shown to be significant. We observed a decrease of 20% only, whereas in the tests reported by LePage almost no glycogen was present. This difference may be explained by the fact that our samples were analyzed at a predetermined time when all rats were surviving and were not moribund.

The decrease of brain glycogen is in agreement with the impairment of the Pasteur reaction. Decreased phosphorylating processes in brain may contribute to the glycogen deficiency as was also shown for muscle and liver after shock. Low oxygen tension has been reported by Gurdijan *et al.*(11) to have decreased the glycogen content of the brain of one morphinized dog in severe hypoxia and cyanide poisoning causes also a highly significant decrease of glycogen in rat brain as seen by Albaum *et al.*(12). Burdette(13), as well as Kovach *et al.*(14) and Takacs *et al.*(15) have also found that in muscle there occurs depletion of glycogen in shock.

Summary. Increased aerobic and anaerobic glycolysis occurred in brains of tumbled (fettered) rats in shock. The Pasteur reaction was significantly decreased. This indicates an increased anaerobiosis in the brain tissue as a consequence of shock with concomitantly decreased yield of high energy phosphate. Glycogen content of brain is also significantly decreased in shock.

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Immunologic Differences Between Murphy-Sturm Lymphosarcoma and Normal Rat Lymph Nodes.* (26272)

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The Murphy-Sturm lymphosarcoma of rats is derived from bloodborne leukemic cells(1), and is thus related to lymph tissue. This relationship was corroborated by the use of fluorescein label technics. We showed previously that rabbit antisera prepared against the ascites form of the lymphosarcoma strongly cross-react with rat lymph node and spleen(2).

We have now been able to show differences in the antigenic patterns of the lymphosarcoma and lymph node (taken as a normal counterpart) by the use of radio-iodine labeled antibody technics. I^{131} labeled globulins from anti-lymphosarcoma and anti-lymph node sera were specifically fractionated by successive adsorption and elution from tumor and lymph node components. It was thus possible to separate the antibodies into fractions of different specificities, indicating the presence of characteristic anti-

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genic components in each tissue. Previously, we had shown that antibodies against lymphosarcoma, either the solid tumor or ascites form, also contained antibodies directed against fibrin(2,3), but these appear to be different from the antibodies directed against the tumor cells themselves.

Experimental. Tumors: The Murphy-Sturm lymphosarcoma (ascites and solid form) has been described(4). Ascites lymphosarcoma cells (referred to as ALS) were collected from tumor-bearing rats 5-7 days after transplantation and washed with saline containing 0.5-1% sodium citrate at 5°C, then lyophilized.

Antisera. Anti-lymphosarcoma sera (anti-ALS) were prepared by immunizing rabbits with washed ascites cells in Freund's adjuvants as reported previously(2). Anti-rat lymph node sera (anti-LN) were obtained by injecting rabbits with lymph node homogenates mixed with Freund's adjuvants. The γ-globulin fractions of the sera were prepared by ethanol fractionation according to Deutsch (5). The globulin fractions were heated at 60°C for 30 minutes to remove thrombin activity before iodination. This was done in view of subsequent addition of whole plasma in some procedures. The procedures for iodination of proteins(6) and assay for radioiodine(4) have been described.

Preparation of sediments. Lyophilized ALS cells were suspended in borate-buffered saline containing 1% sodium citrate (pH 8, 5°C) and homogenized in a Potter-Elvejem tissue grinder until no intact cells remained. The sediments were separated by centrifuging at 25,000 RCF for 20 minutes at 5°C. These were washed several times in the same centrifugal fields in the cold, then lyophilized. Frozen rat lymph nodes were treated in the same way with buffered saline without sodium citrate. Sediments were used for adsorption after suspending in borate buffer at pH 8.0 by means of a tissue grinder.

Preparation of purified anti-ALS antibody (A_A) and anti-LN antibody (L_L): The radioiodinated globulin fraction of anti-ALS serum (40 mg) was treated with whole rat plasma (2 ml; antigen excess) to neutralize antifibrin antibodies. After incubation at 37° for 1 hr the mixture was allowed to stand overnight at 5° and centrifuged. The supernate was treated with ALS sediment (30 mg) at 37°C for 1 hr. The sediment was washed and the adsorbed antibodies were eluted at 60° in presence of added bovine serum albumin (10 mg, 5 ml borate buffer pH 8) as carrier to avoid surface denaturation. Then the eluate† was treated twice with 20 mg of finely dispersed rat fibrin to remove any trace of anti-fibrin activity (2% and 1% of the radioactivity was adsorbed). The resulting supernate is the purified anti-ALS antibody, (A_A). The purified anti-lymph node antibody (L_L) was similarly prepared from I^{131} labeled globulin fraction of anti-LN sera by adsorption and elution from LN-sediment.

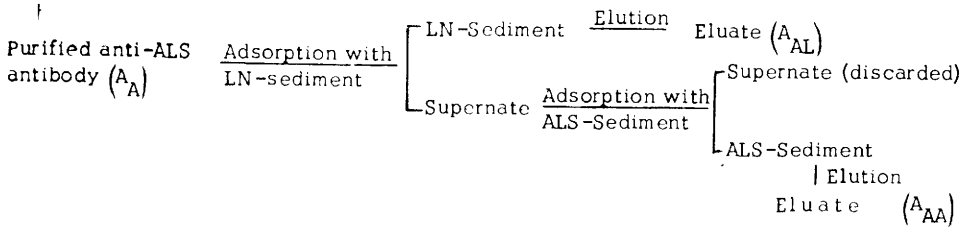
Fractionation of purified anti-ALS antibody (A_A): The scheme of the fractionation is provided in the footnote of Table I. Purified anti-ALS antibody (containing 184 μg radioglobulin and 10 mg BSA) was treated successively with 25, 25 and 12 mg portions of lymph node sediment. 24, 8 and 4% of the original radioglobulin were adsorbed on the sediments. The sediments were pooled after thorough washing and eluted in borate buffer pH 8 containing 0.2% BSA. Fifty-one percent of adsorbed radioactivity was eluted. The eluate was referred to as A_{AL}. The supernate after treatment with lymph node sediments (90 μg radioglobulin) was treated twice with ALS-sediment (30 and 15 mg) removing further 16 and 4% of the radioactivity (on the basis of purified antibody used) respectively. The sediments were combined

† It is known that anti-fibrin antibodies localize in the Murphy tumor when injected into tumor-bearing rats(3). Localizing activity of anti-ALS sera can be removed by treatment with fibrin. When the eluate prepared here was injected into tumor-bearing rats, localization in the tumor was found to be only about 1/10 of that of similarly prepared eluate without use of plasma. Further treatment with freshly formed rat fibrin clot completely removed the tumor localizing activity without affecting localization into normal tissues (e.g. liver). This indicates that only the fibrin-reacting antibody is contributing to the tumor localizing activity of anti-ALS sera and that the antibodies against cell components, dealt with in this report, do not localize in tumor *in vivo*.

TABLE I. Adsorption of Fractionated Anti-ALS Antibodies on ALS- or LN-Sediment.

	1st adsorption*			2nd adsorption* (supernate of 1st adsorption)		
Eluate used	Amt of I^{131} globulin used, μg	Sediment used, mg	% adsorbed	Amt of I^{131} globulin used, μg	Sediment used, mg	% adsorbed
A_{AA}†	4.2	ALS 7.5	40	.9	ALS 7.5	11
				.9	LN 6.3	7
	1.0	ALS 7.5	39			
	4.2	LN 6.3	19	1.4	ALS 7.5	22
				1.4	LN 6.3	9
	1.0	LN 6.3	21			
A_{AL}†	4.2	ALS 7.5	58			
	1.0	ALS 7.5	57			
	4.2	LN 6.3	49			
	1.0	LN 6.3	54			

* Adsorption was carried out at 37°C, 1 hr, pH 8 in 0.1% BSA. Sediments were washed twice with 8 ml borate buffer and counted. Radioactivity on the sediment was expressed in terms of per cent of radioactivity present in original eluate. Portions of the supernate were treated with sediment as indicated under heading of second adsorption.



after washing and eluted (59% eluted). The eluate was referred to as A_{AA}.

Fractionation of purified anti-LN antibody (L_L): The scheme of the fractionation is provided in the footnote of Table II. Purified anti-LN antibody (containing 410 μg radioglobulin and 10% normal rabbit serum) was treated twice successively with 30 mg ALS-sediment each time. Twenty-one and 6% of the radioglobulin were adsorbed. The washed sediments were pooled and eluted (31% eluted). The eluate was referred to as L_{LA}. The supernate after treatment with ALS sediment (295 μg radioglobulin) was treated twice with LN-sediment (25 mg each) removing further 19 and 5% of the radioactivity respectively. The washed sediments were combined and eluted (36% eluted). The eluate was referred to as L_{LL}.

Results and discussion. Purified anti-ALS antibodies (A_A) were first treated with LN sediment, then with ALS sediment and the adsorbed radioglobulin on each sediment was eluted to give 2 preparations A_{AL} and A_{AA} respectively.‡ These eluates were tested for their combining properties with either ALS or LN sediment and the results are shown in Table I.

The antibodies eluted from ALS sediment (A_{AA}) combined with ALS sediment to twice the extent as LN sediment indicating some additional components in the tumor sediment. A_{AA}, after being treated with ALS or LN sediment was further tested for completeness of adsorption by another treatment with ALS or LN sediment. It was found that the primary LN adsorption was not as effective as the ALS adsorption in removing anti-ALS antibody.

‡ In another series of experiments, the supernate after LN adsorption was divided and tested by adsorption with ALS, LN, spleen and liver sediments. ALS sediment adsorbed a much larger fraction of radioglobulin than did any of the other 3 sediments. Among the other 3, spleen and liver sediments adsorbed less than LN sediment suggesting that spleen and liver sediments contain less cross-reacting components to anti-ALS sera than LN sediment.

The adsorptions were carried out using different amounts of globulin (varied by a factor of 4) with the same amount of sediment. The fraction of radioactivity adsorbed was the same for both levels of globulin showing that the pickup was not a function of globulin-sediment ratio in this range.

The antibodies eluted from the lymph node sediment (A_{AL}) were adsorbed on LN and ALS sediments to similar extents, about 55%, indicating the presence of identical or cross-reacting constituents in these tissues.

These experiments show that the antibodies against insoluble components of ALS cells (other than fibrin) are of multiple nature: some are directed against common components of lymph nodes and ALS while others are directed preferentially toward components either unique to ALS or present at very low concentration in lymph node.

The antibodies adsorbed on sediment are directed against the insoluble components of ALS rather than against the soluble parts. This was shown by the fact that the soluble components of ALS do not interfere with adsorption of the antibody by ALS sediment. In fact, when a preparation of purified anti-ALS antibody (1 μg) was treated by 4 mg of ALS-sediment in presence of normal rabbit serum, 36% of the radioactivity was found on the washed sediment. Addition of the soluble fraction from ALS-homogenate (supernate after centrifugation at 78,400 RCF for 60 min; containing 4.2 mg protein) to the system did not significantly affect adsorption (34% adsorbed). The presence of common constituents in the soluble fraction and the sediment would cause such interference.

Similar experiments were carried out with anti-LN antibodies for comparison. Purified anti-LN antibodies (L_L) were first treated with ALS-sediment, then with LN-sediment and the adsorbed radioglobulin on each sediment was eluted to form L_{LA} and L_{LL} respectively. These eluates were adsorbed with both ALS and LN sediments to examine their specificities. The results (Table II) indicate that the antibodies against insoluble components of LN can be fractionated into those with 2 different specificities: one directed against common components of lymph node

TABLE II. Adsorption of Fractionated Anti-LN Antibodies on LN- or ALS-Sediments.

	1st adsorption*			2nd adsorption* (supernate of 1st adsorption)		
Eluate used	Amt of I^{131} globulin used, μg	Sediment used, mg	% adsorbed	Amt of I^{131} globulin used, μg	Sediment used, mg	% adsorbed
L_{LL}†	4.9	LN 6.3	63	1.0	LN 6.3	12
				1.0	ALS 7.5	4
	1.0	LN 6.3	69			
	4.9	ALS 7.5	11	2.0	LN 6.3	59
				2.0	ALS 7.5	9
	1.0	ALS 7.5	14			
L_{LA}†	5.2	LN 6.3	45			
	1.0	LN 6.3	48			
	5.2	ALS 7.5	46			
	1.0	ALS 7.5	48			

* Adsorption was carried out at 37°C, 1 hr, pH 8 in presence of 1 ml NRS. Sediments were washed twice with 8 ml borate buffer and counted. Radioactivity on the sediment was expressed in terms of percent of radioactivity present in original eluate. Portions of the supernate were treated with sediment as indicated under heading of second adsorption.

† Purified anti-LN antibody (L_L) → Adsorption with ALS-sediment → ALS-Sediment → Elution → Eluate (L_{LA}); Supernate → Adsorption with LN-sediment → LN-Sediment → Elution → Eluate (L_{LL}); Supernate (discarded)

and ALS (L_{LA}), whereas the other directed preferentially toward components either unique to lymph node or present at very low concentration in ALS (L_{LL}).

Thus, the fractionation of antisera into fractions of different specificities indicates that lymphosarcoma and lymph node cells each contain a characteristic component(s) either unique to itself or present at very low concentration in the other. The lymph node is taken as the most closely related normal tissue to lymphosarcoma. However, interpretation of the above data is limited to the difference between these tissues. Whether this tumor contains a unique substance or a substance present only in a particular minor cell line in the normal lymph node remains to be seen. The question of the correct normal cells with which to compare tumor cells is an underlying problem in cancer chemistry.

Summary. Rabbit antisera prepared against rat Murphy-Sturm lymphosarcoma (ascites form) and normal rat lymph node exhibit extensive cross reactions with these tissues. However, antibodies in the antisera were separated into fractions of different specificities: some directed against certain characteristic antigen(s) in the tumor or in the lymph node sediment and others against the common (cross-reacting) components in both. This was done by successive adsorptions of the antisera with the tumor and lymph node sediments and elution of adsorbed antibodies from these sediments. I^{131} labeled antibody was used for the studies.

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Latex Agglutination by the Reaction of Human Growth Hormone with Its Antiserum.* (26273)

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The specific antigenicity of human growth hormone has been demonstrated(2,3,4). The present study involves a reaction of human growth hormone with its antiserum and utilizes a simple agglutination technic. The purpose of this article is to report this technic.

Method. The method of antiserum production was a modification of the method outlined by Cohn(5). One mg of human growth hormone† made according to the Raben technic(1) was dissolved in one ml of water which had been adjusted to pH 3.0 with HCl. To this solution was added one mg of heat killed *Mycobacteria tuberculosis* and one ml of a mixture containing 9 parts of Bayol F (Penola Oil Co.) and one part of Arlacel C (Atlas Powder Co.). This mixture was emulsified with the growth hormone solution and injected into the toe pads of a young adult albino rabbit. Three weeks later the same amount of emulsion was prepared without the killed *Mycobacteria tuberculosis* and one-half injected in each of 2 sites intramuscularly. Three weeks later the rabbit blood was obtained by cardiac puncture and the serum

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