the same changes in the blood lipids as those receiving the raw whole pancreas. The unknown pancreatic blood lipid factor or factors referred to by Chaikoff and Kaplan can, therefore, still be considered the subject of further investigation.

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Calcium Factor in Quantitative Determination of Prothrombin.

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The method developed by the author for the determination of prothrombin has on account of its simplicity won wide clinical interest. Its reliability as an index of the bleeding tendency in jaundice has been repeatedly confirmed. Nevertheless, any test based on the use of biological materials presents well recognized difficulties. In the method as originally described,[1] the thromboplastin was a source of trouble because of its variability in potency, but after the writer[2] had perfected a method for making a preparation which was stable and had a high and uniform potency, satisfactory results have been obtained. It was, therefore, surprising to learn that Stewart and Pohle[3] using the method in its present form on normal bloods found: "that the results were extremely variable." They ascribe their difficulties to the concentration of the calcium chloride solution. In the test a 0.025 M solution is used. Assuming that blood contains 10 mg of calcium, a 0.0075 M solution would be equivalent to the concentration of sodium oxalate present in the plasma. One can see that a definite excess of calcium is employed. It was recognized by the author[4] that calcium beyond a certain concentration has an inhibitory action on the coagulation time. The reason a 0.025 M solution was chosen was as follows: The prothrombin test is an outgrowth of the method originally developed at the Fifth Avenue Hospital for the determination of the clotting time of recalcified plasma and in this procedure a 0.025 M solution was found most

1 Quick, A. J., *J. Biol. Chem.*, 1935, **109**, lxxiii.

2 Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

3 Stewart, J. K., and Pohle, F. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 532.

4 Quick, A. J., *J. Immunol.*, 1935, **29**, 87.

TABLE I.
Effect of Concentration of Calcium Chloride on Clotting Time in Determination of Prothrombin.

Molar conc. of $CaCl_2$	*Coagulation time in seconds				
	1	2	3	4	5
.1	42	43	42	45	51
.05	15	15	15	15	17
.033	12	12	12	13	15
.025	11½	11½	11½	11½	12½
.020	11½	11½	11½	11½	12½
.010	11	11	11	11	11
.005	10½	10½	10½	10½	10½
.0025	10	10	10½	10	10½
.00125	11½	12½	10½	11½	12
.000625	18	36	25	20	18

*Blood was obtained from normal human subjects.
The thromboplastin was prepared by mixing 0.3 g of dehydrated rabbit brain (acetone method) with 5 cc of 0.85% sodium chloride solution, and incubating at 50°C for 10 minutes. The supernatant liquid, obtained either by very slow centrifugation or spontaneous sedimentation of the coarse particles, was used.

satisfactory. Since this determination also has clinical value[5] it was considered wise for the sake of simplicity to retain the same calcium chloride solution for both methods.

One can see from Table I that if a satisfactory preparation of thromboplastin is used, the calcium concentration can be varied over a wide range without reducing the clotting time more than one second. No imperative need, therefore, exists for altering the strength of the calcium solution. It must be recognized, furthermore, that the values of the coagulation time in terms of prothrombin concentration have been obtained on the basis of a 0.025 M calcium chloride solution and if a different concentration of this reagent is adopted a new curve must be worked out.

The finding of Stewart and Pohle that the clotting time of the test can be slightly shortened by reducing the strength of the calcium chloride reagent is correct, but they are not justified in attributing their poor results to improper recalcification. They are right when they state that the test can only be reliable if there are no variables except the prothrombin, but they are grossly illogical when in the next statement they propose that for each determination the amount of calcium be so adjusted that a minimal coagulation time is obtained. Calcium is not a variable in the test and must not be made one. The concentration of calcium chloride was arbitrarily fixed at 0.025 M, but a different strength could have been chosen and the values of the clotting time in terms of prothrombin concentration determined for that particular calcium chloride solution.

[5] Quick, A. J., Central Soc. Clin. Res., 11th meeting, 1938.

With the concentration of calcium constant, any increase in coagulation time must be due to a decrease in the prothrombin of the plasma or to interfering factors.

The inhibitory action of excess calcium chloride is probably not due to salt action, but specifically to the calcium ion itself as Aggeler and Lucia[6] suggested. This behavior of calcium deserves more careful study especially to determine the nature of its anticoagulant action. It does not appear to be an antithrombic action, since calcium in this concentration does not inhibit the action of thrombin.

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Influence of Sulfanilamide and Related Compounds Upon Oxidation-Reduction Potentials of the Hemolytic Streptococcus.*

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(Introduced by F. P. Gay.)

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In an effort to secure information as to the mechanism of the action of sulfanilamide upon the streptococcus we have measured the effect of this drug and related compounds upon the oxidation-reduction potentials of the hemolytic streptococcus. An important relation between bacteriostasis and oxidation-reduction levels was pointed out by Dubos,[1] who noted that the growth of the streptococcus and the pneumococcus was inhibited if the Eh of the system was maintained above a critical level by the introduction of suitable dyestuffs, as oxidized indophenols.

The "Holman" strain of hemolytic streptococcus (Gay) was adopted for this investigation. Freshly seeded cultures which contained 280,000 organisms per cc at the beginning of the experiment, were observed over a period of growth for 72 hours. Beef-infusion broth with 1% peptone (Bacto), pH 7.4-7.6, was used throughout. The sulfanilamide was supplied by the Winthrop Chemical Company.

Potentials were measured against 26 gauge platinum-coil electrodes. All cultures and the calomel half-cell were kept at 37.0 ± 0.5°C. A Leeds and Northrup, Type No. 7660, vacuum-tube po-

6 Aggeler, P. M., and Lucia, S. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 11.

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1 Dubos, R., *J. Exp. Med.*, 1929, **49**, 575.