The 2 dogs were treated with nicotinic acid hydrochloride obtained from the Eastman Kodak Company. It was given by mouth, the dosage being approximately 5 mg. per kilo of body weight per day.

There was a prompt resumption of normal appetite in both dogs and an immediate and sustained increase in body weight. The vermilion bands on the upper lips and the reddening of the oral mucosa disappeared and did not return. The watery diarrhea exhibited by one of the dogs disappeared. The body weight curves are shown in Fig. 1.

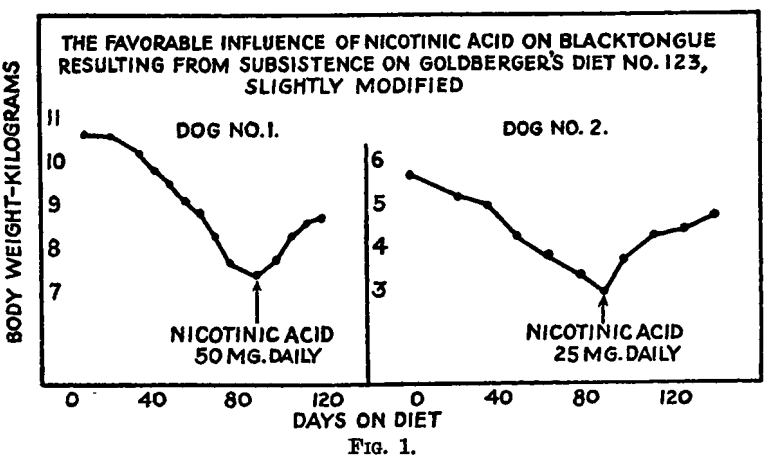

FIG. 1.

Conclusion. The finding that nicotinic acid corrects the deficiency of the Goldberger blacktongue-producing diet is confirmed.

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Alteration in the Immunological Specificity of Fibrinogen by the Action of Fibrinolysin of the Hemolytic Streptococcus.

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It has been shown by Garner and Tillett[1] that if fibrinogen is incubated with hemolytic streptococcus fibrinolysin for a brief period, the fibrinogen can no longer be changed to fibrin upon the

[1] Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 255.

addition of thrombin. However, these authors came to the conclusion that although changes do take place in the fibrinogen when acted upon by the fibrinolysin, the degradation of the molecule is not great. In further studies Garner[2] added that the end product of the reaction could not be differentiated, by immunological experiments, from the original fibrinogen solution.

The possibility of an antigenic alteration of fibrinogen by fibrinolysin was further studied in the following manner.

Human fibrinogen was prepared according to the method outlined by Tillett and Garner,[3] except that the fibrinogen solution was purified by precipitating with 50% saturation with NaCl. It was then centrifuged and the precipitate dissolved in distilled water. The fibrinogen was again precipitated out and dissolved as above, the procedure being repeated 7 times. The fibrinogen solution was then tested by the precipitin reaction against anti-human globulin serum.* The reaction was very faint, indicating a minimum amount of contaminating globulin. This fibrinogen was used in immunizing rabbits. The immune serum produced was filtered through a Berkefeld filter and then inactivated at 56°C. for a half-hour. This inactivation was necessary because the serum contains a small amount of thrombin, and when the fibrinogen is added in the precipitin test a small amount of reactive fibrin is formed which interferes with the test. Inactivating the serum removes this difficulty, as was shown by inactivating normal serum and adding fibrinogen.

Precipitin tests were carried out using a constant amount of antiserum and varying amounts of fibrinogen, and the same amounts of fibrinogen-fibrinolysin solution. Thus the tests were run in two series:

(a) Control Solution: 1 cc. broth (0.05% dextrose) + 2 cc. fibrinogen, incubated for 24 hours prior to the precipitin tests with the antiserum.

(b) Fibrinogen-fibrinolysin Solution: 1 cc. fibrinolysin + 2 cc. fibrinogen, incubated for 24 hours prior to the precipitin tests with the antiserum.

The fibrinogen contained 0.261 mg. nitrogen per cc.; the fibrinolysin was the filtrate of a 20-hour 0.05% dextrose broth culture of hemolytic streptococcus No. 260, obtained from a case of acute pharyngitis. The fibrinolysin lysed the clot of human fibrinogen

2 Garner, R. L., *J. Biol. Chem.*, 1935, **109**, xxxvi.

3 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

* This anti-human globulin serum was kindly furnished by Dr. Forrest E. Kendall of the Research Division for Chronic Diseases, Department of Hospitals, Welfare Island, New York.

and thrombin in less than 20 minutes. Varying amounts of solutions (a) and (b) were added to 1 cc. samples of the rabbit-anti-human fibrinogen serum. The tubes were incubated for one hour at 37°C., and were then placed in the ice-box overnight. The precipitates formed were then washed in distilled water and then diluted to ten cc. with distilled water and analyzed for their total nitrogen content by the Kjeldahl method. The results of a representative analysis are shown in Tables I and II.

TABLE I.
Using Control Solution (a) above.

				Total N of fibrinogen Solution (a) mg.	Total N in Precipitate mg.
1.	1 cc. anti-serum	+ 1	cc. Solution (a)	.174	.31
2.	" "	+ 0.75	" " "	.130	.33
3.	" "	+ 0.50	" " "	.087	.30
4.	" "	+ 0.25	" " "	.043	.21

TABLE II.
Using Solution (b) above.

				Total N in Precipitate mg.
1.	1 cc. anti-serum	+ 1	cc. Solution (b)	.075
2.	" "	+ 0.75	" " "	.075
3.	" "	+ 0.50	" " "	.072
4.	" "	+ 0.25	" " "	.072

From the above results it appears that the fibrinolysin does change the specificity of the fibrinogen, as is evidenced by the change in the quantity of the precipitates when fibrinolysin-fibrinogen mixtures are tested with anti-fibrinogen serum. The next step was to investigate the rapidity of the action of fibrinolysin in bringing about this change in the fibrinogen.

Streptococcus R333, obtained from a case of streptococcus pneumonia, was grown in 0.05% dextrose broth. The filtrate lysed the clot of human fibrinogen and thrombin in 6 minutes. This fibrinolysin was then incubated with fibrinogen at 37° C. At intervals of 15 min., 30 min., 45 min., 1 hr., and 2 hr. samples were withdrawn and added to 1 cc. quantities of the rabbit-anti-human fibrinogen serum. The amount of fibrinogen-fibrinolysin withdrawn was always equivalent to 0.087 mg. of fibrinogen nitrogen; this amount was slightly less than the amount necessary to bring about maximum precipitation, as was shown in the previous experiment. After incubation the tubes were placed in the ice-box over night, and the

following day the precipitates were analyzed. The results are presented in the graph, Fig. 1.

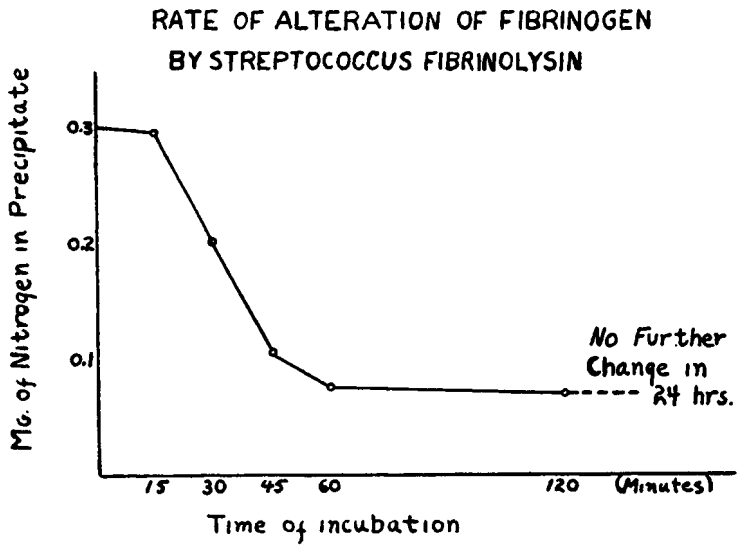

FIG. 1.

The results indicate that there is an initial lag period which is followed by a rapid activity of the fibrinolysin on the fibrinogen, as is shown by the decrease in the amount of the specific precipitate. The reaction is complete in little more than one hour, and there is no further degradation even in 24 hours.

In the work of Garner and Tillett[1] the fibrinogen used probably contained a considerable amount of globulin. This is indicated because in their work when they incubated the fibrinogen with fibrinolysin and then added $(NH_4)_2SO_4$ to 25% saturation they did not obtain any precipitate. However, when they increased the $(NH_4)_2SO_4$ to 35-45% saturation a precipitate was obtained. This latter amount of $(NH_4)_2SO_4$ will precipitate serum globulins. Probably no precipitate was obtained with 25% saturation with $(NH_4)_2SO_4$ because the fibrinogen had been altered and all that remained was the serum globulins. In the experiments completed above the fibrinogen was purified so that it contained only a minimum amount of globulin as was shown by testing with human anti-globulin serum. Fibrinogen prepared by the Tillett and Garner[8] method gave a much greater precipitate with the same anti-globulin serum, showing that it contained a not inconsiderable amount of globulin.

When the fibrinogen-fibrinolysin solution was incubated for from

one to 24 hours the amount of precipitate formed, when the solution was added to the anti-fibrinogen serum, was almost constant. Considering the rapid and progressive change in the fibrinogen within the first hour of incubation with the fibrinolysin it is suggested that the substance remaining in solution, which reacts with the anti-fibrinogen serum, is either a split product of the fibrinogen resulting from the action of fibrinolysin or a small amount of serum globulin.

Another point of interest is the apparent lag period of 15 minutes before fibrinolysin lyses fibrinogen as indicated on the graph. This, however, probably has no significance if we consider it in the following manner:

Assuming that all or most of the antigen nitrogen is present in the precipitate, Table I can be presented as shown in Table III.

TABLE III.

Antigen N mg.	N in precipitate mg.	Antibody N mg.
.174	.31	.136
.130	.33	.200
.087	.30	.213
.043	.21	.167

It appears possible that the maximum antibody nitrogen may have been precipitated by the addition of .065 mg. of fibrinogen nitrogen. Thus the first 15 minutes is not actually a lag period, but it represents the time taken to reduce the amount of fibrinogen to the point where it will precipitate the maximum amount of antibody.

Summary. 1. A fairly pure fibrinogen was prepared and used to immunize rabbits. 2. Precipitin tests were carried out with the anti-serum formed; the precipitinogens used were fibrinogen alone and fibrinogen + fibrinolysin. 3. Analyses of the precipitates indicate that the fibrinolysin does have an effect on the fibrinogen which alters its antigenic specificity. 4. The action of the fibrinolysin is very rapid, and the change it produces in the fibrinogen is accomplished in little more than one hour.