experiments in which the inhibitor was added to the blood plasma and not to be supernatant fluid of the flask cultures the coagulation of the plasma was found to be considerably delayed or completely prevented, even when large amounts of embryo juice was added. These facts suggest that the inhibitor acts on the system involved in the coagulation of the blood plasma as well as on the proteolytic enzymes of the surface of the cells, the desmo-enzymes.

Recently Overman and Wright (*5*) have observed that the trypsin inhibitor decreases the thromboplastic activity of the blood. The study of the effect of the soybean inhibitor on the growth of the cells must therefore be limited to such concentrations of inhibitor as will still allow coagulation of the plasma medium to take place. When the inhibitor, instead of being added to the plasma directly, is introduced into the supernatant fluid of the culture medium, the growth-inhibiting effect is relatively less pronounced.

The figures below illustrate the relative growth inhibition of fibroblasts as a function of increasing amounts of a rather dilute solution of the inhibitor which has been *introduced into the plasma medium* before clotting:

0.17% inhibitor solution	$Q = \frac{\text{Control}}{\text{experiment}}$[1]
0.1 ml	1.01
0.2 "	1.43
0.3 "	2.24
0.35% inhibitor 0.2 ml	2.22

Thus when more than 0.3 ml of a 0.17% solution of the inhibitor is added to the plasma clot, no further suppression of the growth takes place. This seems to indicate that the proteolytic enzymes are completely inhibited by that concentration.

The following results show the effect of a 0.7% inhibitor *added to the fluid phase of the culture medium*:

0.7% inhibitor solution	$Q = \frac{\text{Control}}{\text{experiment}}$
0.25 ml	1.44
0.30 "	1.20
0.35 "	1.18

To establish a certain degree of growth inhibition of tissue cells in a Carrel flask the concentration of inhibitor has to be four times that necessary to obtain the same degree of inhibition when the inhibitor is introduced in the solid phase of the plasma clot.

Two main facts have been observed in this investigation. The soybean inhibitor has been shown to be an important tool in the tissue cultivation technique. An addition of small amounts of inhibitor to the plasma medium stabilizes the clot and makes it possible to cultivate on homologous media tissues with strong inherent proteolytic activity. Thus it is now possible to cultivate malignant cells indefinitely and to make quantitative estimations of the rate of their growth. It will also be possible now, we believe, to study differentiation phenomena *in vitro* by means of suppressing the growth of the cells and slowing down their migration rate.

[1] The tissue area increase is measured by means of a planimeter and expressed in square centimeters, the area being projected with the aid of an Edinger projector enlarging 20 times. Q is a measure for the inhibiting effect.

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Occurrence of Vitamin B_c Conjugase in Human Plasma

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The occurrence in yeast extract of an antianemic principle, different from pteroylglutamic acid, but releasing that substance after acid or enzymatic hydrolysis, was first demonstrated by Binkley, *et al.* (*1*). Pfiffner (*2*) isolated this compound and named it temporarily vitamin B_c conjugate.

In 1947 Schweigert and Pearson (*3*) studied the folic acid content of blood plasma and corpuscles in Mammalia and fowl, before and after treatment with an enzyme, takadiastase, releasing folic acid from its microbiologically inactive forms. They could find only minute amounts of free folic acid, but it seemed from their experiments that vitamin B_c conjugate was present in significant amounts.

TABLE 1

Folic Acid Released after 2-hr Incubation at 37° C of a Mixture of Human Plasma and Takadiastase*

Plasma ml	Takadiastase mg	B_c released mμg
0.5	20	25
0.5	20 (boiled)	25
0.5 (boiled)	20	2
none	20	1.6
0.5	none	0

* Microbiological determination with *Str. faecalis*.

A careful study of the interaction between takadiastase and plasma, both in the native state and after boiling for 10 min, has led to opposite results (*4*). The plasma is deprived of B_c conjugate, but it contains a thermolabile enzyme, which releases folic acid from a conjugate. The role of takadiastase in this interaction is that of a substrate containing vitamin B_c conjugate in significant amounts. Folic acid is set free, see Table 1, after incu-

bation with plasma for 2 hr at 37° C,[1] the pH of the digestion mixture being 4.5.

Striking results were obtained with a crude extract of baker's yeast (containing 3.7 μg B_c per cc in conjugated form) from which 0.2 cc plasma liberated 0.7 μg folic acid in 1 hr at 37° C.

Additional evidence of the enzymatic role of plasma is offered by the use, as substrate, of pure crystalline vitamin B_c conjugate,[2] which gives identical results.

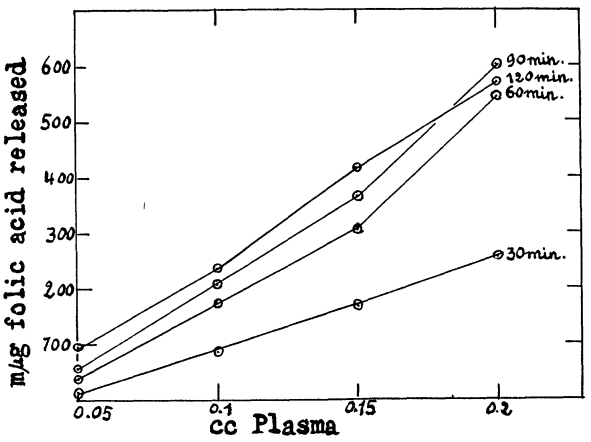

FIG. 1. Liberation of folic acid after various incubation periods at 37° C of 0.5 cc yeast extract + human plasma + 5 cc sodium acetate m/10 at pH 4.5 + water (total volume = 10 cc).

The B_c release as a function of plasma concentration for a constant time of incubation is represented in Fig. 1. There appears to be a linear relationship for plasma concentrations, ranging from 0.05 cc to 0.15 cc.

The study of human plasma conjugase activity in normal and pathological conditions has given the following results: under standard conditions, viz., with incubation at 37° C and pH 4.5, a mixture of 0.15 cc heparinized plasma and 0.5 cc yeast extract made up to a volume of 10 cc, liberated 0.5 μg to 1.5 μg folic acid per cc plasma in 90 min, with a maximum frequency of 0.8 μg to 1.0 μg. No significant variation of conjugase activity could be detected in pernicious anemia or in other pathological conditions, with the exception of asystolic patients, where the amount of conjugase per cc plasma was sometimes considerably decreased and attained values of 0.2 μg to 0.7 μg folic acid.

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[1] In a recent communication published while this article was in press, Simpson and Schweigert came to similar conclusions. (*Arch of Biochem.,* 1949, **20**, 32.)

[2] Crystalline vitamin B_c conjugate used in this experiment was supplied by Parke, Davis & Co. Laboratories, Detroit.

The Effects of Choline and Methionine on Phospholipide Formation in Patients with Liver Disease as Measured by Radioactive Phosphorus[1]

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In the past ten years the importance of nutritional factors in the pathogenesis and prophylaxis of experimentally induced cirrhosis in animals has resulted in a modification of the therapeutic regime in human patients. Of special interest has been the use of a diet high in protein and the administration of vitamin supplements, liver extracts, and lipotropic factors, such as methionine and choline. The efficacy of the above regimen is reflected in the lower mortality figures now being reported in the treatment of advanced instances of cirrhosis (*3*). In general this improvement is ascribed in part to a regenerative action on the liver tissue and in part to the lipotropic action of methionine and choline, resulting in the removal of the fat accumulated in the liver. The latter effect has been interpreted as being due to an increased formation of phospholipides.

In experimental animals with dietary fatty liver it has been shown, by the use of radioactive phosphorus as an indicator, that the administration of choline (*2*) or methionine (*8*) causes an increase in the rate of the synthesis of phospholipides in the liver. Since the plasma phospholipides are synthesized almost entirely in the liver (*5*), it seems that changes in the rate of formation of liver phospholipides would be reflected by corresponding changes in the amounts of newly formed phospholipides in the plasma. Indeed, in dogs on a diet low in protein and high in fat (*7*) a stimulatory effect by choline on the turnover of phospholipides in plasma has been observed.

We now have in progress a systematic study of the turnover of plasma phospholipides in normal human beings and in patients with various diseases. Some preliminary findings have been reported in a summarized form (*4*) and more extensive data will be published elsewhere. We are here reporting only some results obtained in two cirrhotic patients who exhibited a marked response to a single large dose of methionine or choline. These results suggest certain possibilities which might be of considerable interest in the interpretation and treatment of this disease.

One 31-year-old white male (O.A.) and one 36-year-old

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