With thymol blue standards the determination of the P_H of gastric juice may be very easily and quickly made.

Other uses of this instrument are being considered.

42 (1789)

The effect of pancreatic rennet on blood coagulation.

By ALBERT A. EPSTEIN and NATHAN ROSENTHAL.

[From the Department of Physiological Chemistry, Mt. Sinai Hospital, N. Y. City.]

At the last meeting of this society, one of us[1] presented certain observations on pancreatic rennet. Particular stress was laid on the state in which the rennet probably exists in pancreatic extract, and its intimate chemical association with trypsin. In speaking therefore of the pancreatic rennet we wish it understood that it is the rennet-trypsin unit and not the rennet alone that we are dealing with. Mention was also made of the absolute dependence of the milk coagulating function of rennet on the calcium ion.

The theoretical considerations which have prompted the present investigation will be discussed fully at another time and place. Suffice it to say for the moment that rennet (as a class) appears to be widely distributed in nature, in the animal as well as in the vegetable kingdom. In the latter, its native function often seems to be that of a coagulant of the sap of the plant in which it exists, a process comparable, in some respects, to that of blood coagulation.

Our first attempt to discover the effect of pancreatic rennet upon the coagulation of the blood consisted in the following simple experiment. A small quantity of the purified pancreatic rennet was added to a portion of blood freshly drawn from the cubital vein of an hemophilic individual, and the coagulation time noted. We found that whereas the blood in the control tube required 1 hour and 20 minutes for coagulation, the specimen to which the rennet was added clotted in 90 seconds. The result was so striking that we determined to make a careful investigation of the phenomenon.

In a study of the effect of various tissue extracts on blood coagulation, Mills[2] found that a saline extract of pancreas has very

[1] Epstein, A. A., PROC. SOC. EXPER. BIOL. AND MED., 1921, xix, 3.

[2] Mills, C. A., *J. Biol. Chem.*, 1919, xl, 425.

slight coagulating power for blood. The coagulating substance which Mills isolated from various organs other than the pancreas he identified as a protein-lipin. It might be added that thus far we have not been able to demonstrate any lipin element in our pancreatic rennet preparations. Therefore, it appears that our rennet is not identical with the substance which Mills isolated.

To determine the efficiency of pancreatic rennet as a blood coagulant experiments were performed both in vitro and in vivo. The test tube method of Lee and White[3] was used for estimating the coagulation time. In the animal experiments, the individual specimens were withdrawn from different vessels.

We will first consider the effect of refined pancreatic rennet on normal human blood. For this, blood was obtained from the cubital vein of a normal individual in a series of small test tubes, some containing $CaCl_2$, others $CaCl_2$ and pancreatic rennet, and still others containing rennet alone. The two controls which were made, one at the beginning and one at the end of the experiment, showed the coagulation time to be 11 and 12 minutes respectively. The addition of $CaCl_2$ to the blood reduced the coagulation time to a period ranging between 5 and 8 minutes. Pancreatic rennet alone reduced the coagulation time to 1 and 1½ minutes respectively. Calcium chloride and pancreatic rennet, together, short-

TABLE I.

EXPERIMENT 1.

a. Effect of $CaCl_2$ 1 per cent. on the Coagulation of Whole Blood.

Whole blood	1 c.c.	1 c.c.	1 c.c.
$CaCl_2$—1 per cent	1 gtt.	1 gtt.	2 gtt.
Norm. saline	1 gtt.	2 gtt.	3 gtt.
Clotting time	8 min.	7 min.	5 min.

b. Effect of Pancreatic Rennet on the Coagulation of Whole Blood.

Whole blood	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Pan. rennet	1 gtt.	2 gtt.	3 gtt.	4 gtt.
Saline	1 gtt.	1 gtt.	1 gtt.	1 gtt.
Clotting time	1 min.	1 min.	1 min.	1½ min.

c. Effect of $CaCl_2$ and Pancreatic Rennet on the Coagulation of Whole Blood.

Whole blood	1 c.c.	1 c.c.	1 c.c.	1 c.c.
$CaCl_2$—1 per cent	1 gtt.	1 gtt.	1 gtt.	1 gtt.
Pan. rennet	1 gtt.	2 gtt.	3 gtt.	4 gtt.
Clotting time	30 sec.	15 sec.	15 sec.	30 sec.

[3] Lee, R. I., and White, P. D., *A. J. Med. Sci.*, 1913, cxlv, 495.

ened the coagulation time of this blood to 15 and 30 seconds respectively. (Expt. 1, Table I.)

We then tested once more the effect of the pancreatic rennet on the blood of a hemophiliac and the results show (Expt. 2, Table II) that the coagulation time could be reduced from 1 hour and 15 minutes to 2 minutes, by very small, but adequate amounts of the rennet.

TABLE II.

EXPERIMENT 2.

Effect of Pancreatic Rennet on the Coagulation Time of Hemophiliac Blood.

The control tests showed the clotting time of hemophiliac blood to be 1 hour and 15 minutes at the beginning of the experiment and 1 hour and 20 minutes at the end.

Hemophiliac blood	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.
Activated pan. extract—1 per cent	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.
(Rennet content)	0.003 c.c.	0.002 c.c.	0.0015 c.c.	0.001 c.c
Clotting time	2 min.	4 min.	20 min.	29 min.

We next tested the effect of pancreatic rennet on citrated normal plasma. In this experiment, as in that with whole blood, we find that the rennet accelerates coagulation. The clotting time is reduced from approximately 8 minutes to 30 seconds. Two other facts come to light in this experiment, first, that the shortening of the coagulation time is proportionate to the concentration of the rennet, and secondly, that the rennet is capable of coagulating citrated plasma without the addition of calcium chloride (see Table III, Exp. 3, Column 3). The exact manner in which the rennet accomplishes this result cannot be stated with certainty. In the normal coagulation of blood, calcium is indispensable. It will be recalled that rennet requires calcium for the coagulation of milk. Hence, it must be assumed that rennet causes the clotting of citrated plasma, either in a manner peculiar to itself, or that it accomplishes it by causing a dissociation of the calcium ion from the citrate molecule. The experiment indicates, however, that the addition of extra calcium, in the form of chloride, enhances the coagulating effect of the pancreatic rennet on citrated plasma.

The intravenous injection of large doses of activated crude pancreatic extract, or the purified rennet in rabbits is not accompanied by anaphylaxis or other symptoms. Intravascular clotting

does not occur as is the case with saline lung extracts or other tissue coagulants.

TABLE III.

EXPERIMENT 3.

Diluted citr. plasma	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.
$CaCl_2$—1 per cent	2 gtt.	2 gtt.	0	2 gtt.	2 gtt.	2 gtt.
Pan. rennet—1 per cent	0	0	0.3 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.
Clotting time	8 min.	7 min.	8 min.	¾ min.	½ min.	½min

EXPERIMENT 4.

Citrated plasma	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.
$CaCl_2$— 1 per ct.	2 gtt.	2 gtt.	2 gtt.	2 gtt.	2 gtt.	2 gtt.	2 gtt.	2 gtt.	2 gtt.
Rennet	0	0.125	0.062	0.031	0.015	0.075	0.038	0.014	0.007	0.003
Saline	0.25	—	—	—	—	—	—	—	—	—
Clotting time	6½ m.	2 m.	2 m.	1½ m.	2 m.	3½ m.	4½ m.	5 m.	5½ m.	6 m.

Cats were used in studying the effect of intravenous injection of pancreatic rennet on blood coagulation. Moderate doses of the rennet reduce the coagulation time, but not as strikingly as in the experiments conducted in vitro. Excessive doses prolong the coagulation, without giving rise to intravascular clotting. In this respect also, the pancreatic rennet differs from the tissue coagulants studied by Mills. The underlying mechanism of the shortening and prolongation of the clotting time thus produced is being further investigated.

The specific effects produced by the intravenous injection of activated crude pancreatic extract, and the refined rennet, is illustrated by the following experiments.

EXPERIMENT 5.

18 c.c. of a 3 per cent. activated crude pancreatic extract solution (rennet content 0.0054 g.) were injected into the jugular vein of a cat (under ether anesthesia). The results obtained are shown in the following protocol.

Clotting time before injection.....7 minutes.
Clotting time 5 minutes after injection.....4 minutes.
Clotting time 10 minutes after injection.....2 minutes.
Clotting time 20 minutes after injection.....3 minutes.
Clotting time 25 minutes after injection.....3 minutes.
Clotting time 40 minutes after injection.....3 minutes.

EXPERIMENT 6.

Effect of a Large Dose of Refined Pancreatic Rennet on the Coagulation of the Blood (Cat).

4 c.c. of a refined pancreatic rennet (equal to 38 c.c. of a 3 per cent. sol. of the crude sol.) was used.

Clotting time before injection	5½ minutes.
Clotting time 2 minutes after injection	3 minutes.
Clotting time 5 minutes after injection	5 minutes.
Clotting time 10 minutes after injection	15 minutes.
Clotting time 25 minutes after injection	6 minutes.

Thus we find after an initial diminution of the clotting time there was a prolongation and a return to normal at the end of 25 minutes.

THE EFFECT OF RENNETS FROM OTHER SOURCES ON THE COAGULATION OF BLOOD.

On account of the striking action of pancreatic rennet in hastening of coagulation of the blood in vitro, studies have been undertaken with rennets from other sources, for purposes of comparison. The action of these rennets on blood coagulation may possibly be less potent than that of the pancreatic variety. The isolation of the pure rennet from the stomach has thus far been difficult to accomplish. In one experiment we used a crude pepsin solution of known milk coagulating power and found a distinct delay in the coagulation of the blood (see Exp. 7). This action may possibly be due to the impurities in the crude pepsin-rennet solution.

EXPERIMENT 7.

Citrated plasma	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.
Crude pepsin—5 per cent.	0	1 gtt.	2 gtt.	3 gtt.	4 gtt.
$CaCl_2$—1 per cent.	2 gtt.	2 gtt.	2 gtt.	2 gtt.	2 gtt.
Clotting time	8 min.	7 min.	25 min.	none	none
				at the end of 24 hours.	

CONCLUSIONS.

1. Pancreatic rennet shows great activity as a coagulant for normal and hemophilic blood.

2. Intravenous injection of pancreatic rennet does not produce anaphylaxis.

3. Moderate intravenous doses diminish the coagulation time, and excessive doses increase the coagulation time after an initial shortening.

4. Intravascular clotting has not been observed after intravenous administration of pancreatic rennet.

43 (1790)

Observations on the excretion of sugar in the urine in health and disease.

By LUDWIG KAST and HILDA M. CROLL.

[*From the Department of Medicine and the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York City.*]

The amount of sugar in the twenty-four-hour urine specimens has been determined by the new acetone-picric acid method of Benedict and Osterberg.[1] It was found that a diet rich in carbohydrate increases the amount of sugar excreted over that on a low carbohydrate diet. This method also demonstrated the increase in hourly sugar excretion after meals and after glucose ingestion. In four normal adults studied, the average amount of reducing sugar excreted daily was between 0.59 and 1.14 grams. In ten children from 2.5 to 9 years of age, representing a variety of pathological conditions, the average range was from 0.12 to 0.43 gram sugar daily. To cite a few of the 116 hospital cases studied, in 20 patients diagnosed as neurasthenics, on the average between 0.42 and 1.24 grams sugar were excreted daily; in hyperthyroidism the average range was between 0.46 and 0.98 grams; in one case of hypothyroidism an average of 0.40 gram sugar was excreted; in nephritis 0.41 to 0.89 grams, in hypertension 0.44 to 1.12 grams, in arthritis 0.44 to 1.39 grams, and in various cardiac disturbances 0.51 to 1.39 grams on the average were excreted daily. It appears, therefore, that in the diseases studied, when the patients are on ordinary diets, there is no striking increase nor decrease over normal urinary excretion. In diabetes alone there is an increase, although when by dietary regulation the patient is rendered "sugar free" the amount of sugar excreted is practically normal.

[1] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1921, xlviii, 51.