TABLE 1

CLOTTING TIME OF FIBRINOGEN AFTER INCUBATION WITH EDTA-DISODIUM

Incubation time (min) at 37° C	Concentration of EDTA in mg/ml		
	0.10	0.25	0.50
	Clotting time (sec)		
1	21	20	21
5	21	36	44
10	23	58	67
20	22	85	83
40	—	99	99

tion. The clot formed in the presence of calcium was found to be insoluble in urea. The work of Lóránd (*3*) and Ferry, Miller, and Shulman (*4*) further emphasizes the role of calcium in altering the rigidity of the clot.

Our experiments furnish additional evidence of the important role of calcium in the clotting of fibrinogen and show that calcium has an accelerating effect on this process either alone or as a component of a hitherto unidentified factor. Our experiments also point out the possibility that in the speed of clotting of blood the described role of calcium might be involved.

TABLE 2

WEIGHT OF FIBRIN CLOTS FORMED IN THE PRESENCE OF EDTA-DISODIUM

	Concentration of EDTA-disodium in mg/ml				
	2.0	1.0	0.5	0.25	0
	Weight of fibrin clot in mg/cc				
Without calcium	6.0	5.9	6.4	6.2	6.2
With calcium equivalent to EDTA	5.9	6.2	6.0	6.1	6.3

The fibrinogen solutions used in our experiments contained 2–6 mg/ml clottable protein and 5% imidazole buffer of pH 7.24 and were prepared from bovine fibrinogen; the thrombin solutions were prepared from tropical thrombin. The contents of one vial were dissolved in 25 ml glycerol and 25 ml saline. We diluted this stock solution to about 20–25 u/ml for our experiments.

TABLE 3

CLOTTING TIME OF FIBRINOGEN INCUBATED WITH EDTA-DISODIUM CLOTTED WITH NORMAL AND $CaCl_2$ CONTAINING THROMBIN

Concentration of EDTA in mg/ml	Clotting time (sec)		
	Thrombin (normal)	Thrombin with 0.004 *M* $CaCl_2$	Thrombin with 0.008 *M* $CaCl_2$
0.0	19	18	18
0.1	48	22	19
0.3	200	31	20
0.5	210	35	25
0.7	200	150	28
1.0	215	145	85

The EDTA-disodium salt was prepared from ethylenediamine tetraacetic acid (DPi, Eastman Kodak, Rochester, N. Y.). The clotting times were tested by applying the somewhat modified method recently described by Laki (*5*). We used spot test plates made of Plexiglass put in a constant temperature water bath of 37° C. To 0.2 ml fibrinogen 0.1 ml thrombin was added, and the clotting time checked by a stop watch. The fibrinogen determinations were carried out as described by Laki (*6*).

References

1. DYCKERHOFF, H., MARX, R., and LUDWIG, B. *Z. ges. exptl. Med.*, **110**, 412 (1942).
2. LAKI, K., and LÓRÁND, L. *Science*, **108**, 280 (1948).
3. LÓRÁND, L. *Nature*, **166**, 694 (1950).
4. FERRY, J. D., MILLER, M., and SHULMAN, S. *Arch. Biochem. Biophys.*, **34**, 424 (1951).
5. LAKI, K. *Ibid.*, **32**, 317 (1951).
6. ———. *Studies Inst. Med. Chem. Univ. Szeged.*, **2**, 27 (1942).

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Enzymatic Cytolysis of Epithelium by Filtrates of Feces from Patients with Ulcerative Colitis[1]

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Filtrates of feces from patients with ulcerative colitis contain a factor which has a disintegrating effect upon human epithelium. Fixed sections of human skin and bowel incubated with this factor display loss of intercellular bridges of epidermal cells (acantholysis) and loss of cytoplasmic substance of cells of both skin and intestinal mucosa. To date, this factor has been found only in feces from patients with ulcerative colitis.

Preparation of the active filtrate and the method for producing cytolysis are as follows: 100 g of fresh feces from patients with ulcerative colitis are well blended with 300 ml ice-cold *M*/10 phosphate buffer, ph 7.4, and centrifuged. The supernatant fluid is passed through a Seitz filter and collected in an ice-cold flask. The filtrate is then applied to formalin-fixed sections of normal human skin or bowel at 37° C for 30–120 min. The sections are washed in distilled water and stained with hematoxylin and eosin. The active factor is quite labile; it is destroyed in a few hours while standing at room temperature, more slowly at 4° C (1–4 days), but is preserved for over a month in the frozen state.

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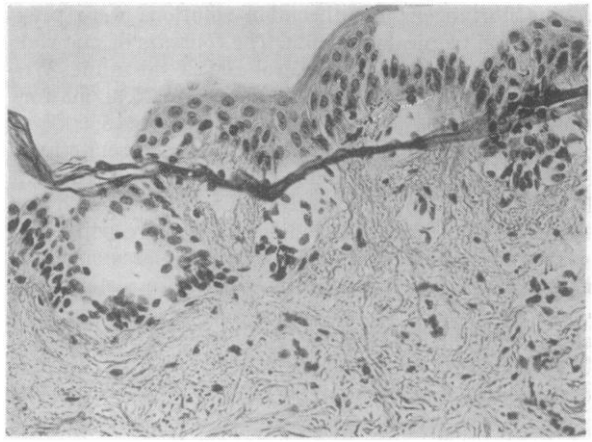

FIG. 1. Human skin after cytolysis by fecal filtrate showing *in vitro* "blister" formation and separation of epidermal cells. The separated horny layer lies across the section. × 225.

In order of appearance, the following changes are observed in and between the epidermal cells (Fig. 1): (1) disintegration of intercellular bridges; (2) loss of eosinophilia of cytoplasm; (3) rents in the epidermis, with "blister" formation; (4) detachment of cells from each other; and (5) swelling and loss of basophilism of nuclei.

The epithelial cells of large bowel lose most of their cytoplasm, leaving relatively normal nuclei (Fig. 2).

The cytolytic factor is most active in *M*/10 phosphate buffer in the pH range 7.2 to 7.8; it is inactive in *M*/10 phosphate buffer below pH 6.4. The factor is destroyed by heating for 10 min at 56° C and is nondialyzable. It is inactivated by human serum, CN^-, Hg^{++}, and Ag^+. Also, suramin sodium and Treburon,[3] both sulfated compounds, are potent inhibitors of this factor.

On the basis of these observations it is assumed that this cytolysis is an enzymatic process. It is not yet known whether the cytolytic factor derives from microorganisms, tissue breakdown, or digestive enzymes.

The following proteolytic and mucolytic enzymes were added to heat-inactivated ulcerative colitis fecal filtrates and incubated with formalin-fixed sections: trypsin (crystalline),[4] chymotrypsin (crystalline),[4] papain, fibrinolysin, lysozyme, ribonuclease, desoxy-

[3] Trade-mark of sodium salt of sulfated polygalacturonic acid methyl ester methyl glycoside. Supplied by Hoffman-LaRoche, Inc., Nutley, N. J.

[4] Worthington Biochemical Sales Co., Freehold, N. J.

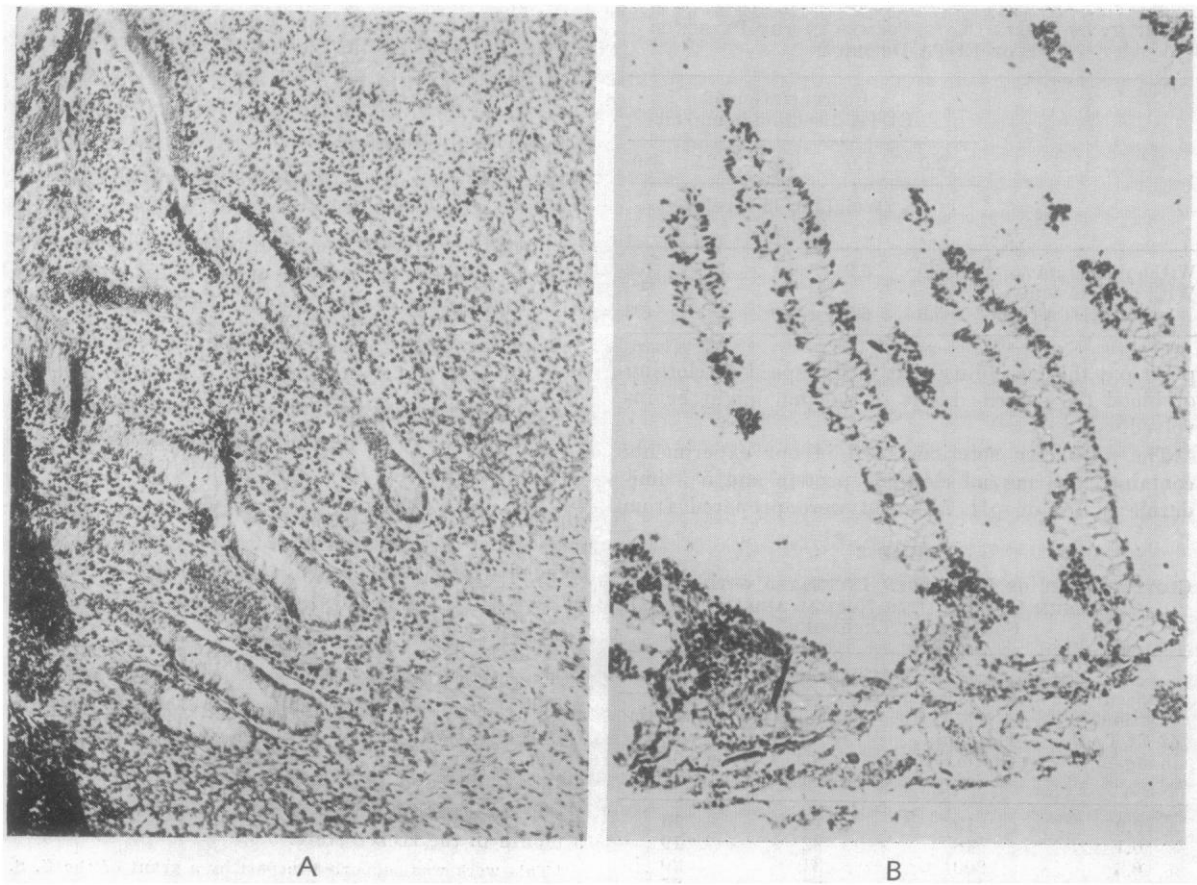

FIG. 2 *A*, human intestinal mucosa after incubation with heat-inactivated fecal filtrate. × 100. *B*, section from same block as *A*. Human intestinal mucosa after incubation with active fecal filtrate showing almost complete cytolysis of epithelium. × 100.

ribonuclease,[4] pectinol,[5] and *Cl. Welchii* type A filtrate.[6] None of these produced acantholysis or separation of epidermal cells. Trypsin in high concentration (100 mg/cc), caused a minimal loss of eosinophilia in the cytoplasm of bowel epithelium.

All these findings refer only to the effect on formalin-fixed sections. After fixation in formalin, tissues were dehydrated, cleared, and embedded in paraffin in the usual laboratory routine. Sections were rehydrated and washed in water before filtrates were applied. If acetone-fixed sections are used as test objects, it is found that the stool filtrates contain a trypsinlike cytolytic factor which, however, clearly differs from the first factor. It is much more stable, is not inhibited by Treburon or suramin sodium, and does not break down intestinal epithelium.

Mild *in vitro* cytolysis resulting from treatment with fecal filtrates yields histologic pictures very similar to that of pemphigus vulgaris. In this fatal, blister-forming disease, the primary histologic change is destruction of intercellular bridges, blister formation being secondary to the acantholysis (*1–4*). Also, some phases of the cytolysis by fecal filtrates are morphologically similar to those described in experimental burns (*5*). It might be worth while to mention that sulfated compounds such as naphuride and Treburon, which inhibit the cytolysis *in vitro,* give promise of being effective in the symptomatic treatment of pemphigus.

References

1. CIVATTE, A. *Ann. dermatol. syphilig.*, **3**, 1 (1943).
2. TZANCK, A. *Ibid.*, **8**, 205 (1948).
3. BLANK, H. Read before 12th annual meeting, Soc. Investigative Dermatol., Atlantic City, N. J. (June 7, 1951.)
4. LEVER, W. F. *Arch. Dermatol. and Syphilol.*, **64**, 727 (1951).
5. LEACH, E. A., PETERS, R. A., and ROSSITER, R. J. *Quart. J. Exptl. Physiol.*, **32**, 67 (1943).

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[5] Supplied by Rohm & Haas Co., Philadelphia, Pa.
[6] Supplied by Lederle Laboratories, Pearl River, N. Y.

Effect of *p*-Chlorophenoxyacetic Acid (ClPA) and 3-Indolacetic Acid (IA) on Certain Dehydrogenase Systems of the Tomato Fruit, *L. esculentum*[1]

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The plant growth regulator *p*-chlorophenoxyacetic acid (ClPA) applied in aqueous solution to flower clusters of the tomato increases the percentage of fruit set and stimulates the development of the tomato fruit. Hsiang (*1*) has shown that stimulation of growth in the orchid flower is usually preceded by an increase in catalase activity and oxygen uptake. The effect of auxins in stimulating growth and respiration in plants has been attributed to the protection of various dehydrogenase enzyme systems against some natural inhibitor (*2*). The interrelationships between the dehydrogenase enzymes, respiration, and growth have been pointed out by Commoner and Thimann in studies on auxin-treated *Avena* coleoptiles (*3*), and by Berger and Avery (*4–6*). A study of the dehydrogenase enzymes of the tomato fruit may provide information on the mechanism of growth stimulation.

It has been pointed out by several workers that hormone treatment as an aid to fruit set of greenhouse-grown tomatoes is most effective when applied during the post-pollination period (*7, 8*). In the present investigation treatment of hand-pollinated fruits was made 6 days after pollination. The optimum concentration of ClPA when used as a flower spray was found by Murneek and co-workers (*7, 8*) to vary between 5 and 25 ppm, depending on weather conditions. In order to insure the same age, individual fruits of the cluster were treated by dipping in aqueous solutions of ClPA. When this method was used the concentrations of ClPA had to be increased approximately forty times that used with the flower spraying method. Responses in fruit set and development were obtained with 200, 1000, and 2000 ppm ClPA similar to those obtained using flower sprays of 5, 25, and 50 ppm.

TABLE 1

MICROGRAMS OF TRIPHENYLFORMAZAN PRODUCED BY DEHYDROGENASE SYSTEMS IN 1 ML TOMATO HOMOGENATE INCUBATED 20 HR AT 38° C FROM FRUITS TREATED WITH 0, 200, 1000, AND 2000 PPM *p*-CHLOROPHENOXYACETIC ACID (ClPA). TESTED 28 DAYS AFTER TREATMENT

Substrate	Control	Concentration ClPA		
		200 ppm	1000 ppm	2000 ppm
	Triphenylformazan (in μg)			
Glutamate	406	340	260	123
Succinate	280	215	191	173
Fumarate	197	360	444	127
Malate	145	127	460	150

Dehydrogenase activity was measured by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) in the presence of various substrates. The method used was that of Kun and Abood (*9*) as modified by Isenberg *et al.* (*10*) for plant tissue. Ten per cent fresh tissue homogenates were prepared, using a glass homogenizer. The principal substrates were 0.2 *M* solutions of sodium succinate, sodium fumarate, sodium malate, and sodium glutamate with pH adjusted to 7.4. Other substrates were included in the study, but the above four gave the most consistent and reproducible results and will be the only ones reported on here. The reaction tubes contained 0.5 ml of 0.2 *M* monopotassium phosphate buffer pH 7.4, 0.5 ml substrate, 1 ml 10% tissue homogenate, and 1 ml 0.1%

[1] Journal Series Paper No. 1295, approved by the director of the Missouri Agricultural Experiment Station.