FIG. 2. Larger end of the egg, with parts of the crust removed and others showing its two layers. The fragment turned over shows the smooth undersurface of the dense inner layer.

centrifuged down, it had a slightly green color and the supernatant solution was faintly pink. It gave a strong red fluorescence with ultraviolet light, and spectrophotometric analysis with a Beckman quartz spectrophotometer showed that the absorption bands were located at 600, 557, and 407 mμ. These figures agree with those given for protoporphyrin by Lemberg and Legge (*4*). It was concluded that the brown pigment was that substance, probably contaminated with protein and calcium carbonate.

The surface area of the egg, as calculated from four different formulas (*2*), was estimated to be between 78 and 85 cm². Taking the average area of 82.9 cm² and the average weight of the air-dried crust as 144.8 mg/cm² (av of two determinations), it was calculated that the total amount of the encrusting material was a little over 12 g. Although this figure is only an estimate, it seems probable that such an egg would have been considerably more useful to Fischer and Kögl (*3*) than the 300 gulls' eggs from the shells of which they managed to extract for their analyses 30 mg of the crystalline dimethylester of "oöporphyrin."

This egg must have been retained for several weeks in the uterus of the hen's oviduct. This is attested not only by its amazing accumulation of protoporphyrin, but also by the caseous state of the contents. Eggs with fully formed shells that are not laid when they should be are sometimes returned up the oviduct and dropped into the body cavity. Others return until they meet an outbound yolk and then return to the uterus with that yolk, the whole being eventually laid as a double egg, or *ovum in ovo*. The unduly thick shells of some of these enclosed eggs indicate that they have been held overlong in the uterus (*5*). In the present case, the uterus of the unfortunate hen was unable either to expel the egg or to send it back whence it had come. It is interesting to note that, in this abnormal situation, the deposition of shell was eventually stopped, or greatly reduced, whereas the deposition of shell pigment continued.

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The Accelerating Effect of Calcium on the Fibrinogen-Fibrin Transformation[1]

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The anticoagulant effect of ethylenediamine tetraacetic acid was first investigated by Dyckerhoff *et al.* (*1*). This substance by its powerful deionizing propperty binds the calcium ions involved in blood coagulation. Investigating the action of ethylenediamine tetraacetic acid, or EDTA, on the coagulant action of snake venom on plasma and fibrinogen (results of these experiments will be published in another paper), we found that the action of EDTA is not only involved in the first but also in the second phase of blood coagulation; i.e., this substance hinders the fibrinogen-fibrin transition. This impediment is related to the time of incubation of the fibrinogen solution with EDTA.

Table 1 shows how the clotting time increases when fibrinogen is incubated with different amounts of EDTA for various lengths of time.

This inhibiting effect is not due to a possible alteration of pH by EDTA, since no significant change in pH was found when fibrinogen was incubated with EDTA in concentrations varying between 0.1 and 1.0 mg/ml. The inhibiting effect must be a direct one on the fibrinogen-fibrin transformation, since no prothrombin contamination was detected in the fibrinogen preparations.

Neither can the increase in clotting time be due to destruction of fibrinogen, for, as Table 2 shows, the amount of clot is the same irrespective of the magnitude of inhibition.

Since EDTA is a powerful binder of calcium it was concluded that the inhibition is due to the removal of calcium. The experiments in Table 3 show that the inhibiting effect of EDTA can be completely reversed by the addition of calcium, thus strongly indicating that the inhibiting effect is due to the binding of calcium.

It has been found by Laki and Lóránd (*2*) that calcium plays a part in the fibrinogen-fibrin transi-

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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*).

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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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