hepatic disease using intravenously administered methionine.[6] The female patient with ulcerative colitis presented a fairly normal curve except for the delayed peak reached at 6 hours.

In studies of abnormal subjects using the solution dose (Table II), certain definite deviations from normal were noted. In a case of carcinoma of the gall bladder, extremely high plasma levels of methionine were reached with a rapid fall between 120 and 180 minutes followed by a secondary rise at 240 minutes. In marked contrast, the patient with carcinoma of the pancreas exhibited extremely low plasma levels of methionine with a steady rate of disappearance. A case of ulcerative colitis showed a high initial peak but no other noteworthy abnormality in plasma levels. The last subject with fever of undetermined origin showed a somewhat flat curve suggesting some decrease in intestinal absorption although increased utilization due to an accelerated metabolic rate cannot be excluded.

The abnormal cases presented are not numerous and are intended to serve mainly as comparisons to the normal controls. Further study of this phase of the problem is necessary to establish the validity and significance of the apparent deviations from the normal pattern which have been pointed out.

Summary. 1. The concentration of methionine in the plasma after oral administration of *DL*-methionine has been studied in humans.

2. The results suggest more rapid and more efficient absorption of the amino acid when administered in solution rather than in tablet form.

3. A faster rate of disappearance of the administered methionine from the plasma was noted in the female subjects.

4. Observations on the apparent *D*-methionine levels suggest that the *D* isomer is absorbed at a rate equal to that of the *L* form. However, there is considerable individual variation in the rate of disappearance of the *D* isomer from the plasma.

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Sodium Oxalate as Anticoagulant.

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It is well known that 0.0025 M solution of sodium oxalate does not delay blood coagulation, even when in stoichiometric concentration with calcium. Three times more sodium oxalate than calcium present in blood is required to prevent blood coagulation (Quick[1]). On the other hand the prothrombin time of oxalated human plasma is prolonged because destruction of the plasmatic cofactor of thromboplastin (P.C.T.) starts a few hours after storage.[3] Quick believes that calcium protects this factor because hemophilic plasma from which calcium has not been removed does not alter its prothrombin time after 48 hours.[2] These experiments do not explain satisfactorily why an excess of sodium oxalate is needed to render blood incoagulable and why oxalated stored plasma destroys by oxidation its cofactor of thromboplastin.[3]

We do not believe that the prothrombin time of oxalated human stored plasma is prolonged because calcium is eliminated. Another possibility must be considered. Sodium oxalate may have more affinity for another substrate than calcium and only when an excess has been added does this last reaction take place. It seems logical to suppose the existence of a

[1] Quick, A. J., *The Hemorrhagic Diseases and the Physiology of Hemostasis*, 1942, Baltimore, Thomas, Ed.

[2] Quick, A. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 249.

[3] Honorato, C. R., *Am. J. Physiol.*, 1947, **150**, 381.

TABLE I.
Prothrombin Time of Fresh and Stored Human Plasma, Decalcified with Amberlite. Results of Oxalate and Citrate Treatment on This Plasma Passed Through Amberlite to Eliminate Calcium.

Prothrombin Time (sec.)					
Oxalated fresh plasma	1 day stored	Amberlite plasma	1 day stored Amberlite plasma	1 day stored Amberlite plasma + sodium oxalate	1 day stored Amberlite plasma + sodium citrate
12″	15″	13″	13″	18″	18″

substance in blood which protects the P.C.T. against oxidation. It must be a strong reductor. Its destruction by sodium oxalate would explain the slow but effective alteration of stored plasma.

To test this hypothesis the following experiments were carried out. Human blood withdrawn in silicon syringes (Jaques[5]) was passed through Amberlite IR-100-H (Quick[6]) to eliminate all the calcium without using oxalate. Then 0.1 cc of 0.1 M sodium oxalate or 0.125 M sodium citrate to 0.9 cc of plasma were added. A third sample passed through Amberlite (without addition of any other substance) was stored at 5°C. For prothrombin time determination a modified one-stage method of Quick[4] was used.

The results are shown in Table I.

After 24 hours, no change in the prothrombin time of the sample passed through Amberlite was observed. Thus calcium is not a protective element. On the other hand a very important change in the prothrombin time of plasmas passed through Amberlite, and to which oxalate or citrate were subsequently added, was observed. If no calcium was present, both salts must have altered the plasma by another mechanism than by the elimination of calcium. No difference was observed between oxalate and citrate activity, even when 0.125 M solution for the latter was used (0.125 M citrate preserves plasma better than oxalate 0.1 M, when both are used to withdraw blood samples). We postulate the existence of a protective substance, strong reductor, to preserve P.C.T. from oxidation, which is seriously and specifically altered by sodium oxalate and only subsequently by sodium citrate, 0.125 M, when calcium is present. Without calcium, differences of an affinity of this protective factor disappear.

[4] Honorato, C. R., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 41.

[5] Jaques, L., *Can. J. Med. Assn.*, 1946, **55**, 26.

[6] Quick, A. J., *Am. J. Physiol.*, 1947, **148**, 212.

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Studies on Polymyxin: An Assay Method for Blood and Urine.*

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Extension of the agar diffusion method[1] for the assay of polymyxin in fermentation liquors and concentrates has resulted in useful methods for the assay of polymyxin in blood and urine. The method to be described is rapid, simple and relatively accurate and has been useful in studies on the absorption and ex-

* Grateful acknowledgment is made to Miss M. E. Schlosser and Mrs. Beverly Gordon for valuable assistance.

[1] Stansly, P. G., and Schlosser, M. E., *J. Bact.*, 1947, **54**, 585.