tolerant animals injected with endotoxin is prompt and is still present at 4 hours after the injection. In tolerant rabbits the leukopenia is apparent immediately, but by 2 hours after injection the white cell count has returned to normal levels.

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Received May 15, 1961. P.S.E.B.M., 1961, v107.

Various Types of Calcinosis Induced by Egg Albumen or Yolk Following Sensitization with Dihydrotachysterol (DHT).* (26700)

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In rats pretreated with dihydrotachysterol (DHT), it is possible to induce intense local calcinosis anywhere on the skin surface by topical application of mechanical trauma(1) or subcutaneous injection of $FeCl^3$(2). The lesions so induced are somewhat reminiscent of the calcifying form of scleroderma (the "sclérodermie calcaire" of French dermatologists). Recently we found that subcutaneous injections of either egg white or egg yolk are likewise highly effective in eliciting cutaneous calcinosis, following DHT sensitization, although neither of these substances produces any significant local damage (*e.g.*, inflammation, hemorrhage or necrosis). It appears that the active principles of the egg —like $FeCl_3$—act somewhat like mordants, in that they prepare tissues for subsequent uptake of calcium salts. Even when administered intraperitoneally or intravenously, both egg white and egg yolk (unlike $FeCl_3$) are well tolerated by rats; hence, they enabled us to study the influence of systemically applied vital mordants for calcium upon DHT-sensitized animals.

Material and methods. Fifty female Holtzman rats, with a mean initial body weight of 97 g (range: 92-105 g), were subdivided into 5 equal groups. All animals were given 1 mg of DHT ("Calcamin," Wander), in 0.5 ml of corn oil, by stomach tube, once on the first day. The rats of *Group I* acted as controls and received no other treatment, while those of the remaining groups received solutions of either egg white or egg yolk, 24 hours after the DHT. The solutions were prepared by mixing the albumen or yolk of the eggs of domestic fowl with an equal amount of distilled water. The resulting thick fluids tend to form stringy precipitates upon standing and do not pass readily through ordinary filter paper; hence, they were filtered first through cotton and then through tissue paper ("Kleenex"®). The individual dose was invariably 5 ml, egg white being given intraperitoneally to *Group II* and intravenously to *Group III*, while egg yolk was administered intraperitoneally to *Group IV* and intravenously to *Group V*. The animals were kept on "Purina Fox Chow" and tap water throughout the experiments and all survivors were killed with chloroform after 4 days. At autopsy their organs were inspected with a stereoscopic loupe, representative specimens of various tissues being fixed in alcohol-formol for the subsequent histochemical demonstration of calcium with von Kóssa's silver nitrate technic.

Results. Five animals of the group receiving egg yolk intravenously died during the period of observation, but all the other rats supported these injections well, except that egg white produced the usual anaphylactoid reaction during the first few hours after its administration. Even this response was mild, since it is well known that large amounts of

* Supported by grants from Nat. Institutes of Health, U.S.P.H.S., Geigy Pharmaceuticals and the Gustavus and Louise Pfeiffer Research Foundation.

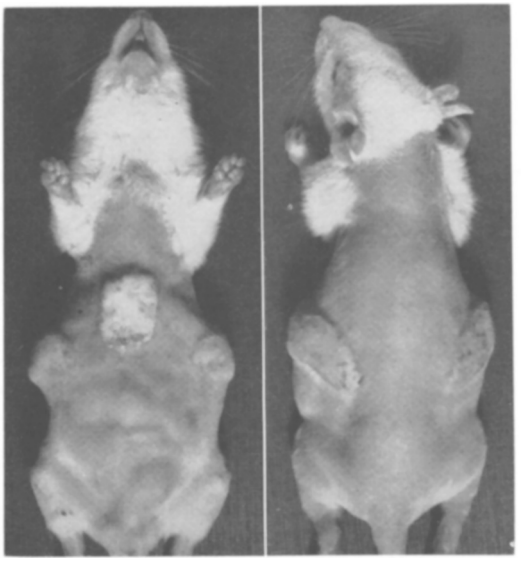

FIG. 1. The 3 typical calcified skin plaques in a rat treated with DHT (p.o.) + albumen (i.p.).

egg white are less effective in this respect than smaller doses(3).

At the dose used, *DHT alone* (Group I) produced only occasional traces of calcification in gastric mucosa, coronary arteries or kidneys.

About 48 hours after *intraperitoneal injection of egg white* (Group II), in all animals, 3 well-circumscribed cutaneous plaques appeared. One patch was situated just above the cartilaginous tip of the xiphoid bone; the other 2, in the costovertebral angles. The lesions were extremely painful to touch and while at first they adhered to the underlying muscle layers, by the fourth day it became possible to move them more or less freely over the subcutaneous tissue (Fig. 1). Histologically, the plaques proved to consist of heavy calcium deposits in the various muscular, fascial and dermal layers between the peritoneum and the epidermis through the entire thickness of the abdominal wall.

The most striking internal lesion was the virtually complete calcification of the pancreatic stroma, which imparted a dense, chalky appearance to the organ. Here, calcium deposition could be demonstrated with particular clarity by fixing the entire loop of duodenum, with the pancreas attached, in alcohol-formol and subsequenlty staining with $AgNO_3$ (Fig. 2A). Histologic examination showed that the calcification is strictly limited to the pancreatic stroma and consists of extremly fine $AgNO_3$-positive granules surrounded by inflammatory changes and intense sclerosis. There was no trace of stainable calcium in the excretory parenchyma, the ducts or the Langerhans islets (Fig. 2B). On the other hand, heavy calcification was also regularly found in the middle layer of the gastric mucosa, the duodenum (especially around Brunner's glands), the retroperitoneal fat and the diaphragm. The kidney exhibited only traces of corticomedullary calcinosis, while the omentum, liver, spleen and adrenal remained unaffected. In the thorax, heavy deposits of calcium were seen on the pleura, the pericardium and throughout the thickness of the thoracic wall, especially around the sternum. The thymus and pulmonary parenchyma showed little if any calcification, but the lymph nodes around the thymus were heavily calcified. Calcium deposition in the coronary arteries was not definitely in excess of the trace amounts produced by this dose of DHT in control animals.

Intravenous injection of egg white (Group III) caused an increase in the intensity of calcification in the coronary arteries. In addition, heavy calcium deposits were often seen in the auricular appendages and the sub-

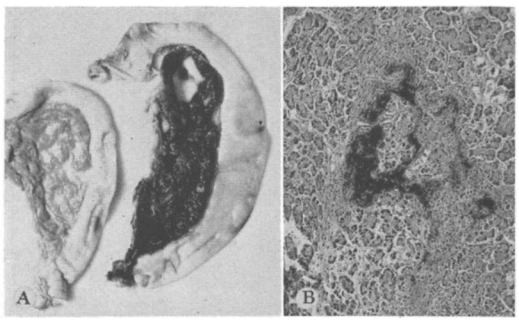

FIG. 2. Pancreatic calcification in a rat treated with DHT (p.o.) + albumen (i.p.). *A*, left: Duodenal loop with pancreas of a rat that received DHT alone, and comparable specimen of an animal treated with DHT + albumen. Both specimens were defatted and stained with silver nitrate to demonstrate calcification. *B:* Histologic aspect of calcification in pancreas of rat shown in Fig. 1. The calcium deposits are extremely fine and are surrounded by a great excess of inflamed connective tissue. (v. Kóssa × 60.)

epicardial portions of the ventricular musculature; these regions never showed calcification in the controls treated with DHT alone.

Following *intraperitoneal injection of egg yolk* (Group IV), there was no calcification of the skin or abdominal wall, although the omentum (which was not affected by intraperitoneal egg white) showed intense calcification. Furthermore, the Kupffer cells and some collagen fibers that surround the hepatic sinusoids likewise exhibited severe calcification. The thoracic organs were only mildly affected, except for the perithymic lymph nodes, which receive lymph-borne substances from the peritoneal cavity. This dissimilarity in the distribution of the calcium deposits produced by albumen and yolk, respectively, is presumably due to differences in their direct and lymphatic spreading. Control experiments in similarly DHT-sensitized animals revealed that, if introduced directly into the subcutaneous tissue, both albumen and yolk induce approximately equal degrees of cutaneous calcinosis.

The most striking effect of *intravenous injection of egg yolk* (Group V) was an intense calcification of the spleen, making the organ whitish, hard and brittle. There was also considerable calcification of the Kupffer cells and of the isolated connective tissue fibers around the hepatic sinusoids. The hepatic parenchyma, blood vessels and biliary ducts remained unaffected. This rather selective distribution of calcification is presumably due to the fact that the lipid-containing particles of egg yolk are preferentially taken up by the splenic and hepatic reticuloendothelium.

Discussion. Following sensitization with DHT, it is possible to produce, by intraperitoneal injection of albumen or yolk, rather selective calcification affecting certain tissues. It is difficult to explain the pathogenesis of the calcification caused by intraperitoneal injection of albumen in all layers of the abdominal wall and particularly in the skin over the xiphoid cartilage and the costovertebral angles. However, the abdominal wall of the rat is especially thin in these locations, which may account for the ready outward spreading of the egg white. The calcification of the thoracic organs may be ascribed to the circumstance that the principal lymphatic channels of the peritoneum ascend along the thoracic wall and, hence, presumably carry egg white upward.

Distribution of the calcification is quite different when egg white is injected intravenously. Yet other types of calcinosis—each with a constant and typical distribution pattern—are produced by intraperitoneal or intravenous injection of egg yolk, which possesses a particular affinity for the reticuloendothelial system. Numerous control experiments (not detailed here) have shown that neither albumen nor yolk causes any trace of calcification in otherwise untreated rats. Furthermore, hypercalcemia produced by other means (parathyroid hormone, sodium acetylsulfathiazole, nephrectomy combined with oral administration of calcium acetate) can similarly sensitize the rat for production of ectopic calcification by "vital mordants". On the other hand, the effect of albumen and yolk can be duplicated by various injectable iron preparations (ferric oxide saccharate, iron dextran). Hence, this type of "vital mordanting" is not limited to the particular pathogenic agents used in the present experiments.

Special importance is attached to the intense inflammation and sclerosis that accompanies and follows calcification in this type of lesion. In many experiments performed in the manner described here, we have seen that, if rats are allowed to survive for 2 or 3 weeks, the fine, dust-like calcium deposits (especially in pancreas, abdominal wall and thoracic organs) are rapidly absorbed and dense sclerotic tissue takes their place. Finely dispersed calcium-dust cannot be detected without the use of special stains; yet it can lead to inflammation and sclerosis. The frequent association of derangements in calcium metabolism and collagen disease (*e.g.*, the hypercalcemia of sarcoidosis, the ectopic calcification in scleroderma) suggests that further studies along these lines may help in elucidation of various systemic diseases affecting connective tissue.

Summary. In rats previously sensitized by a single oral dose of dihydrotachysterol

(DHT), massive and rather selective calcification of the pancreas is induced by subsequent intraperitoneal administration of egg white. Intravenous injection of egg yolk elicits a calcinosis primarily affecting the reticuloendothelial cells of the liver and the spleen. Yet, other types of calcinosis are induced by intravenous injection of egg white or the intraperitoneal administration of egg yolk. It is assumed that albumen and yolk act as "vital mordants," preparing the tissues of suitably sensitized animals for the subsequent uptake of calcium, distribution of the lesions being dependent upon the special distribution patterns of the mordants themselves.

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Received May 15, 1961. P.S.E.B.M., 1961, v107.

Metabolism of Human Amnion Cell Cultures Infected with Poliovirus. II. Utilization of Glucose, Pyruvate and Ribose in Virus Infected Cells. (26701)

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Human amnion cell cultures(1) proved to be a suitable system for investigation of effects induced by poliovirus on the metabolism of the host. It was shown(2) that infected cells utilized glucose by the glycolytic pathway. Levy and Baron(3) showed poliovirus to stimulate glycolysis of monkey kidney cells, and Matzelt(4) found increased activity of the glycolytic enzymes in a similar cell-virus system. This paper is a report of an investigation of the effect of glucose, ribose and pyruvate on virus multiplication. In addition, observations on the synthesis of lactic acid and ribose in infected and uninfected cells are also reported.

Materials and methods. Preparation of cell cultures. Primary human amnion-cell cultures were prepared as previously described(2). Monolayers were formed within 5-7 days.

Virus. A stock of the virus was prepared by inoculating amnion monolayers in milk bottles with the MEF_1 strain of poliovirus type II. After incubation for 72 hours at 37°C the medium was centrifuged, and the supernate containing the virus preserved at −20°C (infectivity titer $10^{7.0}$/ml). Virus titers were determined by inoculating tubes of amnion cells with 10-fold dilutions of the virus suspensions, using 4 tubes per dilution, and following the cytopathic changes microscopically for 7 days. The $TCID_{50}$ of the virus preparations was determined by the method of Reed and Muench(5).

Infection of cell-cultures. The cell cultures were washed with PBS, inoculated with the virus to give a multiplicity of infection of 5-10, and incubated for 1 hour at 37°C; the unadsorbed virus was removed by washing the cell with PBS. Eagle's medium, prepared with glucose, ribose or pyruvate or without any carbohydrate, was added to the cultures, which were reincubated at 37°C.

Chemical determinations. Aliquots of infected and uninfected cells or of cell hydrolysates were used for quantitative determinations of glucose, pyruvate, lactic acid and ribose. Glucose was determined by the Somogyi-Nelson photometric method(6), lactic acid by the method of Barker and Summerson(7), ribose (found in the medium and in the cells) by the method of Drury(8) and pyruvate by reaction with dinitrophenyl hydrazin reagent(9). For determinations of cell-synthesized-ribose, the cells were washed with 3 changes of PBS, released from the