

elaborate efforts to control the methods.

We have had some indication that part of the fluorescence measured as F_2 is related to thiamine; the blank in the thiamine determination, using zeolite adsorption as in the Najjar method for F_2 , increases as the thiamine excretion decreases and this is true even though the niacin intake is constant at about 20 mg per day. Time has not permitted us to investigate this question further.

Summary. F_2 analyses have been made on

the urine of normal young men who were maintained on controlled niacin intakes. The method of Najjar showed no difference in the F_2 excretion of 2 groups whose intakes over a period of 161 days averaged 12.4 and 22.4 mg of niacin per day. There was no difference in the F_2 excretion of 2 groups maintained for 32 days on intakes of 0.12 and 10.12 mg of niacin per day when the method of Huff and Perlzweig was used for the determination.

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Experiments on Erythrocyte Sedimentation Rate*

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In general the rate of erythrocyte sedimentation (E.S.R.) is considered to be dependent on the nature and concentration of the protein components of the plasma (*lit.*¹). However there is some experimental evidence suggesting that E.S.R. may likewise be profoundly influenced by non-protein substances. Fahraeus² demonstrated that gum arabic added to normal blood caused an increased E.S.R. The chemically closely related type III pneumococcus polysaccharide³ was shown to have a similar effect.⁴ Shedlovsky and Scudder several years ago in an unpublished lecture reported an increase in E.S.R. on the addition of viscous extracts of certain sarcomata which presumably contained hyaluronic acid. Sim-

ilar experiments were performed by Kendall (personal communication) and by us (unpublished) with pure hyaluronic acid.

In this paper experiments will be presented which, in combination with published evidence, indicate that probably all highly asymmetrical molecules of large size cause an increase of E.S.R. when added to normal blood. Experiments on the intravenous injection of hyaluronate and hyaluronidase are likewise reported.

Experimental. The first experiments deal with the *in vitro* increase of E.S.R. induced in normal human blood by various substances. E.S.R. in all experiments was measured by the Westergren method, making no correction for cell volume or protein concentration. All blood samples were obtained from healthy young males and, after mixing with the additions indicated in the experiments, were incubated for 30 min. at 37°C. The samples were then drawn up in the sedimentation tubes and read every 15 minutes for 1 hour.

The samples of hyaluronic acid used were prepared from cattle synovial fluid,⁵ from a

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¹ Nichols, R. E., *J. Lab. and Clin. Med.*, 1942, **27**, 1317.

² Fahraeus, R., *Acta Med. Scand.*, 1921, **55**, 3; *Physiol. Rev.*, 1929, **9**, 241.

³ Heidelberger, M., Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1929, **49**, 847.

⁴ Nungester, W. J., and Klein, L. F., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 315.

⁵ Meyer, K., Smyth, E. M., and Dawson, M. H., *J. Biol. Chem.*, 1939, **128**, 319.

	Sample	MM sedimentation			
		15'	30'	45'	60'
2 cc blood + 0.5 cc NaCl		1	2	5	6
" " + 0.4 " " + 0.1 cc Enzyme C (2 mg)		0.5	1	1	2
" " + " " highly polymerized hyaluronate (5 mg) + 0.1 cc NaCl		72	117	120	120
" " + " " " " " " " " " " " " " " " " " "	+ " " " C (2 mg)	5	10	13	17
" " + " " " " " " " " " " " " " " " " " "	(2 mg) + " " " NaCl	29	68	93	113
" " + " " " " " " " " " " " " " " " " " "	+ " " " C (2 mg)	0.5	1	2	3
" " + " " " " " " " " " " " " " " " " " "	(1 mg) + " " " NaCl	13	35	54	69
" " + " " " " " " " " " " " " " " " " " "	+ " " " C (2 mg)	0.5	1	2	3
" " + " " " depolymerized hyaluronate (5 mg)	+ " " " NaCl	1	5	8	13
" " + " " " " " " " " " " " " " " " " " "	+ " " " C (2 mg)	0.5	1.5	2	4
" " + " " " " " " " " " " " " " " " " " "	(2 mg) + " " " NaCl	0.5	2	4	6
" " + " " " " " " " " " " " " " " " " " "	+ " " " C (2 mg)	0.5	0.5	2	2
" " + " " " " " " " " " " " " " " " " " "	(1 mg) + " " " NaCl	1	2	4	7
" " + " " " " " " " " " " " " " " " " " "	+ " " " C (2 mg)	0	1	2	2
" " + " " " Thymonucleate (5 mg)	+ " " " NaCl	5	48	90	110
" " + " " " Gelatin (24 mg)	+ " " " " "	70	112	114	120
" " + " " " " " (6 " ")	+ " " " " "	17	30	32	41
" " + " " " Fibrinogen (25 mg)	+ " " " " "	13	45	72	86
" " + " " " " " (12.5 ")	+ " " " " "	3	12	20	30
" " + " " " " " (5 ")	+ " " " " "	2	5	8	12
" " + " " " " " (2 ")	+ " " " " "	1	2.5	4	6

⁸ Hammarsten, E., *Biochem. Z.*, 1924, **144**, 383.

McClean.⁹ The material must be free of nucleic acid or otherwise an increase in E.S.R. may result. All testis preparations contained, besides hyaluronidase, an enzyme hydrolyzing chondroitinsulfuric acid with the liberation of acetylchondrosaminosulfuric acid-glucuronide. (The action and properties of this enzyme will be reported separately.)

In Table I the effect of the addition of viscous and non-viscous hyaluronic acid, thymonucleic acid, gelatin, and fibrinogen to normal blood is shown, together with the action of testis hyaluronidase. It can be seen that the sample of viscous hyaluronate causes maximal E.S.R. in a concentration of about 0.2%. The increased rate is promptly abolished by incubation with testis hyaluronidase. The non-viscous hyaluronate has no appreciable influence on E.S.R. Thymonucleate about equals the effect of hyaluronate, while the gelatin and fibrinogen samples used had a quantitatively inferior effect, since with the latter much larger concentrations were required to approximate the effect produced by hyaluronate. The specificity of the hyaluronidase was apparent from its failure to decrease the E.S.R. with substances other than hyaluronate. The failure of the testis enzyme preparation to act on the rate increased by fibrinogen is in accord with experiments on the possible proteolytic activity of these testicular extracts. Neither with fibrinogen nor with other proteins could a proteolytic effect ever be detected.

In order to decide what effect hyaluronic acid might have on E.S.R. *in vivo*, 6 guinea pigs and one young rabbit were injected intravenously with hyaluronate in sodium chloride solution. In the guinea pigs the superficial lateral veins of the legs were used. The E.S.R. was determined by the Kato¹⁰ method, using 5 to 10 drops of blood drawn from the marginal vessels of the ear. This was mixed with dried oxalate (equivalent to 0.02 cc of 2% double oxalate solution). The E.S.R. in 2 guinea pigs serving as controls and injected intravenously for several days with 2 cc of 0.9% sodium chloride was never greater than

1%. In Table II a typical experiment is listed. It can be seen that the intravenous injection of hyaluronate leads to a definite increase in E.S.R. which usually reaches its maximum more than 1 hour after the injection. After the injections have been made daily for several days, the E.S.R. may remain elevated for 24 hours. Autopsy in some of the animals after repeated injections revealed no gross pathological changes.[†] The temperature of the injected animals showed no significant deviation from the normal.

The effect on E.S.R. of the intravenous injection of hyaluronidase in animals previously treated with hyaluronate was tested in 2 guinea pigs of 380 and 370 g respectively. In one guinea pig the hyaluronidase was injected immediately after hyaluronate. In the other, the enzyme was injected 4 hours later. In the former animal the E.S.R. remained 1%, while in the latter animal the E.S.R. rose from 0 to 27.5% but had returned to 0, 30 minutes after the injection of enzyme. The animals were apparently in perfect condition after the experiment.

In a similar experiment, the same 2 guinea pigs were injected several days later with 2.2 and 3.6 cc of 2% hyaluronate respectively. The E.S.R. in both guinea pigs before injection was <1 and rose to 12 and 16 respectively one hour after the hyaluronate injection. The second animal received one injection of hyaluronidase 65 minutes after the hyaluronate injection. Samples of blood were taken 10, 30, and 150 minutes after the injection of enzyme. The E.S.R. in these samples remained 1%. In the control animal, which received no enzyme, the E.S.R. 4 hours after the hyaluronate injection had risen to 45%.

Discussion. From literature data as well as from our experiments the conclusion can be drawn that a high degree of asymmetry of the molecules together with high particle weight are the causes of increased E.S.R. Among the known blood proteins fibrinogen, which since Fahraeus² has been thought largely responsible for increased E.S.R. in patho-

⁹ Morgan, W. T. J., and McClean, D., *J. Soc. Chem. and Ind.*, 1932, **51**, 912.

¹⁰ Kato, K., *J. Lab. and Clin. Med.*, 1938, **23**, 980.

[†] We are greatly indebted to Drs. Beatrice Seegal and Emily Loeb, who did some of the preliminary experiments on guinea pigs in 1942.

TABLE II.
Sedimentation Rate in Guinea Pig Injected with Hyaluronate.*

Date	cc inj.	Sedimentation rates in % in 60 min.†		
		Before inj.	45 to 60 min. after inj.	3 to 4 hr. after inj.
6/19	0	2.5		
20	0	1		
21	1.8	0	4	
22	approx. 2		36	
23	1.9		21	
26	2.0	0	27	
27	approx. 1	10	27	47
28	2.0	5		51
29	2.0	27		17
30	1.9	0		54
7/ 1	1.8	54		58
5	1.0	1		46
6	1.8	20		61
7	1.0	9		62
8	1.9	21		
10	0	1		

* 2% solution of sodium hyaluronate in 0.9% sodium chloride.

† It should be noted that the pipettes used in the Kato method are calibrated in percent instead of in mm as in the Westergren method.

logical conditions, apparently fulfills this condition.¹¹

The influence of the degree of aggregation is evidenced from the experiments with hyaluronate. Testis hyaluronidase at a pH of 7.4 leads only to depolymerization and not to hydrolysis as evidenced by the failure of opening of glucosidic linkages.¹²

It is apparent from the animal experiments that the intravenous injection of viscous hyaluronate leads to a considerable increase in E.S.R. similar to that induced by the addition *in vitro*. In contrast with the experiments of Youngner and Nungester¹³ in which the E.S.R. returns to normal in about 40 minutes after the injection of type III pneumococcus polysaccharide, the increase produced by hyaluronate reaches a maximum in several hours, especially if injections are repeated. However we have not succeeded in maintaining a high E.S.R. in animals for prolonged periods. Presumably the substance is enzymatically

depolymerized in the circulation.

Rouleaux formation is generally accepted as the immediate cause of increased E.S.R., although its precise mechanism is still unexplained. Fahraeus² argued against the presence of a "sticky" substance on the surface of the red cells and proposed a poorly defined colloidal phenomenon as the cause of the clumping. Experiments reported in this paper favor the older theory. Furthermore a highly viscous material of great asymmetry has been prepared from stromata. Apparently such highly asymmetrical substances in small concentrations exert a much greater influence on E.S.R. than do the known serum proteins. The occurrence of such substances in small concentrations is indicated by experiments on increased E.S.R. in various diseases, especially in rheumatic fever, where a highly purified testis extract leads to a specific decrease in E.S.R.

Summary. Addition of desoxyribonucleic acid, gelatin, fibrinogen, and especially hyaluronic acid to normal human citrated blood causes a considerable increase in E.S.R. Hyaluronic acid acts only in its highly polymerized form, thus suggesting that molecular weight is an important factor in this reaction, as well as

¹¹ Cohn, E. J., *Trans. and Studies of College of Physicians of Phila.*, 1942, **10**, 149.

¹² Meyer, K., Chaffee, E., Hobby, G. L., and Dawson, M. H., *J. Exp. Med.*, 1941, **73**, 309.

¹³ Youngner, J. S., and Nungester, W. J., *J. Infect. Diseases*, 1944, **74**, 247.

asymmetry of the molecule. Hyaluronidase specifically reverses the action of hyaluronate on E.S.R. Intravenous injection of hyaluronate in guinea pig and rabbit leads to increased

E.S.R. Intravenous injection of hyaluronidase promptly causes the increased rate to become normal.

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Effect of Wheat Germ on Creatinuria in Dermatomyositis and Progressive Muscular Dystrophy.*

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Milhorat, Weber, and Toscani¹ reported a decrease in the creatinuria of 2 patients with dermatomyositis who were given 125 g of fresh whole wheat germ daily. This effect was increased when the wheat germ was incubated *in vitro* with normal human gastric juice.

The present report presents some of the results of investigations carried out in an effort to identify the factor, or factors, in wheat germ having these effects. The subjects were 5 patients with dermatomyositis, and 3 patients with progressive muscular dystrophy. All subjects had extensive muscular wasting, and spontaneously excreted large amounts of creatine. The patients were given a creatine-creatinine-free diet and were maintained in a special metabolism ward where the diet and urinary collections could be rigorously supervised. After adequate periods of control the subjects were given various substances to test any possible effect on the creatinuria. Most of the substances were given daily to 4 or more of the subjects for periods of at least one week and usually for periods of 2 months. Briefly, the observations were as follows: only 3 of the patients with dermatomyositis (subjects A, B, and C), and one with progressive muscular dystrophy (subject D) were able to ingest as much as 125 g wheat germ daily. During the control periods the average daily

levels of spontaneous creatinuria of the 3 patients with dermatomyositis were as follows: subject A, 0.450 g (maximum 0.524, minimum 0.405); subject B, 0.240 g (max. 0.275, min. 0.190); and subject C, 0.150 g (max. 0.240, min. 0.105). The average daily outputs of creatine were unchanged during the first 2 weeks of wheat germ administration, but during the third week they decreased to the following levels: subject A, 0.355 g (max. 0.406, min. 0.279); subject B, 0.082 g (max. 0.090, min. 0.078); and subject C, 0.080 g (max. 0.093, min. 0.072). Subject A was given wheat germ for a total period of 7 months. During the last 2 weeks of this period the average daily creatinuria was 0.310 g (max. 0.365, min. 0.256). Essentially similar results were observed in the patient with progressive muscular dystrophy (subject D). The average daily output of creatine of 0.350 g (max. 0.470, min. 0.338) gradually decreased during the third and fourth weeks of wheat germ feeding to levels of around 0.250 g (max. 0.266, min. 0.218). The excretion of creatine continued at these low levels for the next 2 weeks during which wheat germ was given, and for a period of 3 weeks after the administration had been stopped. The wheat germ given in this experiment had been vacuum packed at the mill and was kept in sealed cans until used. In a subsequent experiment wheat germ which had been stored and received in bulk was given to this patient in the same amounts as in the previous study. No effect on the creatine output was observed during

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¹ Milhorat, A. T., Weber, F. C., and Toscani, V., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 470.