that administration of exogenous ubiquinone in concentrations of 2.5 mg to 25 mg daily for 3 weeks, had no appreciable effect on the number of microbodies or on catalase activity in male rat liver. Although the increase in ubiquinone concentration in liver resulting from CPIB treatment is suggested to be responsible for the inhibition of sterol synthesis and consequent lowering of serum sterol concentration[7,9], the results of the present investigation indicate that ubiquinone per se does not appear to be involved in the microbody proliferation and in the increase in catalase activity that accompanies the administration of CPIB. Since administration of exogenous ubiquinone failed to increase microbody number and catalase activity in male rat liver, but is known to inhibit cholesterol biosynthesis[9,10], it is likely that the hypolipidemic effect and microbody proliferative effect are two independent properties of CPIB with differing mechanisms of action. It is possible that the hypolipidemic effect of CPIB may be mediated through elevation of ubiquinone concentration as suggested by RAMASARMA et al.[7,9], but the microbody proliferative action appears unrelated to increased ubiquinone levels. These results provide further support for the hypothesis that the hypolipidemic effect and microbody proliferative effect may be two independent actions of CPIB[5,6]. However, the possibility that the increase in ubiquinone concentration resulting from the administration of CPIB may not be related to CPIB-induced inhibition of cholesterol synthesis cannot be excluded[13].

Zusammenfassung. Verabreichung von körperfremdem Ubiquinon an Ratten verursacht keine vermehrte Aktivität der Leberkatalase, die mit derjenigen nach CPIB-Medikation vergleichbar wäre. Es wird angenommen, dass die hypocholemische Aktion von CPIB nicht von einer Steigerung der hepatischen Ubiquinon-Konzentration begleitet ist.

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Effect of Fatty Acids on Fetal Rat Bone in Culture

Several recent studies suggest that lipids may influence bone resorption. In our own investigations, we found that an albumin preparation which had been treated with activated charcoal was much less active in causing resorption in culture than the albumin from which it had been prepared[1]. Activated charcoal treatment has been reported to effectively remove fatty acids from albumin[2,3]. In other studies, it has been noted that prostaglandins, fatty acid derivatives, stimulate bone resorption in vitro[4]. Finally, several recent publications indicate that a number of substances alter the lipid composition of bone[5–8]. Although these latter studies do not necessarily implicate lipids in the process of bone resorption, the fact that many of the agents which were shown to alter bone lipids also affect bone resorption raises the possibility of a role of lipids in resorption. To pursue this question further, we have investigated the effects of several fatty acids on bone resorption in vitro.

Details of the methods used have been published previously[9–11]. Pairs of fetal rat radii and ulnae prelabelled with Ca^{45} were cultivated for 72 h. The incubation medium was a modified BGJ[11] containing either activated charcoal-treated bovine serum albumin ('fatty acid free' fraction V, Pentex) or albumin monomer obtained by column chromatography of rat serum albumin on Sephadex G-200. Fatty acids were either converted to sodium salts and complexed to the albumin or dissolved in alcohol and added directly to the culture medium. The pH of the medium after all additions was identical for all media in a given experiment and varied between experiments from 7.4 to 7.5. At the end of incubation the bones were examined grossly for evidence of resorption and then extracted with 0.1 *N* HCl. The Ca^{45} in aliquots of culture medium and bone extracts was determined and the results expressed as percent of bone calcium released into the medium. Statistical significance was estimated by Student's *t*-test[12]. For studying the influence of the fatty acids on calcium binding by the albumins, 1 ml of 50 mg/ml albumin, with or without fatty acid, was dialyzed for 4 h at 37 °C against 20 ml of culture medium to which 0.1 μc Ca^{45} had been added.

Palmitic, oleic and stearic acids, added as their sodium salts complexed to albumin, all stimulated Ca^{45} release from fetal rat bone in vitro (Figure). Significant effects were elicited at a fatty acid concentration of 0.08 m*M* and maximal responses were seen with 0.16 m*M* fatty acid in the medium. At higher concentrations less stimulation was obtained. The increase in medium Ca^{45} was accompanied by gross evidence of resorption. Stimulation of Ca^{45} release was likewise seen when fatty acids were added

Table I. Effects of added fatty acid or triglyceride on Ca^{45} release from fetal rat bone in vitro

Fat added	*N*	Bone Ca^{45} released (%)	*P*
None	22	19.7 ± 0.6	
Oleic acid, 0.1 m*M*	9	21.2 ± 0.9	n.s.
Oleic acid, 0.3 m*M*	4	25.8 ± 1.8	<0.01
Palmitic acid, 0.1 m*M*	4	20.6 ± 1.1	n.s.
Palmitic acid, 0.3 m*M*	9	22.4 ± 0.9	<0.05
Stearic acid, 0.1 m*M*	8	19.1 ± 1.1	n.s.
Stearic acid, 0.3 m*M*	4	27.3 ± 3.0	<0.001
None	4	19.8 ± 1.4	
Tripalmitin, 0.05 m*M*	4	19.6 ± 4.4	n.s.
None	4	15.6 ± 0.5	
Triglyceride emulsion equivalent to 0.3 m*M* fatty acid	4	15.5 ± 0.5	n.s.

All cultures contained 1 mg/ml activated charcoal-treated bovine serum albumin fraction V. *N* values are numbers of bone pairs. Values given as means ± standard errors. *p* values based on comparison with Ca^{45} release from cultures with no fat added.

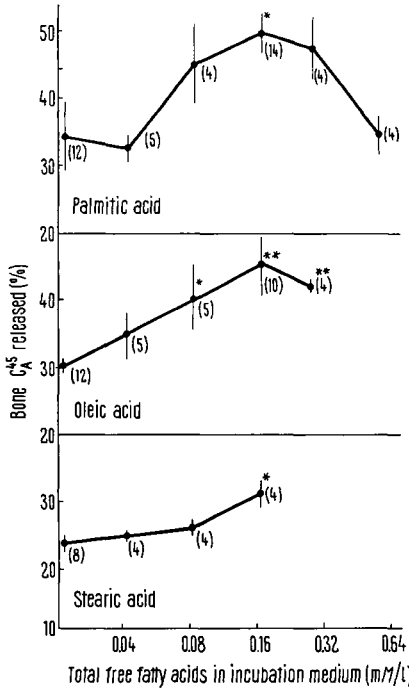
directly to culture medium containing albumin (Table I). Triglyceride, either in the form of tripalmitin or as an emulsion (10% cottonseed oil) had no effects when added at a concentration equivalent in fatty acid content to the active fatty acid concentrations.

In order to determine whether the added fatty acid could be increasing calcium in the medium by binding calcium already present and thus causing release of more calcium from bone, an equilibrium dialysis study was carried out (Table II). The amount of calcium bound by the albumin was not influenced by the content of complexed fatty acid.

Table II. Lack of effect of complexed fatty acid on calcium binding by albumin

	cpm/ml medium
External medium	7,350
Medium inside dialysis tubing	
albumin, 50 mg/ml	15,381
albumin, 50 mg/ml + oleic acid 0.7 m*M*	13,408
albumin, 50 mg/ml + oleic acid 1.4 m*M*	14,124
albumin, 50 mg/ml + oleic acid 2.8 m*M*	13,684

1 ml of 50 mg/ml activated charcoal-treated albumin was dialyzed for 4 h at 37 °C against 20 ml of culture medium BGJ to which 0.1 μc Ca^{45} Cl_2 had been added.



Effects of palmitic, oleic and stearic acids on Ca^{45} release from fetal rat bone in vitro. Fatty acids were added as the sodium salts complexed with albumin. The albumin concentration was 5 mg/ml for all the studies shown. Values are given as means ± standard errors. Values in parentheses are numbers of radius + ulna pairs. *significant increase from Ca^{45} release with no fatty acid added, $p < 0.05$. **significant increase from Ca^{45} release with no fatty acid added, $p < 0.01$.

Free fatty acids appear to be capable of producing bone resorption in vitro. The effect is similar to that seen in vitro with dibutyryl-3′, 5′-AMP[13] but differs from the resorption caused by parathyroid hormone[9], prostaglandins[2] or albumin[1], in that it is biphasic. A small increase in concentration beyond the maximally effective level resulted in a diminution of the response. In a few experiments in which albumins with marked bone resorbing activity were used, the addition of fatty acids inhibited resorption. Most often, however, no effect of fatty acids was seen when highly active albumins were used for preparing the culture medium.

Activated charcoal treatment has been reported to markedly reduce the free fatty acid content of albumin[3,4]. Since the treatment also can reduce the bone resorbing activity of the albumin, it is possible that free fatty acids are one of the factors responsible for the bone resorbing activity of certain albumins. However, there are several reasons which make it unlikely that fatty acids are the sole factor responsible for the resorption produced by albumin. Increasing concentrations of albumin produce further bone resorption[1], rather than the biphasic response obtained with the fatty acids. Also, in other studies (STERN and EARHART, unpublished observations) no correlation was found between the fatty acid content and bone resorbing activity of a number of albumins.

Although heparin has been shown to stimulate bone resorption directly[14,15] the bone-resorbing activity of the fatty acids could be an additional factor in the osteoporosis seen with chronic heparin treatment[16,17], since activation of lipoprotein lipase by the heparin would elevate serum free fatty acid levels[18].

Zusammenfassung. Nachweis, dass Fettsäuren (Palmitin-, Stearin- und Ölsäuren) in sehr niedrigen Konzentrationen die Kalziumabgabe und die Knochenresorption in fötalen Ratten in vitro fördern.

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