K and after Dicumarol is due to a decrease of prothrombin.

1. Quick, A. J., *Am. J. Physiol.*, 1947, v151, 63.
2. Dam, H., *Nature*, 1948, v161, 1010.
3. Sørbye, O., Kruse, I., and Dam, H., *Acta. Chem. Scand.*, 1950, v4, 549.
4. MacMillan, R. L., *Science*, 1948, v108, 416.
5. Lein, J., and Lein, P. S., *Am. J. Physiol.*, 1948, v155, 394.
6. Mann, F. D., Mann, J. D., and Bollman, J. L., *J. Lab. and Clin. Med.*, 1950, v36, 234.
7. Quick, A. J., and Hussey, C. V., *J. Clin. Invest.*, 1951, v30, 903.
8. Quick, A. J., and Collentine, G. E., *Am. J. Physiol.*, 1951, v164, 716.
9. Kapsinow, R., Engle, L. P., and Harvey, S. C., *Surg. Gynec. and Obst.*, 1924, v39, 62.
10. Quick, A. J., *The hemorrhagic diseases and the physiology of hemostasis*, Ch. C. Thomas, 1942, Springfield, Ill., p37.
11. Collentine, G. E., and Quick, A. J., *Am. J. Med. Sci.*, 1951, v222, 7.
12. Quick, A. J., and Stefanini, M., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 232.
13. Owen, C. A., Magath, T. B., and Bollman, J. L., *Am. J. Physiol.*, 1951, v166, 1.
14. Quick, A. J., and Stefanini, M., *J. Lab. and Clin. Med.*, 1949, v34, 1203.
15. Quick, A. J., and Hussey, C. V., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 732.
16. ———, *Fed. Proc.*, 1951, v10, 235.

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Effect of Certain Enzyme Inhibitors on Hemolytic and Hemagglutinating Activity of Mumps Virus.* (19297)

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The hemolysin of mumps virus is a labile property with many of the characteristics of an enzyme(1-3). Studies of the effects of certain enzyme inhibitors on the hemolysin were undertaken to obtain further indications of its probable nature and of its similarities to various types of known enzymes. The action of these inhibitors on the hemagglutinating capacity of the virus was tested simultaneously to examine the relationships of the hemagglutinin and the hemolysin or their separate natures.

Materials and methods. A strain of egg-adapted mumps virus was used. 0.1 ml of dilutions of infected egg fluids were injected into the allantoic or amniotic sacs of 6-8-day-old, white, embryonated eggs. The eggs were incubated at 35°C and the infected fluids were harvested 4-6 days later. Allantoic fluids stored at −40°C were dialyzed for 24 hours at 4°C with phosphate buffered saline (pH 7.0-7.2) before use in these experiments. Tests for hemolytic and hemagglutinating activities of the virus were carried out with the methods previously described(2). The enzyme inhibitors were dissolved in phosphate-buffered saline and the pH was adjusted, as necessary, to 7.0-7.2 before addition to the diluents employed in the hemolysin and hemagglutinin titrations.

Results. Urethane(4-7) is a known inhibitor of a variety of enzymes, including several blood esterases. Its effect on the mumps hemolysin is shown in Table I. The inhibition of hemolysis noted with urethane is more marked with the higher concentrations. Using 10% hemolysis as the lowest significant degree of hemolytic activity, 0.1 M urethane decreases the hemolytic titer about 16 times. The 2-fold decrease in the hemagglutinin titer is not significant.

A variety of enzymes, including catalase, are inhibited by hydroxylamine(4-6). The effect of this substance on the hemolytic agent of mumps is shown in a typical experiment in Table I. Hydroxylamine, in a concentration of 0.01 M, was markedly inhibitory for the

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TABLE I. Effect of Various Concentrations of Enzyme Inhibitors on the Mumps Hemolysin.

Inhibitor	Hemolysis — Dilution of fluid — 2	4	8	16	32	64	128	Control	Hemagglutination titer
0	48*	38	33	29	22	16	13	0	1024†
.1 M urethane	21	15	11	9	8	6	4	0	512
.05 M	29	21	19	14	11	7	5	0	512
.01 M	44	25	27	20	18	14	11	0	512
0	40*	39	37	33	34	15	9	0	512†
.01 M hydroxylamine hydrochloride	39	22	12	6	5	1	2	0	512

* % cells hemolyzed determined colorimetrically.
† Reciprocal of highest dilution of virus producing hemagglutination.

TABLE II. Enzyme Inhibitors Which Produced No Significant Effect on the Activity of the Mumps Hemolysin.

Inhibitor	M conc. of inhibitor used
Sodium fluoride	.1; .01; .05; .001
Potassium cyanide	.01; .05; .001
2,4-dinitrophenol	.01
Sodium pyrophosphate	.01
Cystine	.001
Iodoacetic acid	.1; .01; .05; .001

TABLE III. Effect of Reducing Agents on the Mumps Hemagglutinin.

Reducing agent	Diluent	Hemagglutination titer
0	Phosphate buffer	64*
.01 M cysteine	" "	4096
.05 M glutathione	" "	4096
0	Saline	128
.1 M cysteine	"	4096
.05 M glutathione	"	4096

* Reciprocal of highest dilution of virus producing hemagglutination.

hemolysin in all dilutions greater than 1:2. It reduces the significant hemolysin titer more than 8-fold. No effect was noted on the hemagglutinin.

Another group of substances which affect a variety of enzymes was tested for their effects on either the hemolysin or hemagglutinin of mumps virus as listed in Table II. They were without effect on either property of the virus under the conditions of the tests and in the concentrations noted.

Since the virus rapidly loses its hemolytic activity with ordinary methods of handling, it was thought that aeration might play a role in the process, but oxygenation of virus-containing fluids with pure oxygen for 75 minutes at room temperature was without effect on the hemolysin. To study the effects of reduction of the O/R potential on the viral hemolysin, various reducing agents, such as cysteine and glutathione, were added to the diluting fluids at the time of the titration for hemolytic and hemagglutinating activity. In these experiments, the tubes were tightly stoppered to insure maintenance of reducing conditions. Evidence was obtained that the hemolytic component was somewhat inhibited by 0.05 M glutathione but 0.01 M, 0.05 M, and 0.001 M cysteine generally produced some increase in hemolytic action. However, both reducing agents produced a marked enhancement of hemagglutination as noted in Table III. Neither cysteine nor glutathione had any effect on the erythrocytes *per se* with the exception of causing some reduction of the hemoglobin. Other reducing agents, such as 0.1 M hydroquinone and 0.06 M sodium sulfite had a similar enhancing effect on the hemagglutinating activity of the virus.

Discussion. In certain of its properties, the hemolysin of mumps virus resembles the hemolytic agent, lecithinase A(4-7). Both the mumps hemolysin and lecithinase A are inhibited by urethane(4-6) and calcium and magnesium ions(5,8). At an optimal pH range 7.0-7.2 and a temperature of 37°C, the action of either of these agents results in the lysis of red blood cells. Neither agent is affected by potassium cyanide or sodium fluoride. However, lecithinase A is heat stable whereas the mumps hemolysin is relatively heat labile(2).

Inhibition of mumps virus hemolytic activity without a corresponding decrease in the hemagglutinating potency by urethane and hydroxylamine hydrochloride gives further evidence for the separate identity of the

hemolysin and the hemagglutinin. The striking enhancement of the hemagglutinating capacity of mumps virus by reducing agents which may actually suppress hemolytic activity also suggests that they are distinct entities. Lowering the O/R potential apparently provides optimal conditions for the agglutination of erythrocytes by the virus.

Summary. Mumps virus hemolysin is inhibited by hydroxylamine hydrochloride and urethane without affecting the hemagglutinin. Cysteine increases both the hemagglutinating and hemolytic activity of mumps virus while glutathione, which has a marked, potentiating effect on the hemagglutinin, inhibits the hemolytic activity. Other reducing agents, such as hydroquinone and sodium sulfite, increase the hemagglutinating capacity of the virus. The different effects of these various substances on the hemolysin and the hemagglutinin suggest that they are distinct entities. The possibility that the hemolysin of mumps virus is a lecithinase is discussed.

1. Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. Exp. Med.,* 1948, v88, 503.

2. Chu, L. W., and Morgan, H. R., *J. Exp. Med.,* 1950, v91, 393.

3. Chu, L. W., and Morgan, H. R., *J. Exp. Med.,* 1950, v91, 402.

4. Sumner, J. B., ed., Myrbäck, K., *The Enzymes: Chemistry and Mechanism of Action,* Academic Press, New York, N. Y., 1951.

5. Sumner, J. B., and Somers, G. F., *Chemistry and Methods of Enzymes,* Academic Press, New York, N. Y., 1947.

6. Lardy, H. A., ed., *Respiratory Enzymes,* Burgess Publishing Co., Minneapolis, Minn., 1949.

7. Baldwin, E., *Dynamic Aspects of Biochemistry,* Cambridge University Press, Cambridge, Eng., 1949.

8. Morgan, H. R., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 276.

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Saphenous Circulation Time Test with a Radioactive Tracer. (19298)

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In January, 1947, routine post-operative stimulation of calf muscles was instituted at Crile V.A. Hospital, Cleveland, with the object of reducing the incidence of thrombosis and embolism(1). In order to find a possible explanation of the apparent good results obtained with this treatment, tests were done to ascertain the effect of sinusoidal stimulation of calf muscles upon circulation time. With the use of sodium cyanide as a test medium it was shown that sinusoidal stimulation of calf muscles reduces circulation time(2). However, the methods of study of circulation time have in the past necessitated the entry of the test medium into the systemic circulation. The test being described in this report, through the use of radioactive iodine and a scintillation counter, permits the study of circulation time in a vein or even part of a vein. The present report deals with circulation time studies in the great saphenous vein.

Test solution. Radioactive iodine (I-131) was chosen as being particularly suitable for this procedure. It is readily available, being supplied by the Oak Ridge National Laboratories and flown to the hospital at frequent intervals for use in the diagnosis of thyroid disorders. Its half-life of 8 days is long enough for an adequate stockpile to be maintained. The dose presently employed, 20 microcuries, is strong enough to permit recording by a scintillation counter, and small enough to allow as many as 5 tests at one session without exceeding the maximum total dose considered desirable in such a diag-

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