When the regression lines had been fitted to each replicate the position of the GUPTA estimate relative to the fitted line was examined (Table IV). In most instances the GUPTA estimate fell above the line and, as these were the higher doses, the effect of using these estimates was thus generally to increase the slope of the line compared with the value that would have been obtained if the doses at which censoring occurred had been omitted. When censoring occurred at more than one dose, it was essential to estimate the response at these doses and the results show that omission of the highest dose, when it alone was associated with prolonged narcosis, would generally have led to reduced estimates of potency. When regression lines had been obtained for each replicate, they were compared before a combined line, corrected for individual control values, was computed for each drug. In only one drug was incompatibility of replicates detected and in all drugs where censoring without death occurred the combined estimate indicated that the drug was active.

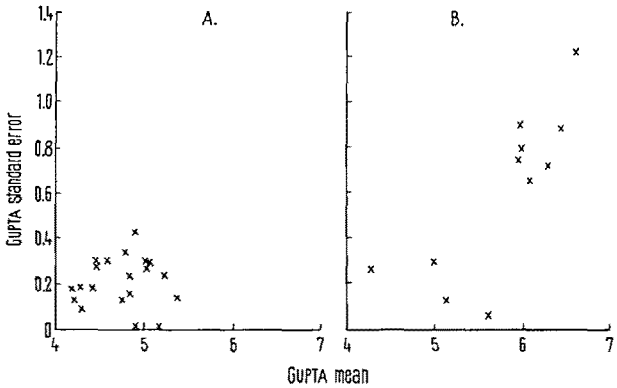

Fig. 2. Relationship between means and standard errors computed by GUPTA method. a) 7 observations from sample size 8. b) 3 observations from sample size 8.

Table IV. Distribution of 61 GUPTA means in relation to fitted linear regression line

	Number of sleeping times observed						
	2	3	4	5	6	7	Total
Dose 5							
Above line	2	7	6	4	3	7	29
Total	3	9	6	5	3	10	36
Doses 3 and 4							
Above line	2	1	4	2	3	5	17
Total	2	2	4	3	4	10	25

In general, the replicated results obtained using the GUPTA method for censored samples were comparable, even when censoring only occurred in some replicates. In some drugs where censoring did not occur over the selected dose range but individual replicates showed a consistent slight trend with dose, repeated replication was necessary before the slope could be established as significant at the 5% level. This suggests that in experiments of this type the experimenter can obtain unequivocable results with fewer replications by using a dose-range that produces some censored samples.

It may thus be concluded that the GUPTA method of computing means for censored samples was satisfactory, leading to estimates that were reasonably distributed about the cut-off point and plausibly related to adjacent dose estimates. The effect was in general to increase the slope of the fitted linear regression which may have indicated that the log response-log dose relationship was in some instances non-linear at these doses. The purpose of the experiments was, however, to relate the potency of a series of drugs over the same dose-range and for these purposes significant potency could be more rapidly determined when the upper doses of the range lead to censored samples in some or all replicates.

Zusammenfassung. Es werden Methoden der Dosis-Effekt-Analyse verglichen, die es erlauben, nicht mehr gemessene Werte in höheren Dosisbereichen zu schätzen. Obwohl die Daten eng beieinander liegen, ergab die von GUPTA[5] 1952 entwickelte Methode zur Bestimmung von Mittelwerten befriedigende Schätzungen, verifiziert durch die Übereinstimmung innerhalb der Gruppen.

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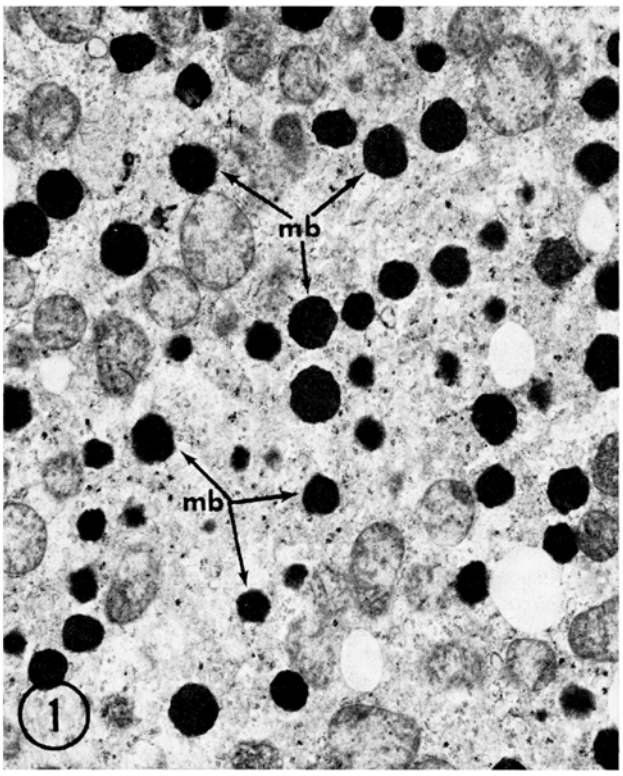
Ethyl-α-*p*-Chlorophenoxyisobutyrate Induced Hepatic Microbody Proliferation in Rat Liver and Ubiquinone Concentration

Male rats fed ethyl-α-*p*-chlorophenoxyisobutyrate (CPIB), a hypolipidemic drug, show a significant increase in the number of hepatic microbodies (peroxisomes) together with a two-fold increase in the content and activity of catalase protein, one of the principal enzymes of microbodies[1–4]. The precise mechanism by which CPIB elicits the hypolipidemic effect and microbody proliferative response is not understood. Accumulated experimental evidence suggests that the hypolipidemic property and the microbody proliferating effect are possibly two independent actions of CPIB and may not interrelate with one another[5,6].

The work of RAMASARMA et al.[7–10] demonstrated 1. that CPIB and ubiquinone (co-enzyme Q) have remarkably similar effects on inhibition of hepatic synthesis of cholesterol and lowering of serum sterol concentration, 2. that CPIB increased ubiquinone concentration in the liver to the same extent as did feeding with exogenous ubiquinone and 3. that the catabolism of ubiquinone was lowered with CPIB administration. From these results it



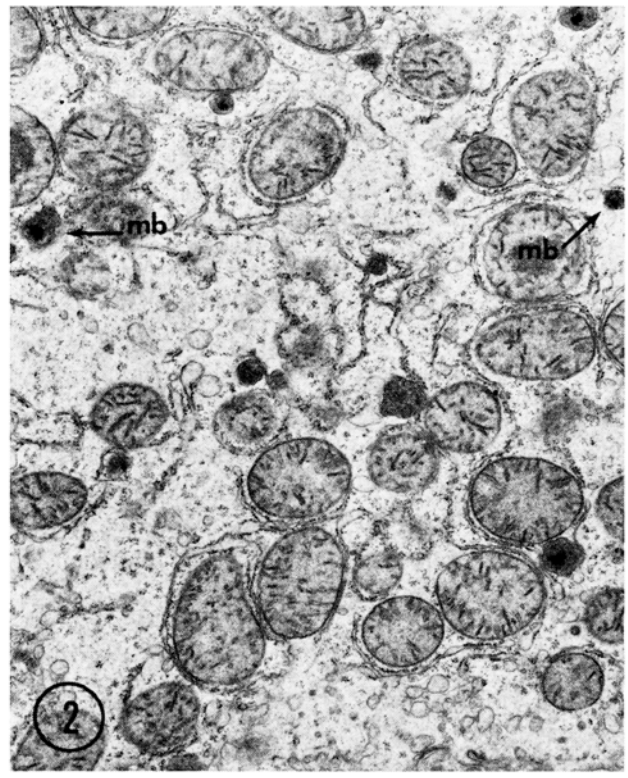


Fig. 1. Numerous microbodies (Mb) are seen in liver parenchymal cells of male rats treated with CPIB for 3 weeks, ×9,600. Fig. 2. The microbody (Mb) number appeared unchanged in livers of rats treated with varying doses of ubiquinone. ×9,600.

was suggested that CPIB possibly exerts its action by elevating the concentration of ubiquinone in the liver, which is then responsible for the inhibition of sterol synthesis[9].

The present studies were undertaken to determine the relationship, if any, of increased ubiquinone levels in the liver to enhanced catalase activity and microbody proliferation resulting from the administration of CPIB. Groups of male F-344 rats (A. R. Schmidt Co., Madison, Wisconsin) weighing 125–150 g were administered ubiquinone-45 (a gift from Prof. O. WISS, Hoffmann-LaRoche., Basel, Switzerland) orally by stomach tube in daily doses of 2.5 to 25 mg, for 3 weeks. The administration of exogenous ubiquinone will ensure high levels of ubiquinone concentration in the rat liver, comparable to the levels attainable by feeding CPIB[8, 10]. Liver biopsies were obtained at 1, 2 and 3 weeks, fixed for 1 h in 2% osmium tetroxide buffered with S-collidine at pH 7.4, and were processed for electron microscopy[2]. Hepatic catalase activity was determined by the method of LÜCK[11] from 5% liver homogenates prepared according to GANSCHOW and SCHIMKE[12]. The morphologic and biochemical findings are compared with animals fed CPIB (gift from Dr. JEROME NOBLE, Ayerst Laboratories) at a concentration of 0.25% in diet for similar periods.

The liver parenchymal cells of male rats treated with CPIB for 3 weeks revealed a significant increase in the number of microbodies (Figure 1). In contrast, however, the microbody number in the liver cells appeared unaltered in rats treated with varying concentrations of ubiquinone for 3 weeks (Figure 2). CPIB treatment significantly increased the catalase activity in the liver, closely paralleling the increase in microbody number, but the administration of ubiquinone did not increase the catalase activity significantly (Table). It is apparent therefore,

Liver catalase activity in male rats treated with ubiquinone and CPIB for 3 weeks

Group	No. of rats	Catalase Activity (Units/mg protein) Mean ± standard error
Untreated control	5	40 ± 1.65
Ubiquinone (25 mg/daily)	4	45 ± 2.18[a]
CPIB (0.25% in diet)	6	87 ± 4.75

[a] Similar values of catalase activity were observed when ubiquinone was administered in daily doses of 2.5, 5 or 10 mg.

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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.