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Experimental calcification.

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In 1905[1]–6[2] one of us was interested in the process of pathological calcification and indicated that in the human the condition was directly related to an antecedent fatty change in the tissues. From the studies then made it was demonstrated that the main factor leading to calcification of diseased organs was the accumulation of fatty materials in necrotic areas. The fat in these areas was liberated by the death of cells or was attracted to the areas of necrosis by the products of disintegration. The latter has been further demonstrated in the accumulation of fat by hyaline and amyloid substances.

Further proof of the relationship of fatty materials to the process of calcification is offered in a series of experiments where fats have been inoculated intravenously into rabbits. Two rabbits have been inoculated with pure olive oil and three with a mixture of cholesterin and olive oil (1–15) by the ear vein. Doses of 0.5 to 1 c.c. were given over a period of from one to eight weeks. The animals withstood the inoculations very well and showed no loss in weight. Temporary respiratory difficulty was sometimes observed for the first hour. The cholesterin mixture was usually warmed before giving.

The best results were obtained with the cholesterin-olive oil mixture after a period of four weeks. The major quantity of the oily injection was filtered out by the lung capillaries and only relatively small amounts reached the distant organs in its original condition. Reactions were recognized in the lung tissue at the end of two weeks when active proliferation of the endothelial lining of the capillaries frequently occluded their lumina or distorted the channels of the arterioles. The proliferating endothelium phagocyted the oil globules and frequently split the

[1] *Journal Exper. Med.*, 1905, VII, p. 633.

[2] *Journal Exper. Med.*, 1906, VIII, p. 322.

larger masses into small fragments which then were scattered through the protoplasm of these cells. The intracellular position of the fat could be observed during all stages of the experiment. In other instances, the phagocyted fat was removed from the blood channels and passed to the lymphatics. Such fat then appeared to lie free and not within cells. In the arterioles the fat appeared both in the proliferating endothelial cells as well as in deeper portions of the intima and media. In the latter structure it was commonly observed as an extracellular deposit. It was not uncommonly seen that the endothelial cells contained both fatty masses and cholesterin crystals. These crystals lay in clefts quite apart from the oil globules.

By staining with sudan all the oil masses were equally colored. Sections stained with Nile blue sulphate showed the presence of neutral fats, fatty acids, and intermediate mixtures of these. With the polarizing microscope some anisotropic globules could be seen, while free cholesterin plates were also demonstrated. The majority of the fatty acid globules were intracellular. These were usually smaller than the globules of neutral fat. The fatty acid racidal was also demonstrated by the Fischler method.

Associated with the intra- and extra-cellular fatty acid globules, was found the deposition of calcium salts. The calcium became precipitated in the borders of these fatty acids and gradually irregular calcium precipitates encroached upon the center of the acid globule. This deposit took place in the protoplasm of the large phagocytic cells as well as in the fatty deposits which were lying free in the tissue or in the lymph channels. At the end of eight weeks we found the lung substance filled with these minute calcareous masses lying in areas of cell proliferation looking not unlike small tubercles. The calcareous process was also recognized in the walls of the blood vessels. In the latter, the process occurred in the deep intima and media where fatty deposits were also demonstrated. These calcium salts were recognized by staining with hematoxylin and their phosphatic radicals with silver nitrate. They could be removed by treating the tissue with hydrochloric acid.

By the method here employed one is able to demonstrate the intimate association between the abnormal presence of fats and

the process of calcification. It is furthermore shown that the endothelial cells of the capillaries of the lungs have the property of splitting fats and liberating the fatty acid radical. By microchemical means the stages in the process may readily be followed.

As the fat within the endothelial cells has been phagocyted and lies in vacuoles in the protoplasm where it is acted upon by lipolytic secretions of the cell, it differs but little, in relation to cell activity, from fatty deposits which are extracellular and are acted upon by lipases present in the serum. In other words, the phagocyted fat of endothelial cells has an entirely different bearing to the cell from the accumulation of fats in fatty degeneration.

Our present findings are in perfect accord with the views we have formerly expressed upon the process of pathological calcification.

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Tumor-like growths in rat stomach following irritation.

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Fibiger's announcement of the production of carcinoma in the rat stomach through the agency of nematodes has not as yet been controverted. We wish briefly to record the fact that somewhat similar pictures can be produced by other means of irritation. By suspending in the stomach cavity woolen balls saturated with chemical irritants or by injecting the chemical irritants into the wall itself, polypoid growths of stratified squamous epithelium can be produced. By using celluloid balls with spinous processes these polypoid growths can be made to reach considerable dimensions. When these irritants are applied to the glandular portions of the organ, marked localized thickenings of the mucosa are produced. The chemical irritants cause a marked downgrowth of stratified epithelium resembling the cancroid type described by Fibiger, while the mechanically induced proliferations are characterized by a marked overgrowth of the cornified layers and relatively slight downgrowth. In the glandular por-