Fluorescence of Bone after Quercetin Ingestion. (25485)

WILLIAM E. RIBELIN, MERLE S. MASRI AND FLOYD DEEDS
Western Regional Research Laboratory, Albany, Calif.*

Preferential deposition of many substances in bone is well-known, but induced fluorescence of bone is uncommon, and to our knowledge has been reported only for tetracycline antibiotics(1). The present report describes the phenomenon in quercetin-fed rats of fluorescence of excised bone when viewed under ultraviolet light (UV). Quercetin (3,5,7,3′,4′ pentahydroxyflavone) is widely distributed in the plant kingdom as either glycoside or aglycone(2). It exhibits weak dull-orange fluorescence under UV. The metabolic fate of quercetin has been described(3,4,5). One of its, as yet unidentified, urinary metabolites exhibits brilliant golden-yellow fluorescence under UV. Lack of toxicity of quercetin has been reported(6).

Methods. Quercetin is commercially available, but the present product was prepared by acid hydrolysis of its rhamnoside, quercitrin, isolated from lemon flavine(7) a commercial dyestuff derived from bark of black oak tree. Quercetin was mixed at level of 1% in diet having the following percentage composition: degerminated yellow corn meal 73, casein 10, linseed oil cake meal 10, ground alfalfa 2, bone ash 1.5, sodium chloride 0.5, and cod liver oil 3. Groups of weanling, adolescent, and adult albino rats from our colony were fed the quercetin-containing diet for various periods, then sacrificed and the excised bones examined grossly, under UV, and microscopically.

Results. When 21-day old rats were fed quercetin-containing diet for 3 days their bones had an intense golden-yellow fluorescence under UV. Maximum fluorescence was apparently achieved by 10th day of feeding. Fluorescence persisted at least 6 weeks after withholding quercetin from the diet. Persistence of fluorescence for longer periods was not studied. Color and intensity of fluorescence appeared similar to that described for rats and rabbits receiving tetracycline antibiotics(8). Rats fed quercetin did not differ from controls in growth and development(6,9), or in gross and microscopic bone structure. Some, if not all, fluorescence persisted after extraction of bone calcium with 5% nitric acid for 3 days. Fluorescence was lost in preparation of paraffin sections. It diffused slowly from bony tissue into formalin solutions when allowed to stand for several weeks. Fluorescence was not seen in non-osseous tissues. Comparable experiments with adolescent and adult rats showed fluorescence was least in old rats and intermediate in adolescents.

Discussion. The nature of the fluorescent substance in bone and mode of its deposition are yet to be elucidated. Its possible identity with the fluorescent urinary metabolite of quercetin merits consideration. The greater concentration in young growing bone suggests a relationship to bone growth rather than mere deposition. Persistence after decalcification of bone suggests a combination other than simple chelation.

The value of induced bone fluorescence for autophotographic studies of bone development, dyscrasias, and neoplasms is obvious. It may be applicable in endocrine studies for assay of growth hormone by measurements of the epiphyseal plate.

Summary. Feeding 1% quercetin in the diet induces fluorescence in bones of rats when such bones are viewed under ultraviolet light. Fluorescence appears the third day of feeding and persists at least 6 weeks after cessation of feeding. Nature of fluorescent substance and mode of its deposition are not clear. Magnitude of fluorescence appears to be related to bone growth. Possible uses of this phenomenon are suggested.

* Laboratory of Western Utilization Research and Development, U. S. Dept. of Agric.

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Constitution of Bile Proteins.* (25486)

TAKETO KATSUKI, HIDEHIKO SHIMURA, CHARLES G. JOHNSTON AND HIROSHI MIYAKE†
Dept. of Surgery, Wayne State University College of Medicine and Detroit Receiving Hospital, Detroit, Mich.

It is unlikely that the gallstone problem will be completely resolved before the biochemistry and physiology of bile are thoroughly understood. Unfortunately little is known about the nature of bile proteins even though several investigations concerning bile proteins have been reported(1,2,3,4,5,6). In this study various bile protein fractions are identified, classified and further characterized with respect to their physico-chemical properties.

Materials and methods. Female sheep weighing 60 to 100 lb and dogs weighing 40 to 50 lb were used. Gallbladder bile was obtained by puncture of gallbladder followed by cholecystectomy. Hepatic bile was obtained from T-tube inserted into the common duct. The long end of the T-tube was plugged with a glass rod and fixed under the skin between bile collections. Subsequent collections of bile from the T-tube were made by incising the skin under pentobarbital anesthesia and connecting a plastic tube to the long end of the T-tube. Bile samples were concentrated to approximatey 1/10 vol. by dialysis with the use of 50% (w/v) gum arabic solution in barbital buffer (pH 8.6, ionic strength 0.1)(7). Paper electrophoresis of concentrated bile samples was performed in barbital buffer of ionic strength 0.1, pH 8.6 for 15 hours at 200 volts and at 8°C using horizontal strip type apparatus(8). Approximately 0.1 ml of concentrated bile was used on filter paper strip (4.5 x 34.0 cm) of Whatman 3 MM filter paper. Since bile pigments and phospholipids are also stained with bromphenol blue and interfere with quantitative evaluation of bile protein fractions, filter paper strips were washed overnight in a mixture of ethanol: chloroform : concentrated hydrochloric acid (2:1:0.01). Proteins on filter paper strip were then stained with bromphenol blue(9). The stained filter paper strips were immersed into melted paraffin at approximately 60°C and color intensity measured with Photovolt densitometer model 525, using a filter of 570 mμ. The faster migrating A-fraction (see Results) was isolated electrophoretically as follows: A small amount of buffer was placed in a cellophane bag set in the anodal buffer vessel of paper electrophoresis apparatus. The anodal end of filter paper strip was placed in this buffer and the A-fraction was allowed to migrate into this buffer by means of electrophoresis and collected. Buffer was removed from the A-fraction as much as possible by dialysis and electrodialysis, then the A-fraction was concentrated by the ultrafiltration method of Mies(10). For paper chromatographic analysis of amino acids, the A-fraction was hydrolyzed in a sealed glass tube with 8 N hydrochloric acid at 100°C for 24 hours. An n-butanol : acetic acid : water

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† Professor of First Dept. of Surgery, Kyushu Univ. Faculty of Med., Fukuoka, Japan.