5	10	10	10	6	10	51	57
Number of days *before* inoculation autolyzed liver injected.	10	5	3					
Number of days *after* inoculation autolyzed liver injected.				9	9	12		
Number of rats without growth or with small abortive nodules.	2	7	5	7	4	9	34	6
Number of rats with tumors.	3	3	5	3	2	1	17	51
Per cent. of takes.	60%	30%	50%	30%	33%	10%	34%	85%

favorably with the results obtained by L. Michaelis with the injection of liver emulsion, as is apparent from the second diagram, or

	Treated Animals.	Controls.
Number of mice inoculated with tumor	20	18
Number of mice without growth or with small stationary tumors	14	5
Number of mice with tumors	6	13
Per cent. of takes	30 per cent.	72 per cent.

even with Ehrlich's statement, that thirty mice immunized with a non-malignant hemorrhagic tumor took the subsequent inoculation of cancer in from 30 to 50 per cent., while the 30 control animals took in 100 per cent. Only Bridré claims to have obtained an absolute immunity with injection of normal spleen, but his conclusions are based on experiments on eight animals only.

It seems then possible to produce in rats a certain amount of resistance to growth of tumor by treatment with tissue, of which the cells are killed, but some endocellular enzyme-like substances may not have been injured in the process.

If we turn now to analyze the investigations of those who have worked with normal tissue, it seems feasible to interpret the results obtained by them in a similar way. It is hard to suppose that when a piece of tissue or an emulsion is injected under the skin, all the cells remain alive and are capable of normal function ten or twelve days later. It seems more probable that the majority of the cells die and undergo some changes similar to the autolytic

process. Very instructive in this connection is the work of Woglom. The spleen extirpated and then inoculated subcutaneously into the same animal induces resistance against growth of tumor. Extirpation of the spleen does not induce any resistance. If resistance is caused in this case by the live functions of the cells of the spleen, then they can act most effectively when the spleen is in situ, and the mice ought to have been naturally resistant. The explanation forces itself on one's mind that the spleen transferred under the skin is autolyzed or undergoes some other similar change.

That autolysis may be one of the means to which the organism resorts in order to elaborate protective substances, is shown by the very interesting investigations of Blum. He demonstrated that products of autolysis of normal tissue possess the power to neutralize tetanus and diphtheria toxins and cobra venom, and it is possible to save animals from death by injecting the products of autolysis subsequent to the injection of toxin.

This investigation is still in its beginning. Different tissues are tried and different methods employed to liberate the endocellular ferments. But the view-point, while new, seems to be correct and capable of stimulating further research, and it is therefore deemed advisable to give this short preliminary report of the present state of this investigation.

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The inhibitory effect of magnesium upon indirect and direct irritability of frog muscle and the antagonistic action of sodium and calcium upon this effect.

By **DON R. JOSEPH** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Laboratories of the Rockefeller Institute for Medical Research.*]

Several series of experiments were carried out on frogs. In the first series magnesium chloride was injected into a lymph sac and subsequently nerve and muscle were stimulated at various times by induction shocks. Of the results obtained two will be mentioned: One is that indirect irritability gradually disappeared completely while direct irritability remained practically unchanged,