Folic Acid Conjugase in Normal Human Plasma and in the Plasma of Patients with Tropical Sprue

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The occurrence in yeast extract of an antianemic material which was not pteroylglutamic acid, but which released pteroylglutamic acid after enzymatic hydrolysis, was first demonstrated by Binkley et al.¹ Later, Wolff et al.² found that plasma contains a thermodurable enzyme that releases folic acid from a folic conjugate.

Toennies et al.³ showed that the bulk of folic acid active compounds in the blood measured with Lactobacillus casei do not exist as such, but result in vitro from the interaction of precursors in red blood cells with a factor in plasma. These workers showed that the red blood cells contain the conjugate and confirmed the finding of Wolff that plasma contains a conjugase. They suggested that the folic acid conjugate is a storage form of folic acid active material which is sequestered in the red cells and becomes accessible to the action of plasma conjugase as the red cells undergo normal destruction.

By chromatographic separation with DEAE resin, Toennies and Phillips,⁴ Usdin⁵ and Noronha and Aboobaker⁶ have shown that the conjugase releases from the red cell folic conjugate not only pure pteroylglutamic acid, but also a large number of other folic acid active compounds, including methyl folates.

Previous studies⁷ have shown that the diet of Puerto Ricans with low income is not deficient in folic acid activity and that there is no deficiency of intestinal folic acid conjugase in tropical sprue.

The present study was undertaken to determine whether the low whole blood folic acid activity present in untreated patients with sprue⁸ was due to deficiency of the conjugase in the plasma or to a reduction in the amount of conjugate stored in the red cells.

EXPERIMENTAL PROCEDURE

Plasma Conjugase Activity for Pteroyl Polyglutamates of Yeast

Collection of Blood. Ten milliliters of blood were collected in a syringe containing 100 μg of heparin. The blood was poured into a tube containing 0.1 ml of 2.3 N NaOH and 41 mg ascorbic acid. The tube was inverted to mix the solution well. The blood was centrifuged for 30 minutes in a refrigerated centrifuge at 2°C. The plasma was removed and stored in a sterile tube in the freezer until needed.

Preparation of Yeast Solution. Ten grams of "Bacto" Yeast Extract (Difco Certified) were made up to 100 ml with distilled water. The solution was autoclaved at a pressure of 15 pounds for 15 minutes and kept frozen until needed.

Enzyme Estimation. It was found that incubation of normal human plasma with a substrate of yeast yields products which support both L. casei and Strep. fecalis to approximately the same extent (Fig. 1). Because nearly maximal activation occurred with 0.1 ml of plasma in the incubation mixture, this amount was chosen in the tests for the

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estimation of conjugase activity in plasma from different subjects; minor reductions in activity might be detected at this range but missed in the flat range of the curve.

The enzyme content of plasma was estimated by the method of Wolff, Drouet and Karlin modified as follows:

Tubes contained 1 ml. of yeast solution equivalent to 0.1 gm. of dry material, which was mixed with 4 ml. of distilled water. Five milliliters of acetate buffer, pH 4.3, were then added to each tube. These solutions were autoclaved at a pressure of 7 pounds for 15 minutes. After cooling to room temperature 0.1 ml. of plasma was added to each tube under sterile conditions and incubated for 90 minutes at 37°C. The samples were autoclaved again; and after cooling to room temperature were centrifuged for 5 minutes. One milliliter of each supernatant was diluted to 20 ml. with distilled water and different aliquots were used for the folic acid activity assay with Strep. fecalis.

**Procedure for Measuring Plasma Folic Acid Conjugase Using Red Cell Substrates**

The following procedure was used for measuring plasma folic acid conjugase.

**Collection of Blood.** The same procedure as for whole blood folic acid assay is used for the collection of the blood.

**Preparation of the Plasma and the Red Blood Cells.** Blood was centrifuged in a refrigerated centrifuge at 2°C. for 30 minutes, plasma was removed and kept in a refrigerator until needed. Red blood cells were washed three times in a centrifuge, with three times their volume of 0.5 M potassium ascorbate in 0.14 M saline.

**Hemolysis.** Two milliliters of washed red cells were mixed with 2 ml. of 0.05 N NH₄OH and allowed to stand at room temperature for 60 minutes.

**Dilutions.** All dilutions were made with 0.5 per cent potassium ascorbate in 0.05 M phosphate buffer.

**Assay.** Two milliliters of red cell hemolysate were diluted to 100 ml.

This solution was used as a source of red cell folate. One milliliter of plasma was diluted to 100 ml. This solution was used as a source of plasma folate or enzyme.

Two milliliters of red cell hemolysate plus 1 ml. of plasma were diluted to 100 ml. (here, red cells could be mixed with plasma from different donors). The samples were incubated, autoclaved and filtered as for whole blood folic acid. After filtering, a 1 to 1 dilution was made and the samples were assayed for folic acid activity using L. casei as the organism.

**RESULTS AND COMMENTS**

The ability of plasma from various sources to activate the conjugated folic acid of yeast is presented in Table 1. The folic acid content of yeast is thought to exist as pteroyl heptaglutamate, which is not active in supporting the growth of S. fecalis unless split to the monoglutamate form. The activation of yeast folate by plasma (Table 1) is evidence for the existence of normal amounts of conjugase in the plasma of the patients with sprue. Additional evidence that the plasma activating factor is enzymatic was derived from the observations that activity is destroyed by heating to 50°C. for 5 minutes or by boiling.

**Table 1**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Range</th>
<th>Mean ± S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>378–503</td>
<td>442 ± 41</td>
</tr>
<tr>
<td>Treated sprue</td>
<td>11</td>
<td>263–563</td>
<td>431 ± 88</td>
</tr>
<tr>
<td>Untreated sprue</td>
<td>8</td>
<td>325–755</td>
<td>443 ± 92</td>
</tr>
</tbody>
</table>

**Fig. 1.** Folate activity after incubating yeast extract with plasma. Open circles represent L. casei activity, closed circles Strep. fecalis.
Table II presents the results of a series of experiments designed to test the ability of plasma from three anemic patients with sprue to activate the latent folate activity in erythrocytes from four different donors. In this procedure, red cells are separated from their plasma, washed and recombined with either the original plasma or plasma from a test subject. Because of these manipulations, it is not always possible to obtain complete recovery of folate or recombination as in the whole blood procedure which provides for hemolysis without separation of cells and plasma. The results demonstrate that plasma from anemic patients with sprue is capable of releasing or activating folate from the red cells to the same extent as the original plasma. Similar results were observed using plasma from two treated subjects with sprue who were not anemic. The results are in accord with the observations of others that the folate activity is an intrinsic property of the red cell. However, these data indicate further that the activating enzyme systems of the plasma are normal in the anemic patient with tropical sprue. In this light, the low whole blood folate activity for L. casei described in cases of tropical sprue must be due to a reduction in the amount of folic acid stored in the red cells, and not to failure of activation.

**SUMMARY**

No abnormality could be detected in the activating enzyme of plasma from patients with sprue as compared with normal plasma, using a substrate of hemolyzed red blood cells. Likewise, no abnormality was observed in conjugase activity of plasma from patients with sprue as judged by its ability to activate a yeast extract for the growth support of Strep. casei.

**REFERENCES**


