of low ionic strength. This has not been completely successful since the amounts of DNA appearing in the supernatant of sediment I have been found to be about 5 per cent of the total DNA recovered in the typical preparation. Similar observations have been reported, for example, in the fractionation of ascites tumour cells[9]. Although DNA-histone is found to be less than 0·1 per cent in our most active preparations, we cannot yet rule out the nucleus as the source of some antigenic material.

The material described by Davies[4] which is isolated from ascites tumour fluid is a lipoprotein that is active in the hæmagglutination inhibition system of Gorer. More recently, Manson *et al.*[3] have reported on a material isolated from a strain-specific mouse spleen cell and lymphosarcoma, grown in cell culture, which also appears to be a lipoprotein. It is reported to be active in a transplant system, but differences in the assay system that they have used and our assay system preclude a comparison of the two preparations.

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'Refuin': a Non-cytotoxic Carcinostatic Compound proliferated by a Thermophilic Actinomycete

'REFUIN' is a high-molecular fraction of a fermentation beer produced by a thermophilic actinomycete. The fraction is recovered by precipitation with ammonium sulphate and by fractional ice crystallization. Although the fermentation temperature is 50° C, the active principle is heat-labile, losing all activity after 6 h at room temperature. Electrophoretic analysis reveals a ninhydrin staining positively charged band.

The morphology and general physiology of the *Thermoactinomyces* sp. producing 'Refuin' was determined by methods previously described[1].

'Refuin' was evaluated for antibiotic activity, *KB*-cell line tissue culture toxicity, and for carcinocidal or carcinostatic activity on four mouse tumour systems: sarcoma 180, carcinoma 755, leukæmia 1210, and Ehrlich ascites.

Activity against Gram-negative organisms was not noted. The Gram-positive activity was highly specific. Although *B. subtilis* was not inhibited and *S. aureus* 209 *P* was only slightly inhibited, *Mycobacterum smegmatis*, *Torulopsis albida* and *S. aureus* respiratory deficient mutant culture *AT CC UV* 2–13680 were strongly inhibited.

The anti-tumour activity in the sarcoma 180, adenocarcinoma 755, and leukæmia 1210 was conducted according to the National Institutes of Health protocols 1 : 301–302 of June 1962. A daily intraperitoneal injection of 50 mg/K of 'Refuin' causes a 75 per cent inhibition of the sarcoma 180 tumour and a 60 per cent inhibition of the adenocarcinoma 755 tumour. No leukæmia 1210 activity was observed.

The Ehrlich ascites assay results are summarized in Table 1. 10^6 ascites cells were inoculated intraperitoneally and immediately followed by a subcutaneous injection of 50 mg/K of 'Refuin'. Although the subcutaneous route is not reported to be effective for administration of other anti-tumour agents, 'Refuin' showed significant activity when administered either subcutaneously or intraperitoneally. Although the intraperitoneal route gives better results, the subcutaneous route permits more critical evaluation of our system. Of special interest was the effect of 'Refuin' in converting the fluid ascitic cells to a small solid tumour in 45 per cent of the treated mice. Upon continued administration of 'Refuin', necrosis and ultimate disappearance of this tumour resulted in many of these mice.

Table 1. ACTIVITY OF 'REFUIN' ON EHRLICH ASCITES MOUSE TUMOUR SYSTEM WHEN ADMINISTERED SUBCUTANEOUSLY

	Treated group	Control group
Number of mice	100	47
Number of mice showing conversion of ascites to solid tumours	45	0
Average survival of those mice showing progressive development of ascites	19·9 days	9·8 days
Number of mice showing total disappearance of ascites and tumour	18	0

Mortalities or excessive loss of weight due to administration of 'Refuin' were not noted. A LD_{50} on unimplanted mice was not obtained since the maximum dose that could be conveniently administered intravenously (700 mg/K) proved non-lethal.

In the *KB* cell line tissue culture system 'Refuin' caused negligible inhibition. This would suggest that the oncostatic activity of 'Refuin' is dependent on the host defences. This lack of tissue culture activity, the lack of systemic toxicity, and its very limited antibiotic spectrum differentiates 'Refuin' from the other anti-tumour agents known at present.

All drugs other than hormones and steroid that are of value in cancer therapy have shown considerable toxicity. As stated by Moore[2] toxicity is a prerequisite for anticancer activity. Indeed, since the drugs presently used for the palliation of neoplastic disease exert their greatest effect on rapidly dividing cells, they also induce toxic symptoms in bone marrow, gastrointestinal mucosa, skin, liver, and lymph nodes. The clinical evaluation of 'Refuin' now in progress has confirmed the lack of systemic toxicity of this drug and gives promise of confirmatory clinical evidence of anti-tumour activity.

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IMMUNOLOGY

Preparation of an Antiserum Specific to a Spontaneous Mouse Leukæmia after the Induction of Artificial Immunological Tolerance to Normal Mouse Tissue

THE feasibility of preparing antiserum to the transplantable Ehrlich ascites tumour after the induction of immunological tolerance to normal mouse tissue antigens in the neonatal rabbit has been reported previously from this laboratory[1,2]. Such tolerant anti-Ehrlich ascites tumour sera, when tested *in vivo*, were toxic for the tumour and relatively innocuous for the murine host.