

jects (Table II). In all these the increase in temperature in the toes was significantly greater with glycine than after alcohol (2 ounces of whiskey in basal state). The average change in temperature at the toes was $+3.8^{\circ}\text{C}$ with glycine, $+1.2^{\circ}\text{C}$ with alcohol; at the fingers $+2.3^{\circ}\text{C}$ with glycine, -0.2°C with alcohol; and at the forehead $+2.4^{\circ}\text{C}$ with glycine and $+1.2^{\circ}\text{C}$ with alcohol.

Oscillometric Readings. The amplitude of oscillometric pulsation in the calf increased in 8 of the 11 subjects with no peripheral vascular disease. The average change for this group of 11 subjects was an increase in pulsation of 25%. No increase in oscillometric pulsation was observed in 3 cases with peripheral vascular disease although significant rises in skin temperature occurred in 2 of

these subjects. In the forearm an increase in amplitude of pulsation following glycine was noted in 6 of these 11 subjects. The average increase in this group of 11 cases was 33%.

Summary. The effect of glycine on peripheral blood flow was studied in 15 subjects by means of skin temperature and oscillometric readings. The blood flow to the extremities was augmented in 11 normal subjects and in 3 of the 4 cases with peripheral vascular disease. The increase in peripheral blood flow is an accompaniment of increased heat production resulting from the specific dynamic action of glycine. The ingestion of glycine provides a simple means to accomplish an effective and sustained increase in peripheral blood flow.

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Relationship of Antibody Content of Normal and Malignant Lymphocytes.*

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The demonstration of antibodies in normal lymphocytes^{1,2} raised the question of whether lymphocytes in lymphosarcoma also could contain antibody. At the present time there are no morphological or physiological characteristics which clearly differentiate normal from malignant lymphocytes. Since one important function of lymphocytes has recently been demonstrated to be the carrying of antibodies,^{1,2} it was possible to compare malignant to normal lymphocytes in this regard.

The data reported in this communication establish the presence of antibody in the

lymphocytes of lymphosarcoma, the relationship of the quantity of this antibody to that of normal lymphocytes, the capacity of tumor cells to obtain antibody from normal lymphocytes, and finally, the ability of tumor cells to carry antibody into a succeeding transplant.

Materials and Methods. Male mice 60 to 80 days old of the CBA strain (Strong) were used. The animals were fed Purina Fox Chow supplemented with calf meal; food and water were available at all times. The tumor used was obtained from Dr. W. U. Gardner. This tumor arose in an estrogen-treated mouse of the C₃H strain and has been transplanted for many successive generations. The transplant of the tumor takes in 100% of CBA mice. The transplanted tumor grows rapidly and kills the host in 2 to 3 weeks. This tumor was selected for the present experiments because it does not metastasize. This was of importance for the study because it was desired to compare the antibody content of tumor and

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[†] Alexander Brown Cox Memorial Fellow.

¹ Dougherty, T. F., Chase, J. H., and White, A., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 295; 1945, **58**, 135.

² Harris, T. N., Grimm, E., Mertens, E., and Ehrlich, W. E., *J. Exp. Med.*, 1945, **81**, 73.

TABLE I.

Comparison of *Staphylococcus* Antitoxin Concentration in Blood and Extracts of Normal and Malignant Lymphocytes.

	No. of mice	Mg nitrogen/ml sera or extract			Inhibition titers			Mg nitrogen in inhibiting dilution		
		Serum	Lympho- cyte extr.	Tumor extr.	Serum	Lympho- cyte extr.	Tumor extr.	Serum	Lympho- cyte extr.	Tumor extr.
Exp. 1	2	5.83	3.85	—*	1:20	1:40	—*	0.276	0.096	—*
Immunized mice	1	6.44	2.58	—*	1:40	1:80	—*	0.162	0.034	—*
given tumor trans-	1	4.88	2.80	—*	1:80	1:40	—*	0.064	0.070	—*
plant; antigen con-	5†	—	—	—	1:40	1:80	1:80	—	—	—
tinued	3	7.06	4.64	8.49	1:20	1:160	1:80	0.35	0.03	0.10
Exp. 2	1	5.62	3.84	—*	1:160	1:160	—*	0.034	0.026	—*
Antigen adminis-	1	5.76	3.48	—*	1:80	1:80	—*	0.075	0.045	—*
tration started at	3	5.50	3.96	10.67	0	1:40	1:320	0	0.099	0.032
time of tumor	1	4.22	0.74	2.08	1:160	1:20	1:40	0.026	0.037	0.053
transplant	1	4.62	2.00	2.28	1:160	1:40	1:40	0.027	0.051	0.058
Exp. 3.	1	4.58	3.26	—*	1:80	1:80	—*	0.059	0.042	—*
Immunized mice	1	5.72	2.34	—*	1:40	1:80	—*	0.142	0.030	—*
given tumor trans-	2	6.22	3.54	8.19	1:20	1:40	1:160	0.31	0.088	0.049
plant; no further	1	6.10	2.40	3.50	1:10	1:80	1:80	0.61	0.032	0.045
antigen										

* The absence of data for tumor extracts indicates control mice with no tumor but otherwise treated the same as tumor-bearing animals.

† Nitrogen analyses not done in this experiment.

normal lymphoid cells in the same animal. The tumor has been described in detail³ and is composed of large cells which resemble reticular cells. Tumor transplants were made by implanting small pieces of the tumor subcutaneously.

Since the mice with this tumor transplant live only 2 to 3 weeks, it was necessary to use an antigen which would produce significant antibody titers within this period. The antigen chosen was a filtrate of a 24-hour broth culture of *Staphylococcus aureus*. The toxin solution was injected subcutaneously in each mouse. Sera and extracts of normal and malignant lymphocytes were obtained from single or from groups of mice and were titrated for their antibody content. Tumor and normal lymphoid tissue, with the exception of the inguinal and axillary nodes nearest the tumor, were treated as previously described.¹ Nitrogen analyses of sera and extracts were made by the micro-Kjeldahl method.

Immediately before each titer estimation the minimum hemolytic dose of the toxin was determined. The antisera and the ex-

tracts were diluted according to the doubling dilution method, and the minimum hemolytic dose of toxin was added to each dilution. Then 0.2 ml of a 5% suspension of fresh, washed rabbit erythrocytes was added to each tube. The tubes were incubated in a water bath at 38°C for one hour and the titers were read. Readings were checked after the tubes had remained overnight in the ice-box. The titers were read as that dilution of serum in which hemolysis had been completely prevented.

Extracts of the tumor used in this study were shown to have no hemolytic activity when incubated with rabbit erythrocytes.

Experimental and Results. The several types of experiments which were conducted are the following:

Experiment 1. Antigen was administered to each animal on alternate days in increasing doses beginning with 0.1 ml and increasing to a maximum of 0.5 ml. At the end of 16 days the experimental mice each received a tumor transplant. Antigen was continued in both control and experimental animals for a further period of 15 days, at the end of which time all mice were bled from the heart and

³ Gardner, W. U., Dougherty, T. F., and Williams, W. L., *Cancer Research*, 1944, 4, 73.

TABLE II.
Antibody Content of Transplanted Tumor Cells.

Mouse No.	Days after tumor transplant	Inhibition titers		
		Serum*	Lymphocyte extract	Tumor extract†
A-2	13	0	1:20	1:40
B-1	13	0	1:10	1:40
B-2	15	0	1:80	1:160
B-3	15	0	1:20	1:160
B-4	15	0	1:40	1:40
B-5	15	0	1:20	1:80
A-3	17	0	1:40	1:80
A-4	17	0	1:80	1:160
A-5	17	0	1:80	1:160
A-6	17	0	1:80	1:80
A-7	17	0	1:160	1:320
A-8	17	0	1:40	1:160

* The lowest serum dilutions titrated were 1:10.

† Tumor transplant in A series had a titer of 1:80; in B series 1:40.

lymphoid and tumor tissue obtained for extraction.

Experiment 2. Antigen administration was started at the time of tumor transplant and continued for a 15-day period; the animals were then sacrificed.

Experiment 3. The mice in this experiment were immunized as described in Experiment 1. After 16 days of immunization each animal received a tumor transplant, and at this time antigen injection was stopped.

The data for the above 3 experiments are presented in Table I. In Experiment 1 the cells of the tumor contained antibody. Since histologically this tumor consists almost entirely of lymphocytes, the immune globulin must have been present in the malignant lymphocytes. The extract of normal lymphocytes contained more antibody per unit of nitrogen than that of tumor cells. In Experiment 2 antigen administration was begun at the time of tumor transplant. Consequently, there was equal opportunity for antibody appearance in both normal and malignant lymphocytes. Under these circumstances the data show that in one experiment with sera and tissues pooled from 3 animals the rapidly growing lymphosarcoma cells had a higher antibody content than did the normal lymphocytes. The other 2 animals in this experiment were moribund at the time they were sacrificed. It is interesting to note that these animals showed highest titers in the serum.

Experiment 3 illustrates the ability of

tumor cells to obtain antibody from normal lymphocytes. Inasmuch as antigen was not administered following tumor transplantation into the immunized mice, the tumor cells could have obtained their antibody only as a consequence of the presence in the animal of previously existing immune globulin.

The data in Table II demonstrate that antibody-containing tumor cells will transfer immune globulin into a succeeding transplant. The mice in the A series received a tumor having a titer of 1:80; in the B series the tumor transplant had a titer of 1:40. Therefore, extracts of tumor transplants showed titers higher than those present in the tumor at the time of its transfer. The new lymphocytes developing from the transplanted cells during the growth of the tumor contained antibody. Hence, there was a transfer of immune globulin to daughter cells arising by mitosis from the labelled cells of the transplant. Moreover, the data show the presence of antibody in normal lymphocytes of the animals in this experiment. Inasmuch as there was no immune globulin present in these mice prior to their receiving the tumor transplant, the normal lymphocytes could have obtained the antibody only from malignant lymphocytes. These data taken together with those in Table I, Experiment 3, establish a reversible exchange of antibody protein between normal and malignant lymphocytes.

Discussion. This study establishes the presence of antibody in malignant lymphocytes.

In this respect, therefore, malignant lymphocytes of the particular type used resemble normal lymphocytes. In most of the experiments tumor cells contained more antibody per unit of nitrogen than did the normal lymphocytes. Also, the malignant cells were able to obtain significant quantities of antibody protein from normal lymphocytes.

The high degree of titer in tumors growing from transplanted, antibody-containing malignant lymphocytes indicates that labelled globulin is carried into the daughter cells. As the growth of the tumor proceeds, increasing numbers of antibody-containing lymphocytes are formed. Thus, the total quantity of available antibody in lymphoid structures is dependent upon the number of antibody-containing lymphocytes present. This type of antibody production, *i.e.*, multiplication of pre-existing cells containing immune globulin, may be a prominent mechanism of antibody production in the normal organism.

The data also show that antibody may be transferred from malignant to normal lymphocytes. This transfer may have occurred as a result of the liberation of immune globulin by degenerating, malignant lymphocytes. It is

also possible that although evidence of metastases was not observed, some tumor cells may have infiltrated the normal lymphoid structures. This latter possibility is unlikely, however, since in the absence of gross metastases, it would be difficult to assume the presence of enough tumor cells in the normal lymphoid tissues to account for their antibody titers.

Summary. Staphylococcus antitoxin titers have been obtained from extracts of lymphocytes secured from a rapidly growing mouse lymphosarcoma. Comparison of these titers with those of the sera and normal lymphocytes of the same animals suggested that tumor tissue had a slightly higher antibody content. Tumor cells were capable of obtaining antibody from some other source in the body, presumably normal lymphocytes. The growth of an antibody-containing tumor transplant in normal mice was accompanied by the development of antibody-containing malignant cells. The normal lymphocytes of the host animal receiving this transplant contained antibody. There is a reversible exchange of antibody protein between normal and malignant lymphocytes.

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Sulfonamide Blood Levels Following Deposition Under the Bladder Peritoneal Reflection.

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Numerous reports on the rate of sulfonamide absorptions from various portions of the body have been published but information was not found on the blood levels following deposition under the bladder peritoneal reflection at the time of cesarean section operation.

In an effort to reduce the morbidity incidence, 5 g of a sulfonamide were placed above the uterine fascia just before the incised area was covered by suturing the bladder peritoneal reflection to the uterus. This tissue is essentially a loose areolar type. Blood levels were taken at 4-hour intervals from the fourth

to the twenty-fourth hour basis following the cesarean section. Because of limited personnel and because the sixteenth and twentieth hours came late at night, only 2 tests for these times were made on the 32 patients.

Every patient had a test at the fourth hour while 30 had at the eighth and 26 at the twelfth hour, only 25 tests were completed at the twenty-fourth hour interval. Marshall's¹ method was used for these determinations.

¹ Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263; Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.