in alkaline media and yellow in acid media. The color change with the purple oil, serving as an indicator in aqueous acidimetric titrations, occurs in the pH range 3.8 to 4.4; the color is yellow at pH 3.8, pink at 4.0, and purple at pH 4.4. The color transition with the blue-gray oil, in methyl alcohol, occurs at an apparent pH of 8.8 to 9.2. The ultraviolet absorption spectra for both forms of each of these indicators, in methyl alcohol solution, are recorded in Fig. 2.

The orange and yellow oils and the yellow forms of the purple and blue-gray oils all show a green fluorescence in ultraviolet light.

Although proofs of structure of these products will be reported elsewhere, it may be stated here that the evidence is that the purple and blue-gray oils are hydroxy paraquinones, the chroman ring in the tocopherol molecule has been ruptured in the case of the purple oil, that the orange is an ortho quinone, and that, with the exception of the yellow product, which is the well-known tocoquinone, the production of the quinones involved the elimination of methyl groups on the aromatic ring of the tocopherol molecule.

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Manuscript received February 4, 1952.

An Egg Encrusted with Protoporphyrin

F. B. Hutt and J. B. Sumner[1]

Cornell University, Ithaca, New York

The egg shown in the accompanying illustrations was found by a farmer's wife in the oviduct of a hen being prepared for the table. Fortunately it was saved and eventually turned in to one of us for an explanation of its nature and origin. The voluminous literature on abnormal eggs was reviewed by Davaine (*1*) in 1860, and again recently by Romanoff and Romanoff (*2*), but neither in these compilations nor elsewhere have we been able to find any record of an egg like this one. A brief report therefore seems desirable.

When received, some three weeks after its discovery, the egg was completely covered with a rough, granular, dark purplish-brown material. The coating was smoothest around the middle and was rough and pebbled at both ends. The egg measured 6.54 × 4.69 cm and weighed 70 g. When shaken, it sounded like an addled one.

Inquiry revealed that it had been removed from the uterine region of the oviduct of a 4-year-old hen, a hybrid from the cross Barred Rock ♀ × Rhode Island Red ♂. The hen had been a good layer and was in good health when killed for the table about Oct. 1, 1951. Because of the age of the hen and the season of the year, one would expect the ovary to be inactive at that time. The owner reported that the only other eggs in the hen were small follicles, attached to the ovary. When freshly removed, the abnormal egg was more purple in color than it appeared after drying.

Contents of the egg were carefully blown out by A. A. Allen. On drilling the hole, nothing could be blown out until water was forced in through the blowpipe. The first material to come out was yellow and caseous. Eventually, by blowing in water and air alternately, the contents were removed. No bad odor was noticeable.

The soaking necessary to blow out the egg loosened the pigmented coat, which cracked in many places as it dried (Fig. 1). It became clear that under the dark encrustation there was a light-colored eggshell, calcareous in nature and apparently normal. Overlying it, the dark material formed a discrete crust composed of two layers (Fig. 2). The inner of these was dense, almost glassy, and about 1 mm thick. The outer layer was thinner and less dense. The average thickness of the crust, including both layers, was 1.6 mm.

FIG. 1. The encrusted egg, showing cracks that developed in the crust after it was blown and dried out. The white spot at the top shows the underlying shell where a fragment of the crust was removed.

Because the hen was derived from two breeds that normally lay brown-shelled eggs, it was suspected that the crust might be composed of protoporphyrin, the pigment causing the brown color of the shell in the eggs of many birds (*3*). Chemical tests by one of us (J. B. S.) verified this assumption. The amount of iron was only 0.007%, which showed that hemoglobin was not present, and hence that there was no blood in the crust. Fragments of the crust were finally dissolved in 25% HCl. When the insoluble material was

[1] We wish to thank Arley Bever for determining the location of the three absorption bands.

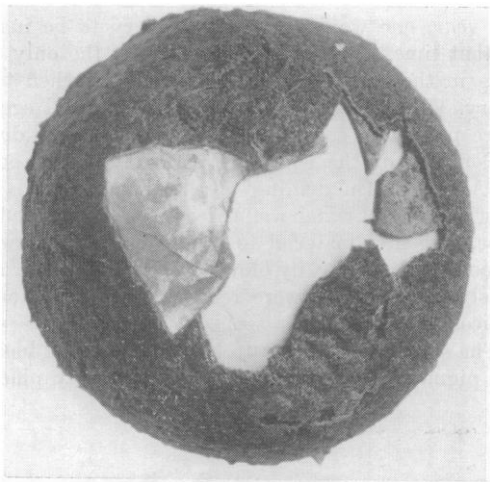

FIG. 2. Larger end of the egg, with parts of the crust removed and others showing its two layers. The fragment turned over shows the smooth undersurface of the dense inner layer.

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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Manuscript received February 4, 1952.

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

G. Rosenfeld and B. Jánszky[2]

Instituto Butantan and *Instituto Pinheiros, São Paulo, Brasil*

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-

[1] This study was supported by the Anastacio Paschoal and M. Pedro Franco Fellowship.

[2] The authors would like to thank Rocha e Silva (Instituto Biologico, São Paulo) for the EDTA sample, Armour & Co., Chicago, for the bovine fibrinogen, and Parke, Davis & Co. do Brasil for the thrombin used in the experiments.