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Influence of Bacterial Pyrogen on Transplantable Tumors. (26119)

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Crude filtrates from microbial cultures have been shown to be effective oncolytic agents and numerous investigators have observed spontaneous regression of tumors following acute infections. The historical significance affixed to microbes and microbial products in malignancy has been reviewed by other authors(1-4).

During studies in this laboratory on potentiation of the immune response by bacterial pyrogens(5), spontaneously occurring tumors of mice were noted to regress after administration of pyrogen. The work reported here was undertaken to determine the effect of these pyrogens on mouse transplantable tumors. Of the 3 tumors studied, only the subcutaneous transplants of the sarcoma S-37 tumor responded to treatment, as evidenced by tumor regression and a decreased mortality rate.

Materials. The mouse tumors utilized in these experiments were a sarcoma strain S-37, an adenoma strain EO771, and a leukemia strain 1498. The sarcoma S-37 was maintained in this laboratory by serial transplantation in the ABC strain of mice. Likewise the adenoma strain EO771 and the leukemia strain 1498 were both maintained by periodic transplantation in C57BL mice. The ABC and C57BL strains of mice used in the subcutaneous and intraocular homologous transplant experiments were purchased from Roscoe B. Jackson Memorial Cancer Inst., Bar Harbor, Maine. The Swiss albino mice employed in the intracerebral heterologous transplant experiment were obtained from a local source. All animals were allowed free access to their food supplies of Purina Laboratory Chow and water.

The tumor-bearing animals were treated with various doses of the bacterial pyrogenic substances PC-3 and/or PC-6 which were fractions derived from the parent pyrogen PC-1 (Piromen).*

Methods and results. Preliminary work suggested that it was advisable to determine the therapeutic value of pyrogen on tumors at different implant sites as well as on different tumor strains. The effect of these pyrogens on both subcutaneous and intracerebral transplants of the S-37 tumor was ascertained.

The other tumors selected for either subcutaneous or intraocular transplant sites were first removed from a carrier animal and minced with scissors in a small quantity of sterile 0.85% sodium chloride solution. Small fragments of tumor tissue were introduced into the animals by the semi-automatic trocar method of Fuson and Eichwald(6). Subcutaneous tumor transplant of the sarcoma and leukemia strains were implanted into the left axillary region of the recipient mice. Intraocular adenoma transplants were implanted into the anterior chamber of the animal's right eye. For intracerebral transplantation, the trocar method proved inadequate; therefore, the S-37 tumors were removed from the carrier mice and the tumors briefly homogenized in sterile saline with a Waring blendor until the large tissue particles were noticeably disrupted. The Swiss albino mice

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TABLE I. Influence of Bacterial Pyrogens on Subcutaneous Sarcoma-37 Tumors in ABC Mice.

Group No.*	Fraction inj.	γ per inj. per wk	Total γ inj.	% survival†	P value X^2 test
I	PC-3	10	30	28	.2 –.1
II	PC-6	20	60	42	.05–.02
III	PC-3 PC-6	10 20	30 60	79	.001
IV	Control	0	0	0	—

* 14 animals in each group.

† % survival at 90 days with no observable tumor reoccurrence in survivors after 3 weekly injections of bacterial pyrogens.

were then inoculated intracerebrally with 0.03 ml of a 1:200 dilution (w/v) of the saline-tumor suspension. Asceptic technics were employed throughout.

The pyrogenic polysaccharides were dissolved in lactate buffer and the treatment injections administered intraperitoneally in 0.2 ml volumes at the following described time intervals. Animals were inspected daily and both mortality and survival time noted.

Fifty-six ABC mice were inoculated subcutaneously with a small transplant of the tumor S-37. The treatment schedule did not begin until 9 days after tumor implantation, at which time palpable tumors, approximately 7 x 4 mm in size, were observed. The mice were divided into 4 groups of 14 animals each, 7 males and 7 females per group. Group 1 received 3 weekly injections of 10 μg each of fraction PC-3, the second group 3 weekly injections of 20 μg each of fraction PC-6, and the third group 3 weekly injections of a combination of both in the same above amounts. The fourth group of mice served as controls and was accorded similar treatment except that they received 3 weekly injections of a corresponding volume of the pyrogen diluent, lactate buffer. The effect of treatment was dramatic. The per cent survival at 90 days after transplant is presented in Table I. A relationship appeared to exist between amount of drug administered and per cent survival rather than a seemingly higher protective value for one pyrogen as opposed to the other. Total amounts of the administered drugs for the 3 therapy groups were 30, 60, and 90 μg, survival being 28, 42, and 79% respectively. Each of the subcutaneous S-37 tumor transplants responded to pyrogen treatment but only those animals survived in which complete elimination of the tumor occurred. No differences in mortality rate or survival time between males and females were noted. Action upon the tumor resulted in the grossly observable sequence of hemorhage and necrosis followed by occasional sloughing. Tumor growth often reoccurred at marginal areas when the response to the pyrogens was incomplete, and surviving tumor tissue continued to spread until ultimately the animal succumbed. Employing the *Chi* square method of analysis with Yates' correction for small numbers, the dose of PC-3 used failed to increase survival time to a significant degree when compared with those animals receiving the lactate buffer. Administration of the PC-6 and the combination PC-3 plus PC-6 resulted in significant protection against the S-37 tumor.

Two hundred and fifty albino mice were inoculated intracerebrally with the S-37 tumor. The tumor-bearing animals were then randomly subdivided into 4 major groups of 25 animals which received no therapy and 3 groups of 75 animals which received treatment either on the first, third, or fifth day following tumor transplantation. Each major group of 75 animals was further divided into 3 sub-groups A, B, and C, for treatment with PC-3, PC-6, or a combination of both as outlined in Table II. Dosages used were the same as those for the subcutaneous S-37 transplantation experiment. Pyrogen administration followed at 4-day intervals from the initial treatment doses. The slight differences in survival time between control animals and those treated with the pyrogen fractions may be seen in Table II. It should be noted, however, that there were no surviving animals. These results show that mice challenged *via* the intracerebral route with the S-37 tumor did not exhibit an increased survival time when treated with these two bacterial pyrogens, regardless of dosage employed or therapeutic time interval.

Adenoma EO771 was implanted intraocularly into sixty C57BL mice and the mice randomly separated into groups of 20 animals each. Groups I, II, and III received a mix-

TABLE II. Effect of Bacterial Pyrogens upon Intracerebral Transplants of Tumor S-37.

Group No.*	Pyrogen fractions/inj.	Time of 1st treatment after transplant, days	Avg survival time, days
I	A 10 μg PC-3	1	12.52
	B 20 " PC-6	1	12.52
	C 10 " PC-3 20 " PC-6	1	13.04
II	A 10 " PC-3	3	12.76
	B 20 " PC-6	3	12.12
	C 10 " PC-3 20 " PC-6	3	13.48
III	A 10 " PC-3	5	13.32
	B 20 " PC-6	5	13.16
	C 10 " PC-3 20 " PC-6	5	13.20
IV	Normal controls	—	13.40

* 25 animals in each group.

ture of 20 μg of PC-6 plus 10 μg of PC-3, a mixture of 10 μg of PC-6 plus 5 μg of PC-3, or a corresponding volume of the lactate buffer, respectively. Four weekly injections of either the pyrogenic fractions or lactate buffer were administered beginning 10 days after tumor implantation. The results are given in Table III. Under the described experimental conditions, the adenoma EO771 also failed to respond to pyrogen treatment. Average survival time for each treated group was approximately that of the control group.

Leukemia 1498 was transplanted subcutaneously into one hundred C57BL mice and the mice randomly subdivided into 4 groups of 25 animals each. Pyrogen administration was initiated at 2 different time intervals and in 2 dosages beginning 48 hours after implantation. The first group of 25 animals received an intraperitoneal injection of 20 μg of fraction PC-6 and 10 μg of fraction PC-3 every 4 days. The second group of 25 animals was accorded the same dosage except that treatment was given every 2 days. Group III received an intraperitoneal injection of 10 μg of PC-6 plus 5 μg of PC-3 every 2 days. The remaining 25 animals constituted Group IV, the tumor controls, and received no therapy. It is apparent from the results (Table IV) that leukemia 1498 did not respond to the pyrogen treatment. Average survival time of the treated animals was slightly less than that of control animals.

TABLE III. Effect of Bacterial Pyrogens on Intraocular Transplanted Tumor EO771 in C57BL Mice.

Group No.*	Pyrogen fraction per inj.	Avg survival, days
I	20 μg PC-6 10 " PC-3	43.2
II	10 " PC-6 5 " PC-3	49.7
III	0	48.0

* 20 animals in each group with each animal receiving 4 weekly injections.

Discussion. Naturally occurring mouse tumors had been noted to undergo regression following administration of a pyrogenic-vaccine combination. This result was attributed to the 2 pyrogens contained in the vaccine and both were tested for their antineoplastic activity on transplantable mouse tumors. These data indicate that only the subcutaneous transplants of the sarcoma S-37 tumors were amenable to pyrogen therapy. Sarcoma S-37 tumors that were not completely eliminated during treatment often became refractory to further treatment and ultimately progressed to kill the animal. Refractoriness to pyrogens has been commonly observed. Animals readily become incapable of eliciting a marked febrile response after repeated pyrogen administration (7-9). The tolerance to pyrogenic bacterial materials has been explained by both humoral inhibitors (10) and accelerated elimination by the reticuloendothelial system(11). Tumor destruction apparently occurred through a vascular response similar to either the hypoten-

TABLE IV. Effect of Bacterial Pyrogens on Transplanted Leukemia 1498 in C57BL Mice.

Group No.*	Treatment interval, days	Pyrogen fraction/inj.	No. of inj.	Avg survival, days
I	4	20 μg PC-6 10 " PC-3	2	12.7
II	2	20 " PC-6 10 " PC-3	3	12.1
III	2	10 " PC-6 5 " PC-3	3	12.6
IV	Control	0	0	13.2

* 25 animals in each group.

sive response of Algire(12) or to the Shwartzman reaction as previously ascribed to other bacterial-derived antineoplastic agents(13-14). Likewise, a refractive state may also be induced toward both the Shwartzman reaction(15-17) and the hypotensive state(12).

Summary. Of the 3 tumor sites tested, only the subcutaneous transplants of the S-37 tumors responded to treatment with the 2 pyrogenic fractions PC-3 and PC-6. Regardless of the treatment regimen, the intracerebral S-37 transplants failed to respond to treatment with pyrogen. Animals bearing either the intraocular transplants of adenoma EO771 or the subcutaneous transplants of leukemia 1498 exhibited no increase in survival time as a result of pyrogen therapy.

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Insulin Antagonism in Serum of Untreated Diabetics and in Previously Treated Diabetics with Ketoacidosis.* (26120)

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It has already been shown that sera of uncontrolled diabetics, who have previously been treated with insulin, neutralize the activity of insulin when tested *in vitro* by the rat diaphragm assay(1). This antagonism disappears when the diabetes is brought under control. As far as we know, no one has tested the effect of added insulin to sera from new and untreated diabetics, where no insulin antibodies are present. The present communication describes studies in which the biological effect of insulin on the rat diaphragm incubated in sera of new and untreated diabetics is compared to its effect when incubated in sera of patients with ketoacidosis, who had been previously treated with insulin.

Materials and methods. Blood was obtained from: 1) recently discovered diabetics with an elevated blood sugar and glycosuria; these patients never received insulin, and 2) patients admitted to the hospital with untreated diabetic acidosis; although they had had no insulin treatment for many hours prior to admission, they all had received insulin for over 2 months in the past. The serum was removed after proper blood clotting and kept at −10°C until time of testing. Sera with a glucose content grossly over 300 mg% were dialysed against a bicarbonate buffer(2) to decrease the glucose content to this approximate range. The same buffer, with glucose added (300 mg%), was used for the bioassay. The specimens were assayed for insulin or

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