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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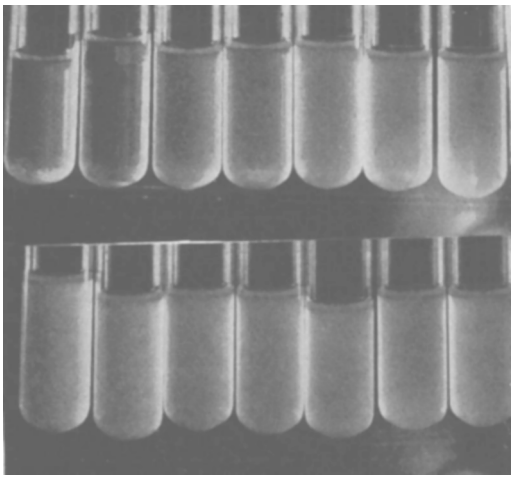

FIG. 1. Latex agglutination by reaction of human growth hormone with its antiserum. Upper row: Rabbit antiserum. Lower row: Control rabbit serum. Both rows: From left to right, tubes 1 through 6, progressive dilutions of human growth hormone 2.8 to 0.086 μg; tube 7, control. Tubes 1 through 4, positive in upper row.

was separated. Globulins were then precipitated from the antiserum according to the method of Deutsch(6) for rabbit serum. This precipitate, labeled Precipitate A, contained all of the serum globulins. A 0.5% stock solution of the lyophilized globulin precipitate was made in .06M Sorensen's phosphate buffer, pH 7.4. A latex-globulin suspension was then prepared by mixing 0.5 cc of 0.5% stock globulin solution, 0.1 ml of Difco Bacto-latex (particle size 0.81 μ) and 9.4 ml of the same phosphate buffer. Control rabbit blood was treated similarly.

Growth hormone(1) 0.5 mg was dissolved in 1.0 ml of water acidified to a pH of 3.0 with HCl. The growth hormone solution was then diluted with 0.06 M Sorensen's phosphate buffer, pH 7.4, to the desired dilution. Serial dilutions of growth hormone with a volume of 1.0 ml were then made with the phosphate buffer in the first 9 of a set of 10 tubes. The tenth tube contained only one ml of buffer. To each of these tubes was added one ml of the latex-globulin suspension. The tubes were then shaken. A similar set of tubes using control globulin was set up. The tubes were heated for 30 minutes at 37°C, then allowed to sit at 4°C for 120 minutes. After warming to room temperature they were incubated at 56°C for 90 minutes; this increased the agglutinating properties of some antisera. They were then allowed to sit at room temperature overnight at which time results were read visually.

Results. Fig. 1 illustrates the agglutination, and Table I shows the reaction quantitatively. This table also shows that agglutination occurs over a restricted range of antiserum/growth hormone concentration and does not occur when antigen concentration is excessive or too little. Table II shows that latex agglutination inhibition occurs if the growth hormone is mixed with the antiserum before a latex-growth hormone suspension is added. This is accomplished by first adding antiserum to progressive serial dilutions of growth hormone and adjusting the volume to one ml and with phosphate buffer. After incubation at 37°C for 30 minutes and sitting at 4°C for 30 to 120 minutes one ml of a latex-growth hormone suspension containing 2.0 μg of growth hormone, 0.1 ml of Difco Bacto-Latex, and 9.9 ml of phosphate buffer is added. The procedure from this point is as described in "Methods" above after addition of the latex suspension.

Discussion. Hayashida and Li(2) showed that human growth hormone antiserum would neutralize the biologic potency of growth hormone; they were also able to produce guinea pig anaphylaxis by the reaction of growth hormone with its antiserum. The results of the experiments of Grumbach *et al.*(4) using precipitating technics give strong evidence

TABLE I. Latex Agglutination* by Immunologic Reaction of Human Growth Hormone with Rabbit Serum Globulin.

Tube No.	Human growth hormone (μg)	Anti-serum globulin	Control serum globulin
1	46.0	0	0
2	23.0	0	0
3	11.5	+	0
4	5.7	++	0
5	2.8	++++	0
6	1.4	++++	0
7	.7	++	0
8	.3	+	0
9	.1	0	0
10	0	0	0

* Degree of agglutination is expressed by No. of plus marks. A negative reaction is expressed by 0.

TABLE II. Latex Agglutination* Inhibition Test by Immunologic Reaction of Human Growth Hormone with Rabbit Serum.†

Tube No.	Human growth hormone (μg)	Row I, antiserum	Row II, control serum
1	3.10	0	0
2	2.66	0	0
3	2.28	0	0
4	1.90	0	0
5	1.52	0	0
6	1.14	+	0
7	.76	+	0
8	.38	+	0
9	.19	++	0
10	.15	++	0
11	.11	++	0
12	.08	++	0
13	.04	+++	0
14	.02	++++	0
15	.01	++++	0
16	.00	0	0

* Degree of agglutination is expressed by No. of plus signs. No reaction is expressed by 0 sign.

† .005 ml of rabbit serum was used in each tube except for tube 16 in each row.

that there is one antigen-antibody reaction in the reaction between the Raben growth hormone product and its antiserum. Read(3) using rabbit antisera to the same product was able to show by a hemagglutination-inhibition technic the presence of an antigen in increased amounts in sera of acromegalic patients and decreased amounts in patients with hypopituitarism when compared to normal individuals. Assuming, then, on the basis of this evidence, that human growth hormone is antigenic, we conclude that latex agglutination as demonstrated by the results of this experiment is a manifestation of the reaction of human growth hormone with its antiserum.

The results in Table I show a zone of optimal concentration for antigen and antibody as manifested by a zone of no agglutination in the region of antigen excess and a zone of no agglutination where antigen concentration is too little. This follows well-known laws of other antigen-antibody systems using rabbit antisera(7).

It is hoped that latex agglutination by the reaction of human growth hormone with its antiserum may be useful for detection of growth hormone in human biological fluids. Experiments are now in progress to test this possibility.

Summary. A method has been described which demonstrated that the reaction between human growth hormone and its antiserum caused agglutination of a suspension of latex.

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Ammonia Detoxication in Liver from Humans.* (26274)

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The recent development of methods for assaying the individual enzymes of the urea cycle in a single homogenate(1) have made possible accumulation of data on ammonia detoxication by this enzymatic mechanism in human liver. With similar data for glutamic acid dehydrogenase and glutamine synthetase, it is possible to calculate the amount of ammonia which *theoretically* could be fixed by these separate mechanisms, and to obtain an over-all estimate for *in vitro* fixation of

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