antirachitic activation of cholesterol with fuller's earth. Bills,[3] and Kon, Daniels and Steenbock[4] concluded several years ago that this active substance was different from vitamin D. However, in working with purer preparations we found that the effect on intestinal reaction, iron reduction and even bone calcification[5] was similar to that of irradiated ergosterol products.

Data with experimental procedures to be reported elsewhere have led to the clarification of the chemistry of the fuller's earth activation of cholesterol. The cholesterol activating constituent of the reactive earth was found to be sulfuric acid or its anhydride. The initial reaction was a dehydration of the cholesterol not only to the dicholesteryl ether, as Bills found, but to the ultimate dehydration product, cholesterilene, of Mauthner and Suida.[6] The final reaction then was found to be sulfonation at the site of the double bond created by the removal of a molecule of water. Actually the concentration of the antirachitic substance was much increased by the treatment of the cholesterilene in carbon tetrachloride with a small amount of sulfur trioxide. This is a well-known method for the sulfonation of aromatic hydrocarbons. Still better yields of the antirachitic substance were obtained when the Friese[7] method for the sulfonation of hydroaromatic hydrocarbons was applied with some modification to the sulfonation of cholesterilene. Using the Shipley technique for the "line" test, protocols were obtained showing degrees of calcification induced by the substance made by various modifications of the Friese method.

If the active substance is a sulfonic acid it should be soluble in water and precipitated by barium. In fact, addition of barium hydroxide or acetate solution to the water soluble acid residue remaining after the evaporation of the acetic acid solvent precipitated a crystalline barium salt. This was filtered off, dried, digested in alcohol, dissolved in carbon tetrachloride and reprecipitated in alcohol. The precipitate was dried at 100° C. The percentage of barium in three such preparations was 13.38, 13.21 and 13.18 and of sulfur, 5.94, 6.16 and 6.28, respectively. The calculated percentages for barium cholesterilenesulfonate $(C_{27}H_{43}O_3S)_2Ba$ are: barium 13.31 and sulfur 6.21. This salt does not melt below 330° C.

Two of these analyzed preparations, 104.4 and 107.2, were converted into the free sulfonic acid by digestion with an equivalent of sulfuric acid and "line" tested in a qualitative way for antirachitic potency. A continuous ++ line of calcification was produced by 3 mg of the cholesterilene sulfonic acid.

Since the insolubility of the barium salt in dilute acid or alkalis indicated a possible formation of the corresponding calcium salt from the free sulfonic acid and the calcium-containing ration in the digestive tract, a preparation 110 of the calcium salt was also "line" tested. Preparation 110 contained 4.37 per cent. calcium and 6.68 per cent. sulfur. The calculated values for $(C_{27}H_{43}O_3S)_2Ca$ are: 4.27 per cent. calcium and 6.86 per cent. sulfur. This salt melts at 320 to 325° C. Its antirachitic potency compared well with its molecular equivalent of the free sulfonic acid.

The potassium salt was prepared from the barium cholesterilenesulfonate by double decomposition with a molecular equivalent of potassium sulfate. This salt melted at 277° C.

A more direct method for the preparation of cholesterilene sulfonic acid and its isolation as the alkaline earth salt is through the sulfonation of cholesterol by modifications of the Friese method. In a preliminary run a 66 per cent. yield of the crude calcium salt was readily obtained.

The cholesterilene sulfonic acid is almost tasteless, soluble in water and also in oils when dehydrated. The monovalent metal salts are generally soluble. The bivalent metal salts are tasteless, insoluble to slightly soluble in water and soluble under certain conditions in organic solvents and oils. Due to their relatively low antirachitic potency overdosage is difficult. However, marked purgative effects were noted with rats, receiving during a four-day period 125 mg of calcium cholesterilenesulfonate.

It was not an objective of this station project to develop another antirachitic substance and it is proposed through letters patent to protect the interests of the public from a promiscuous substitution of such an antirachitic for vitamin D before its pharmacological action is further investigated.

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