from 6.25 to 100 μg; yet in duodenum almost 90% of the 100 μg dose was absorbed. Chicks fed 5.8 or 10.6 ppm thiamine in stock rations had a similar profile in intestinal tract concentrations with lowest value in middle ⅓ of small intestine. Chicks fed 127 ppm thiamine had progressively increasing thiamine concentrations in sections of small intestine distal to duodenum. The data indicate that thiamine is absorbed primarily in the upper small intestine and when fed at very high levels to chicks is secreted by intestinal mucosa of middle and lower sections of small intestine.

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Effect of Parenteral Heterologous Albumin on Transplanted Tumors in Mice.* (29024)

J-C. GAZET AND B. MCKIBBIN (Introduced by W. H. Cole)
Department of Surgery, University of Illinois College of Medicine, Chicago

The effect of RNase on tumor growth *in vivo* has been described by Ledoux for both ascites(1) and solid tumors(2). Mean survival time of the mice was increased from 15 to 185% depending on the tumor, amount of enzyme injected, and time of inoculation of the tumor cells. Similar results have been obtained by other investigators with other types of tumors.

Gazet and McKibbin† have shown that parenteral administration of heterologous RNA will significantly increase the percentage take of free 180 mouse sarcoma and Ehrlich's ascites tumor cells inoculated subcutaneously into mice. The question has arisen, therefore, as to whether heterologous albumin administered in a similar manner would have the same effect as heterologous RNA. The experiments reported herein were designed to answer this question.

Method and materials. All experiments were preformed on female Swiss mice aged 4 to 6 weeks and weighing approximately 20 g, maintained on Standard Mouse Breeders Purina chow with as much water as desired. In all experiments groups of 10 animals were used. The abdomen was shaved on the day prior to experiment, and all tumor cell suspensions were inoculated subcutaneously in the midline in this area. All drugs were injected subcutaneously in the back.

Two tumors, the mouse sarcoma 180 and Ehrlich's ascites tumor, were employed. In the case of the sarcoma, the cell suspensions were prepared from suitable donors under aseptic conditions as described by Cole *et al*

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(3). The ascites tumor was obtained by paracentesis of donors 7 days after primary inoculation with the tumor.

Total cell counts of 25,000 and 100,000 tumor cells were used in the majority of experiments and were found to produce an average take of 50 and 100% respectively in control animals. All tumor suspensions were so arranged that each animal received the required number of cells subcutaneously in 0.1 ml of Hanks' solution. The final incidence of tumor take was calculated when no further new tumors appeared over a period of one month.

Bovine albumin (Cohn fraction V) obtained from Nutritional Biochemical Corp. was used and shown to be free of contamination with nucleic acids by spectroscopy and orcinol and diphenylamine extraction. Most of it was used as obtained from the Company, but for one experiment a small portion was hydrolyzed by heating sealed ampules containing 5 mg albumin per 2 ml of 6 N hydrochloric acid for 24 hours at 104°C. Following this procedure the hydrochloric acid was removed under vacuum distillation. The amino acids were then dissolved in isotonic saline to give a solution equivalent to 2.5 mg albumin per ml. The pH was adjusted to neutrality by titration with sodium hydroxide. The final molarity of the solution was 0.167 with respect to sodium chloride. The solution was refrigerated between each series of injections.

All drugs were freshly made up in 2.5 mg/ml physiologic saline. The albumin was given in 1 ml doses (125 mg/kg body weight) one hour before inoculation with tumor cells, immediately after inoculation, twice on the second post-inoculation day, and thereafter once daily until either 16 doses had been given, or termination of the experiment.

In a further series of experiments a suspension of 250,000 tumor cells per ml in Hanks' solution was incubated with concentrations of 1 μg to 10 mg/ml albumin, at 37°C for one hour. 0.1 ml of this suspension containing 25,000 treated cells was inoculated into each test mouse.

Two separate experiments were undertaken to establish the effect of albumin on tumor growth. A group of mice was inoculated with a suspension containing a total count of 100,000 sarcoma 180 tumor cells so as to ensure a take of 100% or thereabouts. Five days later when a sufficient number of tumors had appeared, the animals with tumor takes were randomized into 2 groups and the tumors measured. Each mouse was individually ear marked so that tumor size could be followed in each animal. One group of animals was given albumin (2.5 mg/ml) and the other group received saline 1 ml daily until either the death of the mouse or regression of the tumor occurred. There were initially 20 animals in each group. Tumor size was defined as the product of the width and length taken at right angles and expressed in square mm. Tumors were measured daily, and average tumor size calculated for each group. As animals died they were excluded from the average growth table.

TABLE I. Effect of Albumin and Saline on Percentage Take of Sarcoma 180 and Ehrlich's Ascites Tumor in Female Swiss Mice.

Tumor	Agent	No. of animals	% tumor take
Sarcoma 180	Saline	80	59
	Albumin	81	92 ($P < .01$)
Ehrlich's ascites	Saline	70	54
	Albumin	69	91 ($P < .02$)

The experiment was then repeated with two important differences. The mice were inoculated with a total cell count of 25,000 tumor cells only and the albumin or saline was given from time of inoculation. The size of the individual tumors was recorded as they appeared and then daily until either death or regression had occurred. There were approximately 20 animals in each group.

Results. Four separate experiments were performed to compare the effect of albumin and physiologic saline on the percentage tumor take of 180 sarcoma in mice. Table I shows that parenteral administration of albumin produced a marked increase in the percentage tumor take from 59% with physiologic saline to 92% in the treated group. This increase in number of tumors was of the same order as that obtained with RNA by

TABLE II. Effect of Albumin, Its Hydrolysate and Saline on Percentage "Take" of Sarcoma 180 and Ehrlich's Ascites Tumors in Female Swiss Mice.

Tumor	Agent	No. of animals	% tumor take
Sarcoma 180	Saline	77	30
	Albumin	71	55
	Albumin hydrolysate	75	47
Ehrlich's ascites	Saline	59	51
	Albumin	60	90
	Albumin hydrolysate	60	77

Gazet and McKibbin in unpublished experiments, and was statistically highly significant ($P = .01$). The experiment was repeated 4 times with Ehrlich's ascites tumor. The increase in the percentage take rose from 54% in the controls to 91% with albumin and was equally significant ($P = .02$).

These 2 experiments were repeated with hydrolyzed albumin (Table II). With both the sarcoma 180 and Ehrlich's ascites tumor, there was an increase in percentage take with the hydrolyzed albumin over the saline controls but this was not as marked as with the unhydrolyzed albumin.

The question then arose as to whether the effect of parenteral albumin was primarily on the tumor cell itself or on the host in producing this increase in the percentage tumor incidence with inoculated free cancer cells. The effect of the incubation of tumor cells with various concentrations of albumin was then examined. In the concentrations used, albumin not only did not have an enhancing effect on subsequent tumor cell takes, but in general, in the higher concentrations did have a toxic effect on the tumor cells (Table III), suggesting that the effect of parenteral albumin as shown by the increase in precentage incidence *in vivo* was either due to an effect on the host or mediated through the host.

The effect of parenteral administration of heterologous albumin on growth of an established sarcoma 180 tumors was also investigated. Two experiments were performed. When the albumin was not given until the 6th day after an established tumor had developed, it had no effect on growth as compared to the size of the tumors in the animals given saline. Likewise, the regression rate, or death rate was the same. However, when albumin was started on the same day the cells were inoculated, the size of the tumor mass was greater (from the 12th day onward) in the mice given albumin (Fig. 1), although the death rate and regression rate were slightly prolonged.

Discussion. The question arises as to whether or not nutrition has a relationship to the results in the foregoing albumin experiments. Tannenbaum and Silverstone(4) have shown that in mice on diets containing between 9 and 45% casein, there is no difference in the establishment of a tumor, rate of growth, incidence of metastases, or mortality with spontaneous mammary carcinoma or induced squamous cell lesions. Only with protein deprivation serious enough to cause a

TABLE III. Effect on Percentage Take of Incubation of 250,000 Tumor Cells/ml for One Hour at 37°C with Various Concentrations of Albumin in Solution Prior to Inoculation of 0.01 ml Tumor Suspension into Mice.

Tumor	Agent	No. of animals	% tumor take
Sarcoma 180	Control Hank's	40	70
	10 mg Albumin/ml	39	14.6
	1 " "	49	17.3
	.1 " "	48	25
	.01 " "	39	33
	.001 " "	39	48.6
Ehrlich's ascites	Control Hank's	20	45
	10 mg Albumin/ml	20	0
	1 " "	19	15.7
	.1 " "	20	5
	.01 " "	20	20
	.001 " "	20	45

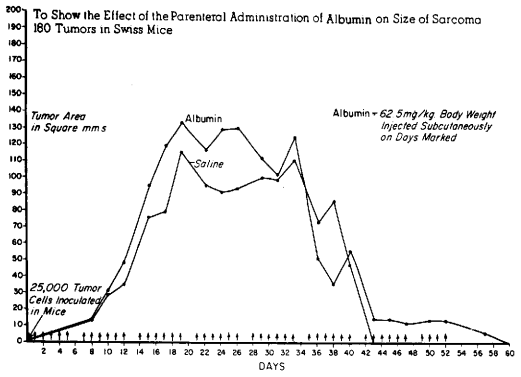

FIG. 1

striking loss of body weight is there significant retardation of the rate of establishment of the tumor and growth of spontaneous tumors(5).

We have no satisfactory explanation for our results. As previously noted, these animals received only Standard Mouse Breeders Purina chow, *ad libitum*. The composition of the substance is kept constant and contains approximately 18% protein. An adult mouse will eat approximately 5 to 8 g of these pellets per day. Thus its protein intake will range approximately from 900 to 1600 mg a day. Injection of 2.5 mg of albumin per day would not appear to significantly increase the protein uptake of treated animals over controls. Thus theoretically, little effect, if any, on tumors or host, would be anticipated on nutritional grounds from its administration of this dosage.

There are 2 possible explanations for the action of parenteral albumin in increasing the percentage tumor takes. If albumin is rapidly absorbed via the circulation and taken up by tumor cells as suggested by Ghose *et al*(6), it would mean that the effect of the albumin is directly on the tumor cell. This would suggest in the experimental state that an increase in circulating proteins or amino acids above a critical level would lead to an increase in tumor cell implantation and growth. In other words, an animal in a catabolic state would have a higher percentage of tumor cell takes after inoculation with free cancer cells than the normal, and one in an anabolic state the reverse.

Certainly neither the *in vitro* incubation nor the reduced tumor cell inoculation experiments performed have supported the theory that the results were due to a direct effect of protein upon the inoculated free tumor cells. Alternatively, an explanation embodying an effect on this host, however mediated, would appear more attractive than a direct effect on the tumor cell.

Foreign proteins may produce other than nutritional effects for example, on the reticulo endothelial system or the endocrine glands. There is evidence that long term parenteral administration of albumin will produce a significant fall in weight of the adrenals and ovaries of female Swiss mice (Gazet(7)). Further, it is well known that cortisone will enhance tumor takes in experimental animals. The effect, therefore, of the albumin may be similar to the operative stress of a celiotomy, which in the experimental animal will increase the percentage take of inoculated tumor cells(8). This stress effect is apparently not the same as Selye stress because it is not related to the adrenals(9).

However, a celiotomy produces a catabolic state with a negative nitrogen balance for a short period post-operatively. Therefore, returning to our original premise, it is possible

TABLE IV. Effect of Albumin and Saline on Percentage "Take" with Reduced Number of Sarcoma 180 Tumor Cells Injected into Female Swiss Mice.

	Saline		Albumin	
Total No. of tumor cells inoculated	No. of animals	% tumor take	No. of animals	% tumor take
100,000	40	80	40	100
25,000	40	55	40	90
2,500	35	31	38	42
250	38	0	40	5
25	38	0	40	0

that albumin stimulates this state and in some unknown way produces the effects described on the host.

Summary. Parenteral administration of heterologous albumin (125 mg/kg body weight per day or 2 g/kg body weight in 14 days) was found to increase the incidence of tumors produced by inoculation of sarcoma 180 cells from 59% in control mice given saline, to 92% in mice given albumin. In the mice given Ehrlich's ascites tumor cells the take was 54% in animals given saline but was increased to 91% in animals receiving albumin. Similar but less significant results were obtained with hydrolyzed albumin.

Prior incubation *in vitro* of albumin with these tumor cells did not produce an enhancement in takes on subsequent inoculation in mice, suggesting that the albumin did not act directly upon the tumor cell. It appears that the effect of the heterologous albumin is more likely to be due to an albumin-host interaction than an albumin-cancer cell effect. This is supported by the study of the effect of albumin on growth of the tumor *in vivo* associated with a decrease in weight of the adrenals and ovaries. This finding is being investigated.

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Acid Ribonuclease Activity in Peripheral Leucocytes of Mice: A New Cytochemical Technique.* (29025)

O. S. ATWAL, J. B. ENRIGHT AND F. L. FRYE (Introduced by S. A. Peoples)
Department of Public Health, School of Veterinary Medicine, University of California, Davis

Using spectrophotometric methods for determination of ribonuclease activity Houck and Berman(1) described the concentration of this enzyme in human leucocytes. This method permits quantitative recovery of the enzyme in the cell. Later, Novikoff(2) described the presence of this enzyme in specific granules of the ploymorphonuclear leucocytes in peritoneal exudates of rabbits. Cohn and Hirsch(3) described the lysosomal nature of these granules. Acid ribonuclease is one of the enzymes associated with the lysosome particles of the cell cytoplasm. Since these subcellular particles are assuming increasing importance in cellular biology, this communication presents a new technique developed during a study undertaken to demonstrate the localization of this activity by cytochemical methods.

The technique is based on the use of ribonucleic acid (RNA) from yeast as substrate and is a modification of the histochemical technique for intracellular localization of acid-deoxyribonuclease in the rat liver described by Vorbrodt(5). Particular attention has been paid in composing the incubation medium that makes it possible to obtain intracellular localizations of acid RNA-ase activity in conformity with those indicated by quantitative data from isolated subcellular fractions described by Houck & Berman (1).

Materials and methods. Blood smears obtained from the tails of Swiss mice of the Webster strain and air dried for 10-30 min-

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