4102

Is Thrombin an Enzyme?

C. A. MILLS AND S. M. LING.

From the Department of Medicine, Peking Union Medical College, Peking, China.

The earlier work on blood clotting, from the time of Schmidt,[1] was done mainly on the assumption that thrombin was an enzyme (fibrin ferment). The first seemingly definite evidence that it was not an enzyme was offered by Rettger[2] and later by the more careful work of Howell.[3] These 2 authors presented data showing a rather definite combining ratio between thrombin and blood fibrinogen when minimal amounts of the former were present. Howell raised a doubt as to the interpretation of the data, however, when he described the formation of repeated crops of fibrin in the serum following the removal of the fibrin masses, upon the weight of which they had based their first conclusions. He himself likened this behavior of thrombin to an enzyme, but still leaned toward the belief that it probably was not such. We have extended Howell's observations and obtained striking results which we briefly summarize below.

To fresh, clear, citrated dog plasma was added $(NH_4)_2SO_4$ sufficient to give 1/7 saturation and the precipitated fibrinogen filtered off, repeatedly washed in 20% NaCl, dissolved in 0.5% sodium citrate solution, reprecipitated with $MgSO_4$, washed and finally redissolved in 0.5% sodium citrate solution again. To the plasma, after the removal of the fibrinogen, $(NH_4)_2SO_4$ was further added to ½ saturation, and the precipitate collected and pressed free of liquid on a filter. This precipitate was stirred into 30% $MgSO_4$ solution, filtered, and the filtrate saturated with $MgSO_4$. The precipitate formed in this last step was collected and pressed free of as much liquid as possible. On treatment with cephalin and calcium it yielded very active thrombin, which deteriorated only slightly during 5 weeks of observation. The portion of the $(NH_4)_2SO_4$ precipitate that was insoluble in 30% $MgSO_4$ was found to contain little prothrombin.

Using these stable solutions of thrombin and fibrinogen we found that one part of thrombin might convert as high as 1,000 parts of fibrinogen to fibrin provided the fibrin was removed in successive

[1] Schmidt, A., *Arch. ges. Physiol.*, 1875, xi, 515.

[2] Rettger, J. L., *Am. J. Physiol.*, 1909, xxiv, 406.

[3] Howell, W. H., *Am. J. Physiol.*, 1910, xxvi, 453.

crops. Furthermore, the clots formed exhibited the appearance of syneresis after 2 to 3 days at room temperature, but on weighing the fibrin at such times it was found that the process was really one of solution. To rule out bacterial action as a cause of the solution of the clots, we sterilized the thrombin and fibrinogen solutions before mixing by passing through a Berkefeld filter and kept the mixtures sterile throughout the period of observation. Under such conditions the clots again showed resolution after 2 to 4 days, those containing most thrombin and clotting most quickly also redissolving most rapidly. The resolution was complete, the fluid being clear and sparkling.

Examination of the sterile fluids containing the redissolved clots showed no fibrinogen coagulating at 55° C., as did the original fibrinogen solution, but instead a heavy coagulum at 75° C. No further change took place on heating to boiling. On comparing this fluid with that from a similar mixture from which the fibrin was removed before it had begun to dissolve, it was found that the coagulum at 75° C. represented in the main the redissolved fibrin. There was no increase in the non-protein nitrogen during this resolution, so that the change was not a digestive proteolysis.

We conclude, therefore, that there are 2 phases to thrombin action, the first being a union with, and precipitation of, the fibrinogen, while the second involves a resolution of this clot with the conversion of fibrinogen into a protein of quite different behavior, coagulating at 75° C. instead of 55° C., and being resistant to further thrombin precipitation. Whether this resolution phase of thrombin action is responsible for the apparent syneresis of clots is a question we are now investigating.

4103

Production of Sugar by Surviving Liver.

A. CARRUTHERS. (Introduced by Hsien Wu.)

From the Department of Biochemistry, Peking Union Medical College.

That the liver can form carbohydrate from certain metabolites, and, in particular, lactic acid, is a view widely accepted. Examination of the experimental evidence nevertheless reveals a large number of contradictory results.

Mandel and Lusk[1] showed that lactic acid when given to the

[1] Mandel, J. A., and Lusk, G., *Am. J. Physiol.*, 1906, xvi, 129.