Effects of Ethyl-α-*p*-Chlorophenoxybutyrate (CPIB) on Total Cholesterol Concentrations of Rat Aorta[1] (34341)

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Ethyl-α-*p*-chlorophenoxyisobutyrate (CPIB; Atromid-S) is an effective hypocholesterolemic agent in both man (1) and laboratory animals (2). The drug is used clinically as a potential preventive agent against the development and progress of atherosclerosis and in the treatment of xanthomatosis. The exact mechanism of CPIB's hypocholesterolemic action has not been clearly elucidated, though there is good evidence to indicate that in the rat the drug inhibits hepatic cholesterol biosynthesis primarily at a step preceding the formation of mevalonic acid (3). Nestel *et al.* (4) have suggested that CPIB reduces serum cholesterol levels in man also by inhibiting the hepatic biosynthesis of this lipid.

An important additional effect of long-term CPIB therapy in man is its apparent ability to diminish cutaneous cholesterol deposits, especially in severely hypercholesterolemic patients (5). The drug is particularly effective in causing a regression of the lipid deposits of xanthoma tuberosum (6), though those associated with xanthoma tendinosum and xanthoma palpebrarum occasionally regress as well (7-9). The CPIB causes a partial regression of the lipemic exudates associated with diabetic lipemic retinopathy (10). These observations have prompted some (11) to suggest that CPIB effects an egress of tissue lipid stores. Thus, it is conceivable that treatment with the drug may not only arrest the atherosclerotic process but also reverse it. The present study was designed to ascertain whether CPIB exhibits an effect on tissue cholesterol levels in rats previously made hypercholesterolemic by a high cholesterol–cholic acid diet.

Materials and Methods. Male albino rats (Sprague-Dawley strain from the Holtzman Co., Madison, Wis.), weighing 150–200 g, were fed a laboratory chow diet (Big Red Diet, formula 5RF, Agway, Inc., Syracuse, N. Y.) containing cholesterol (1.0%; w/w) and cholic acid (0.5%; w/w) for 14 days. This diet was designated "C-C". At the end of this period, the C-C diet was removed, some of the rats killed and the remainder divided into 2 groups, one of which was fed the chow diet alone and the other was fed the chow diet with CPIB at a concentration of 0.25% (w/w) for up to 10 days. At various intervals rats from both the chow fed and the chow plus CPIB fed groups were killed. Samples of sera and sections of abdominal aorta, taken from all the treated rats as well as from a group of "normal" chow control animals, were analyzed for their cholesterol contents by a combination of the methods of Abell *et al.* (12) and Zlatkis *et al.* (13). The sections of aorta were stripped of all extraneous fascial tissue prior to analysis.

In all rats, liver weights were recorded and relative liver sizes (g of liver/100 g of body wt) calculated. CPIB is known to cause a hepatomegaly in rats (14). This parameter was measured to confirm the activity of the drug in the rats used in the present study.

Results and Discussion. The serum cholesterol concentrations of the rats fed the C-C diet for 14 days were significantly higher than those of the chow control rats (Fig. 1).

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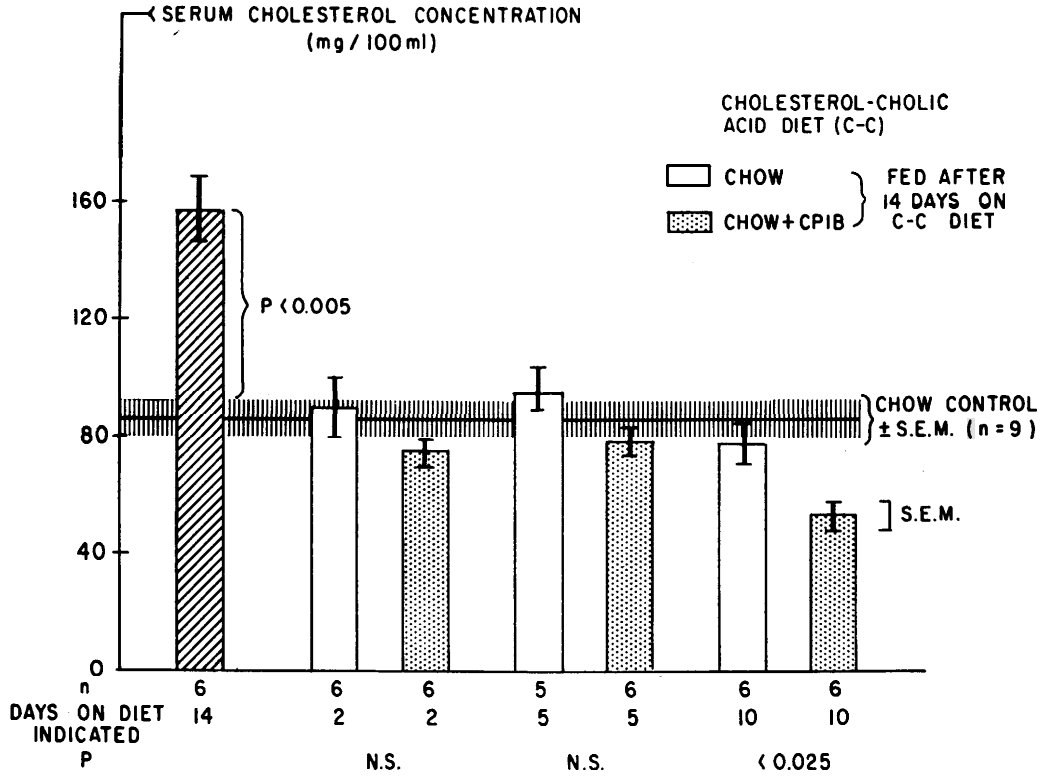

FIG. 1. Effect of the dietary administration of CPIB on serum cholesterol levels of rats returned to a chow diet following 2 weeks on a high cholesterol (1%)–cholic acid (0.5%) containing chow diet (C-C).

In rats fed both the chow diet alone and the chow diet with CPIB subsequent to the withdrawal of the C-C diet the serum cholesterol levels returned to control values after 2 days. The hypocholesterolemic effect of CPIB became manifest 10 days following removal of the C-C diet (Fig. 1). The hepatomegalic effect of CPIB was observed in rats fed the chow diet with CPIB within 5 days after withdrawal of the C-C diet.

Most striking was the effect of CPIB on aortic cholesterol levels. Feeding the C-C diet for 14 days had no apparent effect on these levels (Fig. 2). The aortic cholesterol concentration of the rats fed the chow diet subsequent to removal of the C-C diet remained at control levels for 5 days, but on the tenth day were inexplicably lower than the control values. The aortic cholesterol concentrations of the rats fed the chow diet with CPIB subsequent to termination of the feeding of the C-C diet fell progressively below control values and on the tenth day were significantly lower than those of the rats fed the chow diet alone for the same period (Fig. 2). The relative reduction was 14%. To test the reproducibility of this observation, aortic cholesterol levels were determined in rats that had been maintained on a high sucrose (63%; w/w), nonfat containing diet with or without CPIB at a concentration of 0.25% (w/w) for 2 weeks. These animals were being studied for other reasons that required the sucrose diet. Again, the aortic cholesterol concentration of the rats fed the sucrose diet with CPIB were significantly lower than those of the rats fed the sucrose diet alone (Fig. 3). In this instance, the reduction averaged 26%. Serum cholesterol concentrations were 92.3 $\pm$ 3.8 for the control rats fed the sucrose diet and 50.4 $\pm$ 2.4 for those given CPIB, a significant difference ($p < .001$).

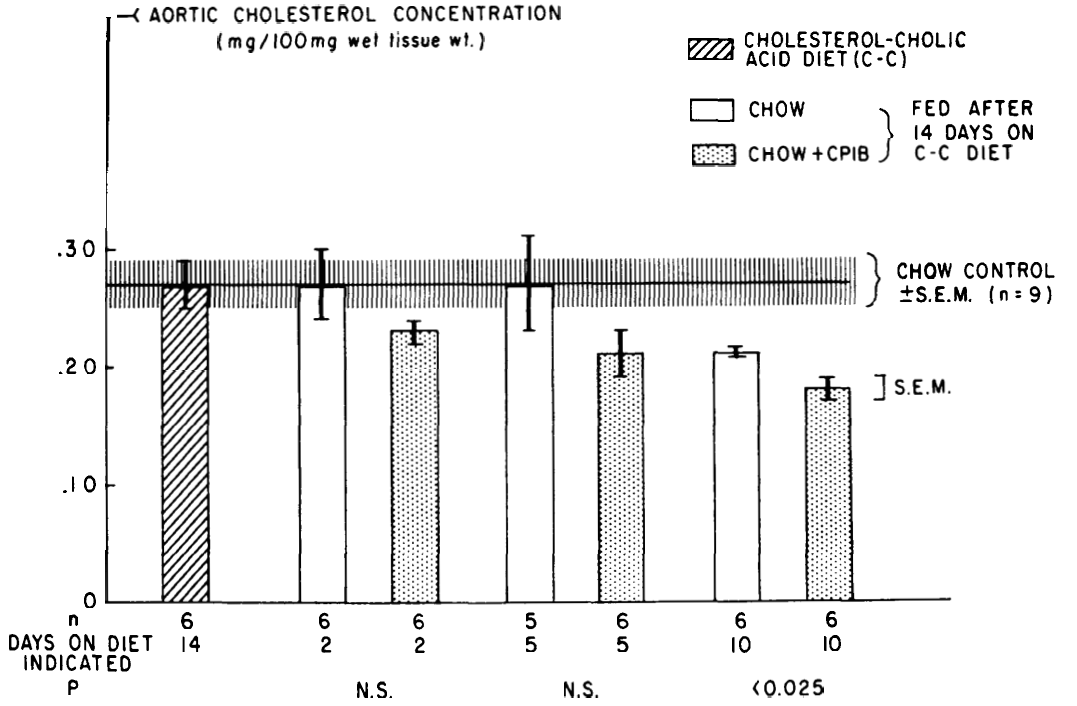

FIG. 2. Effect of the dietary administration of CPIB on aortic total cholesterol concentrations of rats returned to a chow diet following 2 weeks on a high cholesterol (1%)–cholic acid (0.5%) containing chow diet.

These observations are consistent with the suggestion that, in man, CPIB can cause an egress of stored tissue cholesterol (11). The drug is used clinically to lower serum cholesterol (and triglyceride) levels in an attempt to arrest the progress of atherosclerosis. The level of circulating cholesterol has been implicated in the pathogenesis of atherosclerosis [*e.g.*, Ref. (15)] which indicated a correlation between above normal serum cholesterol concentrations and heart disease, a sequel of atherosclerosis. Additionally, it was demonstrated early in this century (16) that the feeding of a high cholesterol diet to certain species of laboratory animals caused hypercholesterolemia and the subsequent formation of aortic atherosclerotic plaques in these animals.

As reported above, CPIB not only lowers elevated serum cholesterol levels in man, but also causes the partial or complete disappearance of cutaneous cholesterol deposits. Whether this latter effect pertains also to the cholesterol deposits present in human aortic

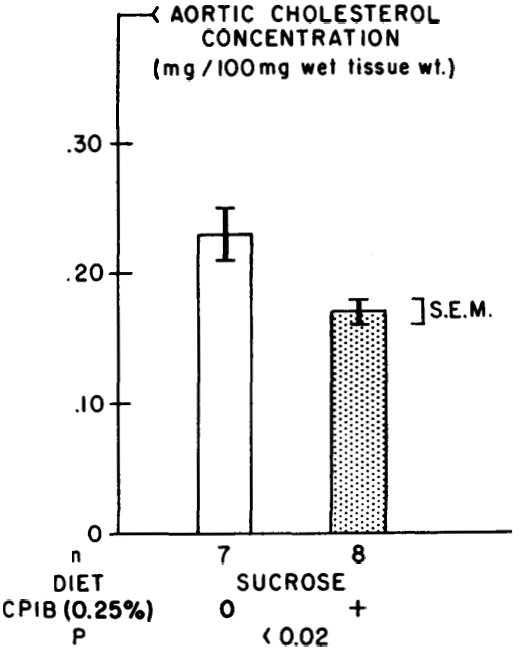

FIG. 3. Effect of the dietary administration of CPIB on aortic cholesterol concentrations of rats fed a high sucrose (63%) diet for 2 weeks.

atheromatous lesions is unknown. The present findings indicated that in the rat CPIB does lower aortic cholesterol concentrations, though it is not clear if such would be the case if the initial cholesterol concentrations of the tissue were high or if similar experiments were performed on other species.

The mechanism(s) of the observed effect of CPIB on aortic cholesterol concentrations remains unclear. It could be due (i) to egress of cholesterol from the tissue, or (ii) to a decreased rate of replacement of aortic cholesterol either on the basis of inhibited synthesis of cholesterol locally or a diminished amount of cholesterol abstracted from the circulating sterol by the tissue. Teal and Gamble (17) and Walsh *et al.* (18) reported that CPIB, added *in vitro*, inhibited the formation of cholesterol from mevalonic acid in a cell-free system from bovine aorta.

Summary. The aortic cholesterol concentrations of rats fed a chow diet containing CPIB (0.25%) for 10 days subsequent to feeding on a high cholesterol (1%) for 10 days subsequent to feeding on a high cholesterol (1%)–cholic acid (0.5%) diet for 2 weeks were significantly lower than those of rats fed the chow diet alone on the same schedule. This finding was repeated in rats fed a high sucrose (63%) diet with or without CPIB (0.25%) for 2 weeks. In the first instance, the reduction in aortic cholesterol levels was related temporally to a reduction in serum cholesterol concentrations in the CPIB-fed rats. This effect of CPIB may be related to a potential antiatherogenic property of the drug.

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