containing organic solvents may be due more to the differences in the sapogenin portion than in the oligosaccharide.

The nutritional effects of triterpenoidal bulk saponins[2], and especially the pronounced physiological effects that can be produced in the ruminant by bulk lucerne saponin[5], stress the need for the isolation of purified constituent saponins from lucerne, and other herbage plants, to facilitate further studies of these effects and, in particular, their role in bloat, since bloat causes considerable losses in animal production in the Commonwealth and elsewhere[6].

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Antitumour Effect of Culture Media from Tumour-immune Spleens

The antigenic activity of tumours has been indicated by several authors using immunological or other *in vitro* methods, and also passive transfer of antitumour agents by serum injections from animals with regressed tumours[1–4].

In the experiments reported here, fragments of spleens of rats (black Walker-resistant strain, eleventh generation of brother–sister mating) were cultivated in roller tubes on glass, one whole spleen in ten 16 mm. × 160 mm. tubes with 4 ml. 50 per cent horse serum–Tyrode medium in each. Abundant outgrowth of amoeboid, giant and fibroblast-like cells was noted. The medium was collected three times a week during three weeks. Spleens were taken either from normal rats, or from rats hyperimmunized by repeated implantations of the Walker 256 tumour; this regresses in resistant rats at first implantation and does not take at further implants.

The medium was collected and thoroughly centrifuged to remove any cells released from the cultures, and then injected without storage on the days of collection in ten doses of about 5 ml. intraperitoneally. The recipients were Wistar rats implanted intramuscularly on the day of the first culture medium injection with Walker 256 tumour-cell suspension. The suspension was prepared overnight by means of an electromagnetic stirrer from tumour fragments at 4° C. in phosphate-buffered saline. In each experimental set every rat was implanted with an equal dose of live tumour cells (estimated by the method of unstained cells count[5]). The doses were about 6 million cells per rat in the first experiment, 1 million in the second, and 8 million in the third.

Table 1

Treatment: culture medium from		No. of rats with tumours	Deaths up to 42 days after tumour implantation		Range of survival (days)	No. of regressions after treatment
				(Per cent)		
I	Normal spleens	5	5/5	100	19–36	0
	Immune spleens	7	4/7	57	28–46	0
II	Normal spleens	8	6/8	75	24–75	0
	Immune spleens	7	2/7	29	37–73	2
III	Normal spleens	9	8/9	89	19–46	0
	Immune spleens	9		44	25–47	3
Total	Normal spleens	22	4/9 19/22	86	19–75	0
	Immune spleens	23	10/23	43	25–73	5

The results of three independent experiments are summarized in Table 1.

Our experience has shown that, as a rule, six weeks is the life-span of susceptible rats implanted with the tumour used. For this reason the number of deaths up to the 42nd day was chosen as a standard for the influence of the treatment. The rats used in these experiments are commercial Wistar, not inbred for the susceptibility to Walker 256 tumour; some individuals from this source can live with the implanted tumour considerably longer without any treatment. Even typically resistant individuals can be selected from this strain, the immune serum of which inhibits the growth of the tumour if injected to tumour-bearing rats (unpublished results). We conclude that the animals in our experiments, which are capable of relatively high spontaneous defence, are those which were cured by the addition of active principle from immune spleens cultivated *in vitro*.

The production of antibodies against bacterial and protein antigens in cultures of immune spleens has been repeatedly demonstrated[6,7]. This was not done, within our knowledge, with tumour antigens, though an inhibition of tumour explants grown together with immune spleen was observed[8].

These results show that passively transmissible antitumour factors are present in a culture medium of tumour-immune spleens. It is not decided here whether the active substance(s) are produced in the cultures as a continuation of the process initiated *in vivo*, or simply extracted from the cultivated spleen fragments. Elucidation of this, the isolation of the active principle, and a trial of active immunization of spleen cells *in vitro* against tumour antigens are being investigated.

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