Table II. Effect of indomethacin on the field stimulated acetylcholine output from Auerbach's plexus of guinea-pig ileum

	Indomethacin concentration (μg/ml) 0	15	30	45
Animal group[b]	Acetylcholine output: (ng/g/min)[a]			
I(6)[c]	51.57 ± 10.61	36.33 ± 5.03		
II(6)	45.20 ± 4.84		36.52 ± 6.68	
III(6)	57.77 ± 14.77			38.10 ± 6.04

[a] Same as in Table I. [b] Same as in Table I, with the following exception: After the 2nd 30 min incubation period the bath fluid was replaced again with fresh fluid containing physostigmine ± indomethacin and a 3rd 30 min incubation was performed. During the 3rd incubation period the tissue was field stimulated supramaximally at 0.3 Hz with 0.4 msec duration. At the end of this 3rd incubation, this bath fluid was assayed for acetylcholine content. [c] Same as in Table I.

ACh liberation, then the effect of PGE synthesis blockade should be visible in the spontaneous ACh output from Auerbach's plexus. This output should be affected because Auerbach's plexus possesses complete neuron, that is, neuron with cell body and processes. And, within the realm of neuron and chemical transmission theory, this would mean that spontaneous ACh output is the result of the activity of the ACh-containing neurons of the Auerbach's plexus. If, therefore, PGE does have any physiological role in ACh liberation, PGE synthesis inhibition should have affected significantly the spontaneous ACh output from ACh-containing neurons. Furthermore, after the inception of these studies, a report by BOTTING and SALZMANN[7] showed that INDO at 10 μg/ml reduced PGE output from whole ileum of guinea-pig to undetectable levels but that INDO in concentrations as high as 20 μg/ml was ineffective in changing ACh output in the majority of cases. Incidentally, these workers did not use proper control in their ACh output measurement.

Thus the findings presented here make it most unlikely that PGE is involved in ACh liberation from Auerbach's plexus of guinea-pig ileum, refute the hypothesis of EHRENPREIS et al.[5], and negate the hypothesis of WENNMALM and HEDQVIST[4] that PGE by a negative feed-back controls cholinergic transmission. If the latter was the case, then after the blockade of PGE synthesis by INDO, the ACh output should have increased. As can be seen (Table I), there is no increase in ACh output after INDO.

Since EHRENPREIS et al.[5] concluded that PGE has a role in ACh liberation on the basis of the effects of INDO on end-organ response (i.e., muscle contractions) induced by field stimulation, the effects of INDO on ACh output caused by field stimulation was also extended. INDO at concentrations of 15, 30, and 45 μg/ml failed to block significantly the ACh output resulting from field stimulation as shown in Table II. The independent *t*-test values, with increasing drug concentrations, are 1.2984, 1.0518, and 1.2343 compared with respective controls and are not statistically significant ($p > 0.05$). The fact that INDO, at a concentration of 45 μg/ml, caused a statistically significant block in twitch tension induced by field stimulation (present study as well as a previous study[5]), and yet failed to alter ACh output caused by field stimulation, clearly indicates how EHRENPREIS et al.[5] could have reached to a wrong conclusion based on end-organ effect of INDO, a PGE synthesis inhibitor.

Summary. The effect of INDO, a PGE synthesis inhibitor, on ACh output from Auerbach's plexus of guinea-pig ileum was investigated. INDO (15–45 μm/ml) failed to alter significantly either spontaneous ACh output or ACh output induced by field stimulation. It is concluded that PGE plays no physiological role in ACh liberation from this tissue.

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Hepatic Levels of Cyclic AMP in Normal and Lead-Sensitized Rats after Treatment with Bacterial Endotoxin

Some recent reports suggest that bacterial endotoxin may alter cyclic adenosine-3′,5′-monophosphate (cAMP) metabolism, thus explaining the variety of cellular and metabolic events which occur during endotoxin shock. For example, it has been shown that injection of endotoxin into rats and baboons causes rapid glycogenolysis in the liver, followed by increased blood glucose levels, and finally, hypoglycemia[1,2]. These observations could readily be explained if circulating endotoxin increased hepatic cAMP levels, thereby causing phosphorylase activation and glycogen degradation. Results reported by BITENSKY et al.[3] provide direct evidence that *E. coli* endotoxin interacts with hepatic cell membranes to increase the responsiveness of adenyl cyclase to epinephrine, and GIMPEL[4] found increases in hepatic adenyl cyclase activity in guinea-pigs treated with endotoxin. For these reasons, experiments were designed to measure hepatic cAMP levels in rats after i.v. injections of endotoxin. Cyclic nucleotide levels were also determined, using lead-sensitized animals, since lead acetate[5,6] is known to markedly sensitize rats to endotoxin.

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Hepatic cyclic AMP levels in rats after injection of saline, lead, endotoxin, and glucagon[a]

Treatment	Time after injection			
	1 min	5 min	15 min	4 h
Sodium acetate + saline (control)	0.57 ± 0.1 (5)	0.63 ± 0.1 (3)	0.83 ± 0.1 (3)	0.82 ± 0.1 (3)
Sodium acetate + endotoxin (3.3 mg/0.5 cm³)	0.61 ± 0.1 (5)	0.77 ± 0.1 (5)	0.64 ± 0.1 (5)	1.1 ± 0.02 (2)
Lead acetate + saline	0.52 ± 0.1 (3)	0.57 ± 0.1 (3)	0.71 ± 0.1 (3)	0.82 ± 0.2 (2)
Lead acetate + endotoxin (0.5 µg/0.5 cm³)	0.51 ± 0.1 (4)	0.79 ± 0.1 (4)	0.81 ± 0.2 (4)	0.95 ± 0.4 (2)
Sodium acetate + glucagon (1 mg/0.5 cm³)	7.2 ± 1.3[b] (2)	4.6 ± 0.5[b] (2)	4.4 ± 1.8 (2)	1.3 ± 0.1 (2)

[a] Data is expressed as picomoles cAMP/mg wet weight of liver. Values represent the mean ± SEM, and the number in parentheses indicates the number of animals per group. [b] $p < 0.01$ vs. control.

Materials and methods. After Nembutal® anesthesia, male Sprague-Dawley rats (170–220 g) were given i.v. injections (0.5 ml) of the following: sodium acetate (4.3 mg); lead acetate (20 mg); endotoxin (3.3 mg or 0.5 µg); phosphate-buffered saline; or glucagon. *Serratia marcescens* endotoxin (Lot No. 582087), prepared by the BOIVIN method, was obtained from Difco Laboratories (Detroit, Michigan). At various time intervals post-injection, rats were killed by submersion in liquid nitrogen. The frozen liver was isolated, extracted, and analyzed for cAMP, using the competitive binding method of GILMAN[7].

Results and discussion. The results, summarized in the Table, show that control injections of sodium acetate and saline caused no change in hepatic cAMP. Glucagon was used as a positive control, and, as expected, produced significant elevations in hepatic cAMP in the 1- and 5-min time intervals, post-injection. However, under the same conditions, rats given a LD_{50} dose (3.3 mg) of *S. marcescens* endotoxin showed no change in cAMP concentrations. Liver samples were examined as much as 4 h post-injection, when some of the endotoxin-treated animals had begun to die. Even at that time, there was no significant alteration in cAMP levels, indicating that control of hepatic cAMP may not be a significant step in the mechanism of endotoxin toxicity. These results, however, do not rule out the possibility of very small, localized changes in hepatic cAMP concentrations, which affect carbohydrate metabolism, but are not detected in measurements of the total hepatic cAMP pool. In contrast, bacterial exotoxins, such as cholera toxin, have been shown to markedly elevate cAMP in the intestinal mucosa[8].

An additional group of rats was treated with lead acetate at a concentration which markedly sensitizes these animals to very small quantities of endotoxin[5,6]. Lead acetate is known to inhibit a number of enzymes, including cyclic nucleotide phosphodiesterase[9], and since this enzyme is important in cAMP metabolism, this would be a reasonable mechanism to explain the lead-endotoxin interaction. In the present study, however, when lead was given alone, or in combination with LD_{50} endotoxin, there was no significant change in endogenous cAMP (Table). Thus, the mechanism of lead acetate sensitization is not by means of marked elevations in hepatic cAMP, and alternative explanations should be considered for the meachnism of endotoxin lethality and lead-sensitization.

Zusammenfassung. Nachweis, dass die Konzentration von zyklischem AMP in Lebern normaler und Blei-sensibilisierter Ratten durch i.v. Injektionen von *Serratia marcescens* Endotoxin nicht beeinflusst wird.

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Influence of Calcium on the Interaction of Anesthetic Drugs with Artificial Phospholipid Membranes

In a previous paper[1], we reported a study on the effect of 2 drugs with local anesthetic properties (tetracaine and hexobarbital) on the electrical resistance of artificial phospholipid membranes (bilayers). Both drugs increase the resistance probably by forming hydrophobic bonds, but ionic interactions may alter drug activity by modifying the local concentration at the lipid-water interface.

It has been proposed[2,3] that calcium and local anesthetics compete with one another with respect to their

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