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.

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Production of Sugar by Surviving Liver.

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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.

diabetic organism was excreted as glucose. This transformation they suggested occurred in the liver. Jannsen and Jost[2] could not show that intravenous injection of lactic acid led to the formation of sugar in the muscles. They supposed the change occurred in the liver. Meyerhof and Lohmann[3] and Takane[4] claim that liver slices bring about a synthesis of carbohydrate when respiring in Ringer solution containing lactic acid. Abramson and Eggleston[5] find, however, that the intact anesthetized dog does not demonstrably synthesize glycogen (in the liver) from sodium r-lactate. Finally, Burn and Marks[6] by perfusing livers previously rendered glycogen free found that there was a production of sugar which was not derived from either lactic acid or from protein. The following results were obtained in some work planned to investigate lactic acid, carbohydrate relationships in normal and abnormal tissues.

Rabbits were starved for 36-48 hours. The animals were stunned by a sharp blow on the head and rapidly bled to death. The liver was excised, chopped with scissors into pieces only fine enough to allow of good sampling (the tissue was not made into a brei), and samples weighed out, fairly accurately, but rapidly. The tissue to be incubated was placed in flasks containing 20 cc. phosphate-Ringer solution—pH 7.4—and the air in the flasks was displaced by oxygen. The tissue was incubated at 37° C. for 3 hours, the flasks being gently shaken all the time. The control samples were placed in either ice-cold 95% alcohol (for glycogen, soluble sugar and lactic acid determinations) or in ice-cold 4% HCl (for the total sugar content). Glycogen was estimated by Pflügers' method; soluble sugars were extracted in 66% alcohol and, after removal of the alcohol, the aqueous mixtures were cleared by the use of the $HgNO_3$ reagent of Patein and Durfan. After removal of excess mercury by H_2S and blowing off of the H_2S crystal clear solutions were obtained. To insure that there was no sugar in the tissue which escaped detection in these methods, samples were also hydrolyzed with 4% HCl for 3½ hours. After removal of proteins by NaOH and $ZnSO_4$ these solutions were also cleared with $HgNO_3$. The method of Hagedorn and Jensen was employed for the estimation of sugar.

The results in Table I show that when the total sugar content of tissue is determined by summing glycogen and soluble sugar frac-

[2] Jannsen, S., and Jost, H., *Z. Physiol. Chem.*, 1925, cxlviii, 41.

[3] Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1926, clxxi, 381.

[4] Takane, R., *Biochem. Z.*, 1926, clxxi, 403.

[5] Abramson, H. A., and Eggleston, P., *J. Biol. Chem.*, 1927, lxxv, 763.

[6] Burn, J. H., and Marks, H. P., *J. Physiol.*, 1926, lxi, 497.

TABLE I.
Carbohydrate content estimated as glycogen and soluble sugars in mg. glucose per 5 gm. liver.

Exp. No.	Before Incubation. Calcul'd as glucose Glycogen	Before Incubation. Calcul'd as glucose Soluble sugar	Before Incubation. Total	After Incubation. Calcul'd as glucose Glycogen	After Incubation. Calcul'd as glucose Soluble sugar	After Incubation. Total	Difference
2	7.7 7.0	8.9 7.8	16.6 14.8	3.4	28.9	32.3	16.6
3	51.5 52.3	74.4 75.4	125.9 127.7	7.1	132.3	139.4	13.6
4	65.3 68.0	68.8 63.6	134.1 131.6	8.5 10.5	140.9 137.9	149.4 148.4	16.1

TABLE II.
Carbohydrate content estimated after acid hydrolysis in mg. glucose per 5 gm. liver.

Exp. No.	Before Incubation	After Incubation	Difference
1	96.3	100.7	+4.4
2	68.3	75.6	+7.3
4	173.8 168.1	171.4	+0.5
5	113.2 115.2	112.8	—1.4

tions (both expressed as glucose) there is apparently a production of sugar during the period of incubation. The results given are typical of what has been observed many times. With large amounts of tissue the differences may be quite considerable. Precisely why there is the difference it is difficult to say, but that it cannot be due to experimental error is suggested by the agreement between the duplicates.

That the production of sugar is only apparent and not real is shown by the results in Table II. The values given by acid hydrolysis of whole tissue show no production of sugar during incubation. It has always been found that the acid hydrolysates give a much higher value than the sum of glycogen and soluble sugar, and this is probably due to the presence of substances influencing the reducing power of the sugar. This discrepancy, however, does not invalidate the results since the agreement between the duplicates is again of a good order.

It is suggested then that when the power of the liver to produce sugar is being investigated it is not sufficient simply to estimate the sugar in terms of glycogen and soluble sugar. Acid hydrolysis of the whole tissue should also be considered.