idoacetate and by M/10,000 fluoride, concentrations too low to inhibit bone phosphatase or to affect the solubility of bone salts. The inhibition by fluoride, currently believed to prevent formation of phosphopyruvate from 2-phosphoglycerate by blocking the action of enolase, indicates that dephosphorylation by phosphatase does not take place prior to that stage in the cycle. Calcification of cartilage, accordingly, involves phosphorylative glycogenolysis at least to the point of phosphopyruvate formation.

An exacting test of the role of phosphorylative glycogenolysis in this connection can be made by determining the effect of various inhibitors upon *in vitro* calcification of cartilage when P is supplied as the several phosphoric esters of the glycogenolytic series. We find that calcification with glucose-1-phosphate, in concentrations of 10 mg per cent. P, is not inhibited by phlorizin but is inhibited by cyanide, iodoacetate and fluoride; calcification with 2-phosphoglycerate is not inhibited by phlorizin, cyanide or iodoacetate but is by fluoride; calcification with α-glycerophosphate is not markedly inhibited by any of these agents. These results are consistent with what is known of phosphorylative glycogenolysis in muscle.[4] If phosphoric esters not in the series but readily dephosphorylated by bone phosphatase (β-glycerophosphate, phenylphosphate) are used, good calcification is obtained, which can not be blocked by inhibitors of glycogenolysis. If phosphoric esters not in the series and not dephosphorylated by bone phosphatase are used, calcification does not occur.

Conclusions. Calcifying cartilage contains an enzyme system for phosphorylative glycogenolysis. This system constitutes part of Robison's "second mechanism of calcification."

The processes of phosphorylative glycogenolysis are essential for *in vitro* calcification of cartilage in solutions containing P only as inorganic phosphate. They provide a mechanism whereby in such a medium lacking preformed phosphoric ester substrate for bone phosphatase, a concentration of P can be effected locally at the site of calcification in the cartilage matrix.

This mechanism probably operates in normal, *in vivo* calcification of cartilage. It could account for the selective calcification occurring in glycogen-rich primary centers of fetal ossification before erythrocytes, the only adequate source of phosphoric esters hitherto recognized, appear at the site of calcification.

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THE MILK-INFLUENCE OF BREAST TUMORS IN MICE[1]

IN this report we wish to present further data on the characteristics of the active milk-influence which plays an important role in the development of spontaneous carcinoma of the mammary glands in mice.[2,3]

Details of the experiments were as follows. Lactating females of the cancerous A strain were killed on 2/6/41 and their mammary glands removed. The tissue was macerated in a tissue-press and forced through a disc having openings 0.045 of an inch. The macerated material was filtered, using a fine wire cloth and 38 cc of milk, etc., was obtained to which was added an equal amount of sterile physiological salt solution. The extract was filtered through a Seitz filter and the filtrate (called filtrate A) was injected subcutaneously or intraperitoneally into 15 mice of the fostered C3H strain. The incidence of breast tumors in breeding females of the fostered C3H strain is about 2 per cent. The injected animals, as were all the mice used in these studies, were four weeks of age at the time of treatment and each received 2 cc of the filtrate. Nine of the injected mice have developed breast tumors, one has died non-cancerous and the others are living. All the females in this and the following experiments were used as breeders.

To the macerated tissue and the unfiltered residue of filtrate A was added 40 cc of distilled water. The extract secured following filtering with a wire cloth was filtered through the Seitz filter. This filtrate (called filtrate B) was injected, as above, into 12 females of the fostered C3H stock. Ten mice have developed breast tumors.

To determine if the unfiltered material contained the active milk-influence, 17 fostered C3H mice were given, in small dishes, the unfiltered residue and the macerated tissue diluted 1:3 with water. The average amount given per mouse was 7 cc, and most of the material was consumed. Fourteen of these females have developed breast tumors, 2 are living and 1 died non-cancerous.

Mice receiving the filtrates developed tumors at an average age of 287 days and the females receiving the unfiltered extract at 244 days.

On 4/17/41, 75 cc of distilled water was added to 15 cc of macerated breast tumors (tissue-press). The extract was given by mouth to 13 mice of the fostered C3H stock and 6 females of the BAF_1 generation (C57 black ♀ × A ♂, normal incidence of breast tumors, 1 per cent.).[3] The average amount given per female was 3.8 cc. Nine fostered C3H and 1 BAF_1

[1] Assisted by grants from the National Cancer Institute and The Jane Coffin Childs Memorial Fund for Medical Research.

[2] J. J. Bittner, SCIENCE, 84: 162, 1936.

[3] *Idem*, Trans. and Studies of the College of Physicians of Philadelphia, 9: 129, 1941.

females have had breast tumors, 1 C3H mouse died at an early age and the others are living.

To the residue of the tumor-tissue was added an equal amount of 50 per cent. glycerin. This was placed in the refrigerator on 4/17/41, shaken daily and filtered through a wire cloth on 4/24/41. Twenty-five cc of filtered substance was obtained to which was added 40 cc of Locke's solution. The extract was injected intraperitoneally into 16 fostered C3H females (2 cc, each mouse) and 8 BAF_1 females (1 cc, each mouse). Five of the C3H females have developed spontaneous breast tumors; the others are living.

On 5/6/41 an extract of 30 cc of macerated tissue of spontaneous breast tumors and 90 cc of 50 per cent. glycerin was placed in the refrigerator, shaken daily until 5/15/41, when it was removed and filtered (wire cloth). Seventy-five cc of filtrate was obtained to which was added 140 cc of Locke's solution. Fifty-five mice were injected intraperitoneally, each receiving 1 cc. As 43 of the injected mice were dead by the following morning, the others were given the extract in small dishes. The average amount given was 4 cc per animal but little of the material was consumed in two days. Five BAF_1 and 7 fostered C3H females survived the treatment and tumors have been observed to develop in two of the hybrid animals.

SUMMARY

Females of the fostered C3H strain and the BAF_1 hybrid generation, having a normal incidence of breast tumors of 1–2 per cent., were given, by mouth or injection, filtrates (Seitz filter) or extracts of glycerinated-treated tissue containing the active milk-influence for the development of spontaneous breast cancer. Sixty-three experimental mice were observed to have an incidence of 41 per cent. Thirty-six mice received unfiltered or untreated material and have had an incidence of 67 per cent. Many of the mice of each group are still living.

Previous studies indicated that the active milk-influence would not become inactive following desiccation.[4] Following ultracentrifugation the active influence appeared in traces, if at all, in the fat fraction and in the final supernatant fluid, and it is possible that the active agent is a colloid of high molecular weight.[5]

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MEANS OF INCREASING THE ILLUSION OF DEPTH IN PHOTOGRAPHS

PHOTOGRAPHS used in scientific illustration generally lack the illusion of depth or three dimensions. The recent introduction and use of film transparencies (translites) has made available a means of obtaining the third-dimensional effect in photographs. "Translites" when held up to the light and viewed from a distance of two or three feet give the optimum of third-dimensional illusion or effect.

An easy, inexpensive means of mounting and viewing the transparencies in front of the laboratory windows is herein described. The films are mounted in metal frames between two sheets of glass. The dimensions of the frames used by us are twenty-two by twenty-nine inches and they hold nine translite films (seven by nine inches) arranged in three rows of three each. The metal frames were obtained from the Multiplex Corporation of St. Louis, Mo.

The frames are held in front of the windows by the following means. Two deeply grooved wooden tracks or window bars are used to keep the frames upright in the lower part of the window. The lower grooved track is permanently fastened to the framework of the window. The upper detachable grooved track is fastened to the framework of the window by means of two bolts passing through holes at each end of the bar and fastened by wing nuts. The upper bar can easily be unfastened to insert the frame containing the translite films. Several frames may be placed in a window and the ventilation or illumination of the room is not appreciably decreased (see Fig. 1 A and B).

Since the films are placed between two sheets of glass, they are not liable to injury by frequent handling and therefore retain their usefulness indefinitely. This method of illustration had been found to be much superior to ordinary photographs and greatly enhances the illusion of the third dimension or depth in photographs.

"Translites" may be made on either translite film or paper (Eastman Kodak). The paper is less expensive than the film, but it does not have the same degree of brilliance or transparency. Each side of the translite film or paper is coated with a photographic emulsion. These emulsions are of different speeds so that when developed the two images are of different densities and are separated from each other by the thickness of the paper or film. The illusion of depth is thus readily obtained when these double-coated, two image films or papers are viewed from a distance of several feet.

Photographic negatives of medium contrast taken in the usual manner are used in making the translites.

[4] *Idem*, SCIENCE, 93: 527, 1941.

[5] M. B. Visscher, R. G. Green and J. J. Bittner, *Proc. Soc. Exp. Biol. and Med.*, 49: 94, 1942.