# Plasma Antithrombin Alterations Following Oral Papain. (26669)

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We have recently found that oral administration of papain* results in therapeutic activity in man of the same character as that resulting from buccal administration(1). Since this therapeutic efficacy was not accompanied by the untoward side-effects sometimes seen with intravenous enzyme therapy, it appeared that the enzyme was probably not absorbed, but that a plasminogen activator, released by the action of papain in the upper gastro-intestinal tract and absorbed from that site, could be implicated. The lack of systemic absorption of papain is further suggested by the fact that the oral mouse $LD_{50}$ of this enzyme is in the neighborhood of 15.000 mg kg(2).

Roche e Silva, Andrade, and Teixeira(3) have shown that intravenous infusion of proteolytic enzymes into animals results in the appearance of an antithrombin-like component in blood plasma. Niewiarowski and Kowalski confirmed this, using streptokinase and plasmin in the cat(4). The latter authors have described the anticoagulant as a high molecular weight product of fibrinogen digestion, which can be destroyed by further proteolysis(4). On the basis of this and their own work(5), Wallen and Bergstrom postulated that the protein fragments formed during fibrinogenolysis, and which are substrates for thrombin, can act as competitive inhibitors in the proteolytic reaction of thrombin on fibrinogen, thus contributing to antithrombin activity.

With this background, the present study has been carried out to ascertain whether or not oral papain administration can produce *in vitro* changes which will signal its activity.

This work does not purport to show that oral papain alters *in vivo* clotting mechanism. In fact, in clinical application, no signs referable to changes in clotting mechanism have been observed with oral papain.

*Methods.* Fifteen hospitalized patients who were recovering from recent fractures served as subjects. Blood specimens were collected with 0.4 ml of 5% potassium oxalate for 5 ml of blood.

For assay of plasma antithrombin, the method of Fletcher, Alkjaersig, and Sherry (6) was employed. This test measures clotting time of a patient's plasma following *in vitro* addition of a standardized thrombin† solution. Euglobulin clot lysis determinations were made according to von Kaulla and Schultz(7).

As a low dose of papain, 10 mg were administered every 4 hours by mouth for 48 hours. As a high dose, 200 mg were administered every hour for 4 hours to the same patients. One control clotting time estimation served for both administrations in each patient. Papain was administered as 10 mg uncoated tablets, since enteric coated papain has been previously found to be clinically relatively ineffective(1).

*Results.* Table I clearly demonstrates that the small dose of papain caused a marked increase in plasma antithrombin activity. The high dose resulted in an equally consistent reduction in plasma antithrombin activity. Both sets of results differ from control levels in a statistically highly significant manner. The reduction in plasma thrombin clotting time below that of the control may be explained by the terminal hydrolysis not only of antithrombin formed from fibrinogen but also of the normally circulating antithrombin present in plasma. It is of further interest that patients with reduced thrombin clotting times showed marked acceleration in euglobulin clot lysis time. This finding implies that under the test conditions reduced thrombin clotting time seems to be related to enhanced proteolysis (Table II).

* Papase—Warner-Chilcott Laboratories.

† Topical Thrombin, Parke-Davis.

TABLE I. Plasma Thrombin Clotting Time (Seconds).

| Reaction mixture: .1 ml plasma + .1 ml thrombin | | | |
|---|---|---|---|
| Patient | Control | After 4 hourly doses of 200 mg each | After 12 doses of 10 mg q.4.h. |
| R.B. | 17 | 10 | 45 |
| D.L. | 16 | 10 | 35 |
| S.K. | 18 | 9 | 35 |
| T.G. | 17 | 12 | 40 |
| R.V. | 17 | 10 | >600 |
| D.L. | 18 | 11 | 24 |
| Y.F. | 17 | 10 | >600 |
| E.C. | 16 | 10 | 27 |
| M.V. | 17 | 9 | 24 |
| S.A. | 17 | 9 | >600 |
| L.D. | 17 | 12 | 30 |
| S.L. | 16 | 11 | 25 |
| R.W. | 19 | 12 | 27 |
| H.J. | 16 | 10 | 26 |
| D.S. | 17 | 10 | 31 |
| Means | 17 | 10 | 25–37* |
| | | p = <.01 | p = <.01† |

* Calculated true mean is between 25–37 at p = .99.

† p = <.01 when values >600 were excluded. p = .02–.05 when values >600 were included. The paradoxical decrease in significance was due to the large increase of stand. error when the >600 figures were added.

TABLE II. Euglobulin Clot Lysis Time after Oral Papain (Hours).

| Dose & frequency | Control | Treated |
|---|---|---|
| 200 mg/hr for 4 hr | 6 | 1 |
| *Idem* | 7 | 3 |
| " | 9 | 2 |
| " | 9 | 3 |
| " | 8 | 2 |
| " | 6 | 2 |
| " | 6 | 3.5 |
| " | 7 | 3 |
| " | 7 | 2 |
| " | 6 | 2 |
| " | 8 | 1 |
| " | 9 | 3 |
| " | 8 | 3 |
| " | 7.9 | 2 |
| " | 6 | 1 |
| Mean | 7.3 (6.7–7.9)* | 2.2 (1.8–2.6)* |

* 95% confidence limits p = <0.01.

More recent studies appear to show that by increasing the quantity of fibrinogen (plasma) in the thrombin-fibrinogen reaction mixture, a prolonged thrombin clotting time is produced with high papain dosage as well as with low doses. This is compatible with the postulate of Niewiarowski and Kowalski (4) that thrombin inhibition is regulated by concentration of fibrinogen-derived polypeptides in this system.

*Summary.* 1. Administration of small oral doses of papain to human patients results in production of a plasma antithrombin agent demonstrated by prolonged thrombin clotting time *in vitro.* 2. Administration of large oral doses of papain to human patients results in a decrease in thrombin clotting time, and an acceleration of euglobulin clot lysis time. 3. These experiments demonstrate that orally administered papain produces a systemic effect, although in all probability it is not itself absorbed.

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