TABLE I. Variations in the Anti-Hyaluronidase Titre with Age and Sex. Total No. cases, 72; males, 35; females, 37.

	Male				Female			
Age, yrs	No. cases	%	S.D.	$S.E._m$	No. cases	%	S.D.	$S.E._m$
0-20	2	21.2	—	—	0	—	—	—
21-30	9	31.2	5.09	1.78	10	32.8	4.16	1.39
31-40	9	24.9	4.00	1.40	8	34.7	8.88	3.36
41-50	6	31.1	5.37	2.40	10	39.8	6.94	2.32
51-60	6	28.7	4.77	2.14	5	39.4	3.64	1.82
61-80	3	34.9	—	—	4	37.5	—	—

Both sexes group 21-30 mean = 32.0% ± 0.997.

Male group, 31-60
Mean — 28.2% ± 1.220
S.D.—3.13
$S.E._m$—1.809%

Female group, 31-60
Mean — 38.0% ± 1.926
S.D.—2.84
$S.E._m$—1.640%

TABLE II. Comparison of Male and Female Titres.

Age, yrs	Sig. ratio	Level of sig., %
21-30	0.770	46.0
31-40	2.694	1.7
41-50	2.610	2.0
51-60	3.816	0.6

Table I. Between the ages of 21 and 30 years, the mean anti-hyaluronidase titre (percent inhibition) is the same. Beyond this age level, a significant difference in titres appears in the serum of the two sexes. The anti-hyaluronidase concentration of the serum of the normal male between the ages of 31 and 60 years remains constant at 28.2% ± 1.220 ($P.E_m.$). However, the titre of the serum from the female between these same ages rises to 38.0% ± 1.926.

Accepting a 5% level of significance, it may be seen that the difference between the 2 titres is statistically significant for males and females above the age of 31. Since the majority of females studied, of the age 31 or over had entered or passed the menopause, it would seem that this endocrinological change is associated with an elevation in the anti-hyaluronidase titre of the serum.

The failure to recognize this difference in the titre between the sexes could easily lead to uncertainty in the interpretation of the anti-hyaluronidase level in serum from pathological conditions.

Conclusions. 1. The anti-hyaluronidase titre of the normal human serum has been determined by the means of the viscosimetric method.

2. The anti-hyaluronidase titre is the same for both sexes between the ages of 21 and 30 years (32.0%). Between the ages of 31 and 60 years the titre in the serum from the normal female (38.0%), is significantly higher than that of the male (28.2%).

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Serum Anti-Hyaluronidase in Human Leukemia and Lymphosarcoma. Effects of Cortisone and ACTH.* (18448)

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The present studies deal with the serum anti-hyaluronidase in human leukemia and lymphosarcoma, and with the changes in the levels following treatment with cortisone, ACTH and other forms of therapy. Follow-

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ing the work of Haas(1) on anti-hyaluronidase in infection, other workers studied the anti-hyaluronidase in malignant disease. Fulton *et al.*(2), found an increased titre in malignancy as compared to the normal. However, he did not separate his normal groups by sexes, nor did he differentiate the type of malignant disease studies. Hakanson and Glick(3), and Kirilik *et al.*(4), found that the anti-hyaluronidase levels were normal in patients with benign tumors, high in patients with cancer and highest of all in those whose cancers had metastasized. Most workers in this field have attempted to associate the spreading factor with the invasiveness of malignant tissue(5). The statement has been made recently that, "there is general agreement to support an association of spreading factors with carcinomas than there is with sarcomas"(6). However, Hakanson, and Kiriluk(3,4), both reported several lymphosarcomas which had the highest titre of all their cases.

There are no studies specifically on the anti-hyaluronidase titres in the blood of patients with leukemia or lymphosarcoma or the effects of cortisone or ACTH on the titre of these diseases. Our data indicate that very high anti-hyaluronidase titres are to be found in both these diseases, and that changes occur following treatment.

It has been shown(7), that the elevated anti-hyaluronidase titre of the serum in rheumatic fever is depressed upon the administration of cortisone or ACTH, and that this decrease parallels the clinical improvement. Dorfman(7), felt that this reflected changes in the tissue hyaluronic acid mechanism. The present data indicate that the serum anti-hyaluronidase parallels the state of the leukemia or lymphosarcoma just as it does in rheumatic fever. In acute or chronic lymphatic leukemia, a decrease in the titre occurs following the administration of cortisone or ACTH. However, in chronic myelocytic leukemia, cortisone causes an exacerbation of the disease process, and with it there occurs a marked rise in the anti-hyaluronidase.

TABLE I. Serum Anti-Hyaluronidase in Leukemia and Lymphosarcoma Untreated Cases.

Group	Sex	No. cases	Mean, %		Range, %
Normals	M	35	28.2		
	F	37	38.0		
Chr. lymph. leukemia	M	7	53.6	55.6	45.0-66.6
	F	4	58.7		48.5-76.5
Chr. myel. leukemia	M	5	49.8	49.7	43.7-53.0
	F	1	49.2		—
Acute myel. leukemia	M	1	52.4		—
Acute lymph. leukemia	M	1	47.6		—
	F	1	61.4		—
Lympho-sarcoma	M	2	71.0		57.5-84.5
	F	1	34.7		—
Hodgkin's disease	M	1	80.6	70.3	60.8-87.9
	F	3	70.1		

Methods. The viscosity method based on Haas(1), was used in preference to the turbidimetric or mucin clot methods since it was felt that viscosity more closely approximates the physiological function of hyaluronic acid.

Results and discussion. The results are summarized in Tables I, and II. It will be noted (Table I) that the leukemias and lymphosarcomas all uniformly show an elevate anti-hyaluronidase titre. The mean titre for 27 cases was 55.0%. This figure is similar to that of Kiriluk *et al.*(4) who found a mean of 57% for 3 cases of lymphoblastoma. It is of interest that the values for the lymphoid diseases are higher than for the myelocytic, and the value for the Hodgkin's-lymphosarcoma group is considerably higher than either form of leukemia (2 cases of the latter with low titres were found in patients whose disease was quiescent). Hence, the titre tends to be highest where the tumor cells invade or metastasize to the blood stream *least*, as in

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TABLE II. Effect of Therapy on the Anti-Hyaluronidase Titre.

Disease	Patient	Sex	Before therapy, %	After therapy, %	Treatment
Chronic lympho-cytic leukemia	L.	M	—	13.1	Urethane, NaPAB
	D.	M	75.7	40.5	ACTH 1.85 g
	R.	F	48.5	20.7	Urethane
	F.	F	69.0	30.8	ACTH
Acute lympho-cytic leukemia	E.	M	47.6	44.8	Amethopterin
	C.	F	61.4	36.4	Cortisone 1.55 g Nit. must. 30 mg
Chronic myelo-cytic leukemia	M.	M	53.0	17.6	X-ray, urethane
	M.F.	M	43.7	19.5	Urethane, nit. must.
	R.	M	49.8	60.0	Cortisone 1 g
	H.H.	F	—	13.8	Urethane
	E.C.	F	—	16.3	Fowler's sol.
	H.	F	49.2	71.1	Cortisone 1.2 g
Lymphosarcoma	N.	M	57.5	53.4	Cortisone 3.45 g
	E.F.	M	84.5	48.5	Cortisone 1.6 g
	B.	F	—	30.2	Urethane
	C.G.	F	34.7	53.2	Cortisone 2.95 g
Hodgkin's disease	A.	F	—	26.6	Testosterone, estrogens
	S.	F	72.5	56.6	Cortisone 1.1 g
	C.R.	F	61.7	39.4	Cortisone 1.5 g
	C.R.	F	62.0	37.0	Nit. must. 30 mg, X-ray

the latter disease. In addition, no correlation could be found between the anti-hyaluronidase titre and the bone marrow picture. Also, no marked differences were noted in the bone marrow when the titre was reduced by therapy. Our data, therefore, fail to indicate any relationship between the serum anti-hyaluronidase and the invasiveness of the tumor cells for the blood stream and bone marrow. The theory that the anti-hyaluronidase titre is a measure of invasiveness must assume, therefore, that Hodgkin's disease is invasive in the same sense as carcinoma. In 8 cases of carcinoma as well as the present studies, the impression is gained that the anti-hyaluronidase may be related more reasonably to the quantity of malignant tissue. In the leukemias and lymphosarcomas the quantity of tissue is great, and undoubtedly greater than in the majority of carcinomas by reason of their widespread distribution in many organs.

Following therapy, the anti-hyaluronidase titre dropped in conjunction with clinical improvement (Table II). Exceptionally low titres were found following the administration of urethane, either alone or in combination with other forms of therapy. This is of interest since urethane has a specific action upon nuclear metabolism. In general, it appears that a drop in the titre to normal or below normal was evidence of an arrest in the disease process.

Of particular interest is the very marked rise in anti-hyaluronidase attendant upon the administration of cortisone to 2 patients with chronic myelocytic leukemia. The titres rose to levels among the highest found in this study. This is indicative of the stimulating effect of cortisone upon the leukemic myelocytic cell.

Conclusions. (1) The anti-hyaluronidase titre of the blood of patients with lymphosarcoma, Hodgkin's Disease, and leukemia has been determined by means of the viscosimetric method. (2) The anti-hyaluronidase titre is elevated in all 3 conditions and varies with the clinical condition of the patient. (3) Cortisone causes an exacerbation of the disease in myelocytic leukemia with a corresponding increase in the anti-hyaluronidase titre. (4) In lymphocytic leukemia and Hodgkin's disease, cortisone, ACTH and other forms of therapy cause a decrease in the anti-hyaluronidase titre corresponding with clinical improvement. (5) There is no apparent relationship between the degree of invasion of the blood stream and the bone mar-

row picture and the changes in anti-hyaluronidase titre. (6) The height of the anti-hyaluronidase titre appears to be related to the quantity of tumor tissue rather than to its invasiveness.

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Heinz Body Phenomenon in Monkey Erythrocytes, a Quantitative Method. (18449)

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Webster(1) has published an excellent review on the Heinz Body Phenomenon in Erythrocytes. In this he states that "further work on the relationship between Heinz body occurrence and the action of the spleen. . . . appears to be necessary in order to extend our knowledge of the role played by the spleen in hemolytic anemias produced by chemical agents." It has been observed that the amount of quinine hydrochloride necessary to produce the acute death of the monkey was less in animals with a severe anemia resulting from malaria than it was in monkeys with an equally severe anemia produced by phenylhydrazine(2).

Several methods have been devised for the demonstration and estimation of Heinz bodies. Cruz(3) and others(4-6) have made use of the turbidity which occurs in the blood to estimate quantitatively the amount of Heinz bodies present. Counts may be done either on fixed stained smears or on supravital preparations(7,8). Fertman and Doan(9) say, "The inclusions could be seen in unstained living blood films as refractile yellow tinted bodies. In dark field examination they appeared highly refractile, globular and irregular, similar in general appearance and distribution to the inclusions seen in bright field." Figge(10) thinks that Heinz bodies can be readily demonstrated in plain dried blood films examined with a high power objective; he found that they disappeared when examined under oil, balsam or other mounting media. Utilizing the fact that Heinz bodies are not hemolysed in distilled water, we have demonstrated that the number of Heinz bodies in the blood of the monkey may be determined by diluting the blood with water in a standard red cell pipette and counting the number of Heinz bodies on a haemocytometer. Observations were made in both the normal and splenectomized monkey.

Methods and materials. Ten *M. mulatta* monkeys, weighing from 2.6 to 4.0 kg, were used. Blood for the erythrocyte counts, smears and Heinz bodies was obtained from the marginal vein of the ears. Smears were stained with a combination of Wright's and Giemsa's stains. The number of Heinz bodies free in these smears was counted per 500 erythrocytes. Red cell counts were made using standard technics and Heinz body counts were carried out by a similar technic. The blood for the latter was drawn to the 0.5 mark on a red cell diluting pipette and the diluting fluid was drawn to the 101 mark, thus giving the count as the number of Heinz bodies in M per cmm. Distilled water and a solution of phloxine dye were used as the

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