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INHIBITORS OF STEROL SYNTHESIS. HYPOCHOLESTEROLEMIC ACTION OF DIETARY 9α-FLUORO-5α-CHOLEST-8(14)-EN-3β-OL-15-ONE*

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Summary: 9α-Fluoro-5α-cholest-8(14)-en-3β-ol-15-one, at a level of 0.15% in a laboratory chow diet, has been shown to have a significant hypocholesterolemic effect in normal rats. The mean serum cholesterol level (mg per 100 ml ± S.E.M.) decreased from 84.5 ± 1.5 to 53.3 ± 3.0, 54.1 ± 3.2, and 60.5 ± 2.6 after 5, 9, and 12 days, respectively, on the steroid-containing diet. The effects of the 9α-fluoro-15-ketosterol on serum cholesterol levels were significantly different from those of either ad libitum or pair-fed controls. The diet containing the steroid caused a significant decrease in food consumption which was associated with a decrease in the rate of gain in body weight.

5α-Cholest-8(14)-en-3β-ol-15-one (I; Figure 1) has been shown to be a potent inhibitor of sterol biosynthesis in L cells and in primary cultures of fetal mouse liver cells (1,2). Moreover, I, and its hemisuccinate and palmitate esters, were found to have significant hypocholesterolemic activity in normal rats (3-6). Oral administration of I in a low cholesterol diet was found to suppress food intake and to markedly lower serum cholesterol levels of normal rats when compared with either ad libitum or pair-fed control animals (4). We have recently prepared, by chemical synthesis, the 9α-fluoro analog of I, 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II), and have demonstrated that this compound is a very potent inhibitor of sterol synthesis in L cells grown in a serum-free medium (7). In these cells, II caused a 50% inhibition of the synthesis of digitonin-precipitable sterols from labeled acetate at a concentration of 5×10^{-7} M (7). A major site of action of the inhibitor in these cells in culture appeared to be at the level of HMG-CoA** reductase since II caused a 50% reduction in the level of activity of this enzyme at a concentration of 2×10^{-7} M (7).

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** 3-Hydroxy-3-methylglutaryl coenzyme A.

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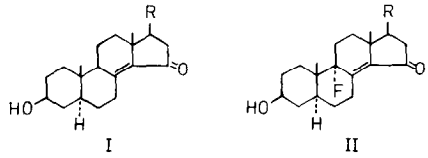

Figure 1. 5α-Cholest-8(14)-en-3β-ol-15-one (I) and 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II).

The purpose of this paper is to report that II, at a concentration of 0.15% in a laboratory chow diet, was found to suppress food consumption and the rate of growth of normal male Sprague-Dawley rats. Moreover, II had significant hypocholesterolemic activity when compared to either ad libitum or pair-fed controls.

Materials and Methods

9α-Fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II) was prepared as previously described (7). Male rats of the Sprague-Dawley strain were purchased from Sprague-Dawley Farms (Madison, Wisconsin). The rats were maintained on a light (7:00 AM - 5:30 PM)-dark (5:30 PM - 7:00 AM) cycle and fed a Purina Formulab Chow (#5008) diet (Purina Company, St. Louis, Missouri) for a period of 8 days prior to the initiation of the experimental period. The rats were then divided into 3 groups (with approximately the same mean serum cholesterol concentrations) and thereafter housed individually in metabolic cages. Compound II (750 mg) was added in small portions to 500 g of the basal diet in a 2 liter glass stoppered bottle. After thorough mixing by extended shaking, the resulting 0.15% diet was stored at 4° C. Prior to use, the diet was allowed to warm to room temperature. The rats were divided into 3 groups: (1), ad libitum group (N=8) with free access to the basal diet and mean body weight of 143.3 g ± 2.1 (S.E.M.); (2), fluoro ketone group (N=8) with free access to the basal diet which contained the 9α-fluoro-15-ketosterol and with a mean body weight of 144.6 g ± 2.8 (S.E.M.); and (3), pair-fed group (N=8) with access to the basal diet but only in the amount consumed by its individual counterpart in the fluoro ketone group on the previous day and with a mean body weight of 145.9 g ± 1.8 (S.E.M.). Blood samples were taken from the tail vein of the rats between 8:15 AM and 10:30 AM and at no time was more than ~0.5 ml

of blood removed. Serum was obtained by centrifugation of blood using Sure-Sep Junior (General Diagnostics, Warner Hambert Company, Morris Plains, New Jersey) for 20 min at 2000-2500 rpm in a table top centrifuge. Serum cholesterol concentrations were assayed by a modification of the Cholesterol Auto Test (Biodynamics, BMC Division; Boehringer Mannheim, Indianapolis, Indiana).

Results

9α-Fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II), when administered to rats at a concentration of 0.15% in a Purina Formulab Chow diet, caused a decrease in food consumption (Figure 2). The difference in mean food consumption between the experimental group and the ad libitum controls was significant throughout the period of study ($p<0.001$ on days 1 through 9, $p<0.01$ on day 10, $p<0.05$ on day 11, and $p<0.02$ on day 12). This reduction in food consumption was associated with a decrease in the rate of weight gain by the rats (Figure 3). The mean values for the mean percentage increase in body weight in the experimental group and the ad libitum control group differed significantly ($p<0.001$ on each day) throughout the study.

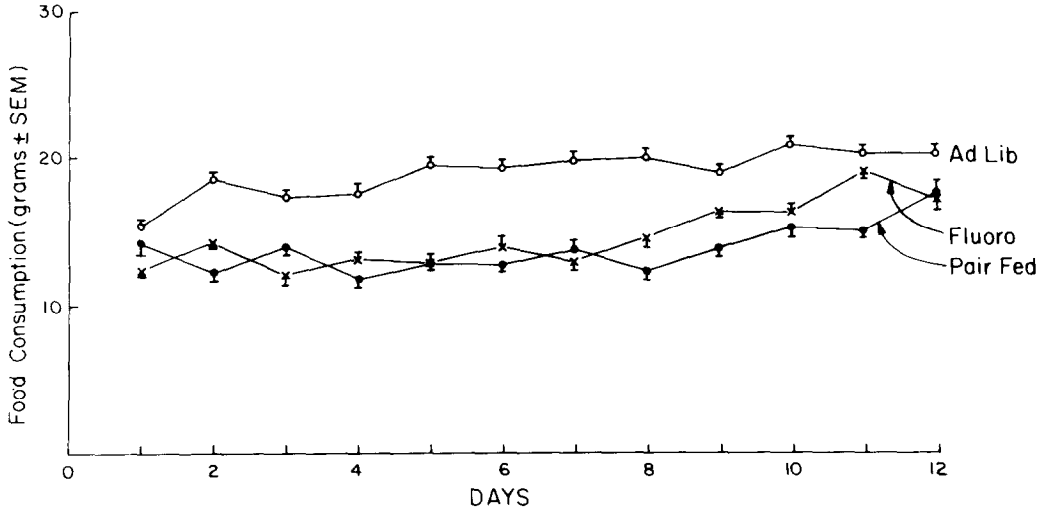

Figure 2. Effect of dietary 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II; 0.15%) in a laboratory chow diet on food consumption. o——o, ad libitum controls; x——x, 0.15% II in a laboratory chow diet; •——•, pair-fed controls.

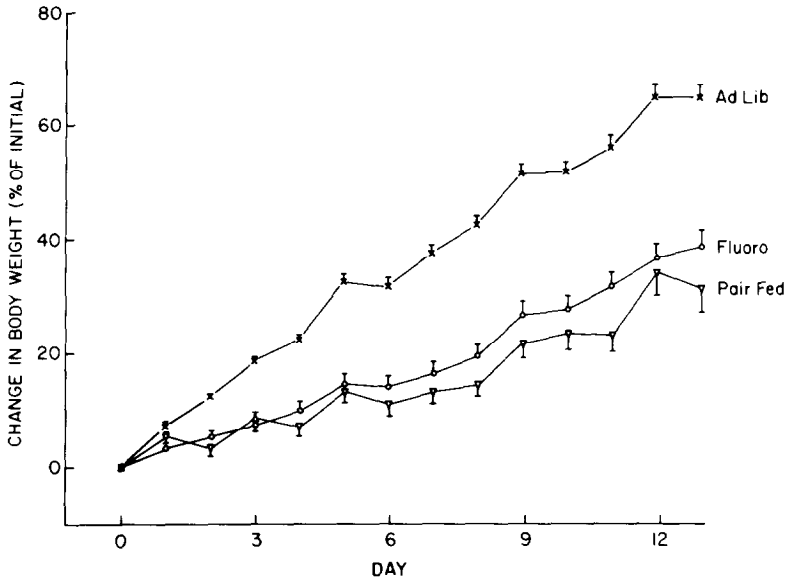

Figure 3. Effect of dietary 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II; 0.15%) in a laboratory chow diet on body weight. ●——●, ad libitum controls; o——o, 0.15% II in a laboratory chow diet; ▽——▽, pair-fed controls.

The mean serum cholesterol concentration of the rats receiving the diet containing the 9α-fluoro-$\Delta^{8(14)}$-15-ketosterol were significantly ($p<0.001$) lower than those of the ad libitum controls on days 5, 9, and 12. Moreover, the mean cholesterol level of these animals also was significantly lower ($p<0.01$ on day 5 and $p<0.001$ on days 9 and 12) from that of the pair-fed control animals (Figure 4).

Discussion

A number of oxygenated derivatives of cholesterol and of other sterols have been shown to be potent inhibitors of sterol biosynthesis in animal cells in culture (1,2,7-19). However, of those oxygenated sterols studied to date, only 5α-cholest-8(14)-en-3β-ol-15-one (I), its 3-keto derivative, and the hemisuccinate and palmitate esters of I have been reported to have significant hypocholesterolemic activity (3-5). A number of other oxygenated sterols with high activity in the inhibition of sterol synthesis in cells in culture, have been found to have no hypocholesterolemic activity in intact animals. These include 25-hydroxycholesterol (20), 7-ketocholesterol (20,21), and a number of ether analogs of 7-ketocholesterol

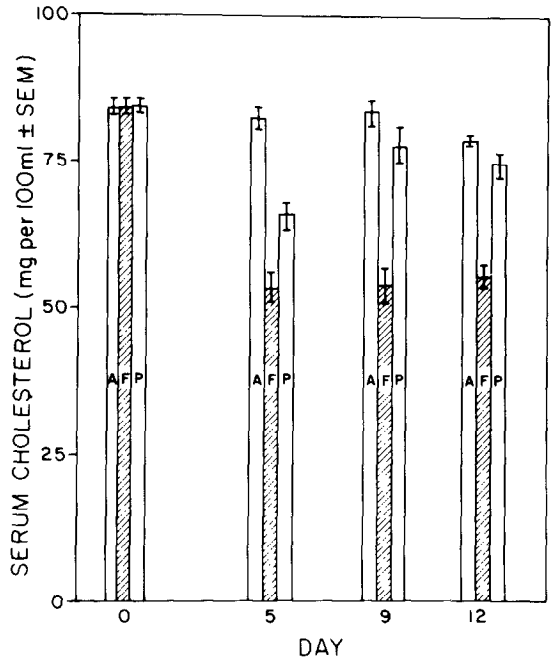

Figure 4. Effect of dietary 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II; 0.15%) in a laboratory chow diet on serum cholesterol concentrations of rats. A = ad libitum controls; F = 0.15% II in a laboratory chow diet; P = pair-fed controls.

(22). The lack of effect of these compounds on serum cholesterol levels of intact animals may be due to their rapid metabolism in tissues (23), by their lack of absorption, or by other factors. The results presented herein demonstrate that 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II) has significant hypocholesterolemic activity when administered to normal rats at a concentration of 0.15% in a laboratory chow diet. This finding and the observed reduction of food consumption and suppression of growth are similar to the previously reported (4) effects of its 9α-protio analog, 5α-cholest-8(14)-en-3β-ol-15-one (I). It is noteworthy that the results of preliminary experiments performed in a similar fashion, with the corresponding 9α-hydroxysterol, 5α-cholest-8(14)-ene-3β,9α-diol-15-one, indicate that this 15-oxygenated sterol, while it had inhibitory activity on sterol synthesis in L cells comparable to that of I and II (7), had no significant effect on serum cholesterol concentration of rats (24).

Further studies of the biological effects of the 9α-fluoro-$\Delta^{8(14)}$-15-ketosterol (II) are in progress.

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