was depressed. Those chicks which received 4% added glycine without vitamin B_{12} suffered excessive mortality. The addition of as little as 3 micrograms of vitamin B_{12} per kilo of diet overcame the inhibitory action of both levels of glycine. These results indicate that vitamin B_{12} functions in the metabolism of glycine.

The authors are greatly indebted to Merck and Co., Inc., Rahway, N. J., for the vitamin B_{12} concentrate and other crystalline vitamins; Lederle Laboratories, Pearl River, N. Y., for folic acid; Allied Mills, Inc., Portsmouth, Va., for soybean oil; Abbott Laboratories, North Chicago, Ill., for Haliver oil; U S. Industrial Chemicals, Inc., New York City, N. Y., for DL-methionine; and Cerophyll Laboratories, Inc., Kansas City, Mo., for Protamone.

Received July 17, 1950. P.S.E.B.M., 1950, v75.

Inhibition of Calcification *in vitro* by Surface Active Compounds.* (18126)

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The observation that some types of pathological lipid deposition are often followed by mineralization suggests that lipid substances may enhance mineral salt deposition. An *in vitro* study of the role of lipids in the calcification of hypertrophic epiphyseal cartilage of the rachitic rat may further our understanding not only of normal calcification, but may also provide a clue to the nature of abnormal mineralization. This method, which enables one to observe the selective formation of new bone salt in rachitic epiphyseal cartilage, depends on the functioning of the complete system essential for calcification(1-3, 6,11,12). This technic has provided, in previous experiments, evidence that lipids are related to calcification. For example, bone formation is inhibited in the rachitic tibia if it has been previously extracted with alcohol, acetone, or chloroform(2). Moreover, the calcifying mechanism is injured by phloridzin and iodoacetic acid; two compounds which are inhibitors not only of phosphorylative glycogenolysis, but also of fat absorption(3,4). That these inhibitions can be overcome with excess inorganic or organic phosphates does not preclude the possibility that a system handling lipids is directly involved in the local deposition of bone salts(5,6). Levine and Follis(7,8) have demonstrated the presence of a lecithinase in cartilage. They postulated that it is part of an independent system for elevating the local concentration of phosphate ions. Another line of investi-

* This investigation was made possible by a grant from the Wendell Willkie Memorial Fund. A preliminary report appeared in *Am. Chem. Soc. Abst.*, 117th Meeting, 1950.

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TABLE I. Inhibition of calcification *in vitro* by Surface Active Compounds.*

Surface active agent	Conc. of agent	Degree of calcification*	Degree of control calcification*
Sodium desoxycholate	1.9×10^{-4} M	1(++++)	1(++++)
" "	$1.9 \times$ "	2(++++)	2(++++)
" "	$4.8 \times$ "	0	1(++++)
" "	$4.8 \times$ "	0	2(++++)
Sodium cholate	0.9×10^{-3} M	2(++++)	2(++++)
" "	$1.9 \times$ "	(+++)	2(++++)
" "	$1.9 \times$ "	(+++)	1(++++)
" "	$2.8 \times$ "	0	1(++++)
Duponal C	$0.3 \times$ "	1(++++)	2(++++)
" "	$0.7 \times$ "	1(++++)	2(++++)
" "	$0.7 \times$ "	1(++++)	1(++++)
" "	$1.4 \times$ "	(++)	1(++++)
" "	$2.8 \times$ "	0	1(++++)
Tween-20	0.04%	2(++++)	2(++++)
"	0.08%	(+++)	2(++++)
"	0.08%	(+++)	1(++++)
"	0.20%	0	1(++++)
"	0.40%	0	1(++++)

* Specimens incubated in the calcifying solution 18-24 hr. Calcifying solution contains: .7 eq/L NaCl, .05 eq/L KCl, .22 eq/L $NaHCO_3$, Ca x P product = 10 mg Ca and 3 mg P = 30. The degree of calcification is indicated as follows: 0 = no calcification; (+) trace; (++) broken thin line; (+++) almost complete thin line across the provisional zone; (++++) complete thin line across the provisional zone; 1(++++) heavy line across the provisional zone including the primary tongues of cartilage; 2(++++) heavy line across the provisional zone including the primary and secondary tongues of cartilage; 3(++++) practically complete calcification of the metaphysis.

gation which indicates that lipids are related to calcification, is the work of Levander(9). He demonstrated the presence of an alcohol soluble factor in embryonic rabbit bone which initiated osteogenesis in rabbit muscle. His results have been questioned by Pfeifer(10).

In the present experiments, the effect of a variety of surface active agents on calcification *in vitro* was studied in an attempt to alter the lipid equilibria of calcifying cartilage. Wistar strain albino rats, 22-24 days of age, were placed on a modified Steenbock-Block diet for 21 days(11). Thin slices of epiphyseal cartilage were cut from the tibia by hand and incubated at 37°C without shaking in sealed flasks in the calcifying solution containing the concentrations of surface active agents described in Table I. The flasks were stoppered so as to prevent gas exchange with the atmosphere. During the 18-24 hour period of incubation, the pH was maintained at 7.35-7.4. The sections were then washed with distilled water and stained with 2% silver nitrate to reveal the newly formed bone salt deposits. Variations in the response of the calcifying mechanism of individual rats were controlled by incubating untreated sections from each animal used in a particular experiment. It was found that the surface active agents used, blocked the formation of bone. As can be seen in Table I, sodium desoxycholate was effective at a concentration of 4.8×10^{-4} M and another bile salt, sodium cholate was effective at a concentration of 1.9×10^{-3} M. Of the 2 synthetic detergents tried, the anionic Duponal C inhibited in a concentration of 1.4×10^{-3} M and the nonionic Tween-20 inhibited at a concentration of 0.08%. By varying the standard technic, a difference in the effect of the above surface active agents was noted, particularly with Tween-20. This modified procedure consisted of shaking rachitic sections with various higher concentrations of these compounds for 7 minutes in an unstoppered flask at 25°C, washing with distilled water, and then incubating the tissue in the calcifying solution in the manner described above. As shown in Table II, with less than 40 times the minimum concentration of the respective compound required to inhibit calcification with the continuous treatment in Table I, various degrees of inhibition were found with sodium desoxycholate, sodium cholate, and Duponal

TABLE II. Inhibition of Calcification *in vitro* by Preliminary Shaking with Surface Active Compounds.

Surface active agent	Conc. of agent	Degree of calcification*	Degree of control calcification*
Sodium desoxycholate	1.2×10^{-2} M	0, 0	1(++++)
" "	$1.2 \times$ "	0, 0, 0	2(++++)
Sodium cholate	$5.8 \times$ "	1(++++), 1(++++)	2(++++)
" "	$5.8 \times$ "	1(++++), (++++)	1(++++)
" "	$5.8 \times$ "	(++), (+++)	1(++++)
Duponal C	$5.3 \times$ "	(+), (++)	1(++++)
" "	$5.3 \times$ "	(++), (+++)	1(++++)
" "	$5.3 \times$ "	0, 0, 0	2(++++)
Tween-20	5%	1(++++), 1(++++)	1(++++)
"	20%	2(++++), 2(++++)	2(++++)

* The incubation period, composition of the calcifying solution, and the coding methods are the same as used previously in Table I. The pH of the preliminary shaking solutions varied between 7.2 and 8.

C. However, with Tween-20 it was possible to increase the concentration 250-fold without observing any trace of inhibition. Previous work has shown that preliminary shaking with high concentrations of sodium chloride for 2 hours will inhibit calcification(12). The variation in sodium concentration in the above compounds, however, can not be correlated to the degree of inhibition.

It is possible that one is dealing with more than one type of inhibition in the above experiments. Not only may bile salts and anionic detergents interact with lipids, but they may also modify proteins and activate as well as inactivate various enzymes(13). In dealing with tissue slices containing a complex biological system, it is difficult to draw conclusions based on detergent action in simple solutions. In the standard *in vitro* procedure described above, the low concentrations in which the bile salts and Duponal C are effective are probably below the critical value required for the denaturation of proteins. Anson(14) treated soluble proteins with from 1/5 to more than equal their weight of detergent to produce denaturation. Furthermore, Hotchkiss(15) has pointed out, "The concentrations of detergent capable of denaturing most ordinary soluble proteins are in a higher range than those necessary for killing bacteria." Therefore, the question as to whether cellular damage occurred which was responsible for the observed inhibitions with low concentrations of these agents must be seriously considered. The properties of the nonionic Tween-20, which inhibited only when present during the actual period of bone salt deposition, strongly suggests that possible cellular damage is not a critical factor in causing inhibitions with low concentrations of detergents. Tween-20, as a nonionic type of detergent does not inhibit bacterial growth(15) and hence would not be expected to injure any cells which may be involved in calcification. Moreover, since proteins and nonionic detergents have not been demonstrated to interact(16), the cause of inhibition by Tween-20 may be related to some alteration of the lipid equilibria essential for calcification. It is possible that the nature of the inhibition by low concentrations of the other agents is similar to that which occurs with Tween-20.

In the modified procedure, the concentrations of bile salts and Duponal C are sufficiently high so that the possibility that intracellular and extracellular lipid and protein components of the calcifying system have been modified exists(13,16). Metabolic studies were not undertaken to determine whether cells were in fact injured. However, the lack of inhibition by Tween-20 under these modified conditions, further indicates the presence of a more sensitive type of inhibition to the

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calcifying system when lower concentrations of surface active compounds are present during the actual process of mineralization. The existence of an essential lipid factor secreted by the cells as calcification proceeds would help explain the observed effect of the above surface active agents on the calcifying mechanism.

Summary. Calcification *in vitro* of the hypertrophic epiphyseal cartilage was found to be inhibited by 4.8 x 10^{-4} M sodium desoxycholate, 1.9 x 10^{-3} M sodium cholate, 1.4 x 10^{-3} M Duponal C, and .08% Tween-20. By preliminary treatment of rachitic sections with higher concentrations of these surface active agents for a short time interval, it was possible to demonstrate inhibition only with the bile salts and Duponal C.

Received April 28, 1950. P.S.E.B.M., 1950, v75.

The Effect of X Radiation on Antibody Formation.* (18127)

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Hektoen(1) conclusively demonstrated that total-body exposure of experimental animals to ionizing radiation suppressed the usual antibody response to antigens injected shortly before or shortly after irradiation. Hektoen's observations led him to ascribe this suppression to the destructive effect of irradiation on lymphatic tissue and bone marrow. These findings have been corroborated by a number of workers and have recently been reviewed by Taliaferro(2). Craddock and Lawrence(3) reported that total-body exposure of rabbits to 250 r X radiation effectively suppressed the formation of antibodies to antigens (typhoid vaccine and washed red cells from sheep) administered 8 hours after irradiation.

This communication relates a study on the capacity of the rabbit to form antibodies after the whole body, except for the spleen or appendix, has been exposed to 800 r X radiation. A preliminary report on this work was described elsewhere(4).

Materials and methods. Young adult rabbits (Swift's snuffle-free), weighing approximately 2 kg were used in this study. The rabbits were divided into groups and the experiments carried out as indicated in Tables I and II.

The rabbits of all groups, including the control animals, were anesthetized with Nembutal (38 mg per kg total-body weight) administered intravenously in the first experiments; later only those were given anesthetic which were subjected to irradiation, surgical exteriorization of the spleen, or both. Under anaesthesia, the spleen of each animal of Groups 3, 7, 8, and 9 was withdrawn from the abdominal cavity through a left upper quadrant incision. In the animals in Group 3, which were not irradiated, the spleen was exteriorized, wrapped in moist gauze, and left for one hour (the approximate time required to deliver the radiation to the exposure groups). In Group 7 the spleens were placed in lead shields during irradiation. The lead shields consist of two parts that fit together by means of overlapping flanges with a baffle type slit 3/16-inch wide and measuring 3¼ inches along the long axis. The wall of the

* Supported in part by a grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and (in part) by a grant from the National Cancer Institute, U. S. Public Health Service.

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