

## Effect of aspirin on normal and cholera toxin-stimulated intestinal electrolyte transport.

R K Farris, ... , D W Powell, S M Morris

*J Clin Invest.* 1976;57(4):916-924. <https://doi.org/10.1172/JCI108368>.

### Research Article

The effect of aspirin on normal and cholera toxin-stimulated electrolyte transport has been investigated in vitro, because this drug appears to inhibit cholera toxin-induced intestinal secretion in in vivo animal models. In the Ussing chamber, 10 mM aspirin decreased the control rabbit ileal potential difference and short-circuit current by 50% and increased conductance by 28%. Bidirectional electrolyte flux determinations showed that aspirin significantly increased both Na and Cl absorption and reduced flux (which probably represents HCO<sub>3</sub> secretion) to zero. This effect of aspirin appears to be identical to that reported to others with catecholamines as determined with similar techniques. However, alpha-adrenergic blockers did not prevent the electrical effects of aspirin, suggesting that aspirin does not have its effect through release of tissue stores of catecholamines. In the presence of aspirin, cholera toxin increased the potential difference and short-circuit current, and decreased the conductance of rabbit ileum in a fashion qualitatively similar to control tissues. However, aspirin reversed cholera toxin-stimulated Na transport from secretion to absorption, inhibited cholera toxin, induced Cl secretion by 58% and partially, but not significantly, inhibited HCO<sub>3</sub> secretion. Thus, the inhibitory effect of aspirin on cholera toxin-induced electrolyte secretion appears to be due to aspirin-stimulated Na and Cl absorption. Although aspirin reduced tissue cyclic AMP concentrations in normal and cholera toxin-stimulated ileum, it also inhibited the electrolyte [...]

**Find the latest version:**

<https://jci.me/108368/pdf>



# Effect of Aspirin on Normal and Cholera Toxin-Stimulated Intestinal Electrolyte Transport

R. KENT FARRIS, EDWARD J. TAPPER, DON W. POWELL, and SARAH M. MORRIS

*From the Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514*

**ABSTRACT** The effect of aspirin on normal and cholera toxin-stimulated electrolyte transport has been investigated in vitro, because this drug appears to inhibit cholera toxin-induced intestinal secretion in vivo animal models. In the Ussing chamber, 10 mM aspirin decreased the control rabbit ileal potential difference and short-circuit current by 50% and increased conductance by 28%. Bidirectional electrolyte flux determinations showed that aspirin significantly increased both Na and Cl absorption and reduced the residual flux (which probably represents HCO<sub>3</sub> secretion) to zero. This effect of aspirin appears to be identical to that reported by others with catecholamines as determined with similar techniques. However,  $\alpha$ -adrenergic blockers did not prevent the electrical effects of aspirin, suggesting that aspirin does not have its effect through release of tissue stores of catecholamines. In the presence of aspirin, cholera toxin increased the potential difference and short-circuit current, and decreased the conductance of rabbit ileum in a fashion qualitatively similar to control tissues. However, aspirin reversed cholera toxin-stimulated Na transport from secretion to absorption, inhibited cholera toxin-induced Cl secretion by 58%, and partially, but not significantly, inhibited HCO<sub>3</sub> secretion. Thus, the inhibitory effect of aspirin on cholera toxin-induced electrolyte secretion appears to be due to aspirin-stimulated Na and

Cl absorption. Although aspirin reduced tissue cyclic AMP concentrations in normal and cholera toxin-stimulated ileum, it also inhibited the electrolyte secretion induced by exogenous cyclic AMP. Thus, if aspirin's stimulatory effect on sodium and anion absorption in normal tissue and its inhibitory effect on cholera toxin-stimulated sodium and anion secretion involves a cyclic AMP-mediated system, the effect must be at a step distal to cyclic AMP production or degradation. The exact mechanism of aspirin's effect on normal and cholera toxin-induced electrolyte transport, and its possible usefulness in the treatment of cholera diarrhea, remains to be determined.

## INTRODUCTION

The hypothesis that prostaglandins might be involved in the mechanism of action of cholera toxin (1) gained support when two separate groups demonstrated that anti-inflammatory drugs such as aspirin (ASA)<sup>1</sup> inhibited cholera-induced intestinal secretion (2, 3). However, subsequent reports have presented significant evidence against this hypothesis. First, it has been demonstrated that the stimulatory effects of cholera toxin and prostaglandins on intestinal adenylate cyclase activity are additive, suggesting that in the intestine there are separate receptors for these two agonists (4). More importantly, it has been shown that ASA and indomethacin, in doses sufficient to inhibit prostaglandin synthesis, do not inhibit the stimulation of cyclic AMP (cAMP) production by cholera toxin in human leukocytes (5) or in rabbit ileal mucosa (6). Finally, Hindi et al. have shown that cholera toxin stimulates cAMP production and intestinal secretion, but not prostaglandin synthesis, in rabbit in-

---

This work was presented in part at the American Gastroenterological Association Meeting in San Antonio, Tex., May 1975. (1975. *Gastroenterology*. 68: 968.)

Dr. Farris was supported by a training grant in gastroenterology (AM 05503) and Dr. Tapper by a National Institutes of Health Postdoctoral Individual Research Fellowship (AM 01879), both from the National Institute of Arthritis, Metabolism and Digestive Diseases. Dr. Powell is the recipient of a Research Career Development Award (AM 70454) from the National Institute of Arthritis, Metabolism and Digestive Diseases.

Received for publication 10 June 1975 and in revised form 1 December 1975.

---

<sup>1</sup> Abbreviations used in this paper: ASA, aspirin; cAMP, cyclic AMP; G, conductance; cGMP, cyclic guanosine 5'-monophosphate; I<sub>sc</sub>, short-circuit current; J, flux; m, mucosal; PD, potential difference; R, residual; s, serosal.

testine (7). Although these studies speak strongly against a role for prostaglandins in the secretory activity of cholera toxin, they do not explain the previously reported inhibitory action of ASA (2, 3). The experiments reported here demonstrate this inhibitory action of ASA in vitro, and suggest that this action may be due, at least in part, to stimulatory effects of ASA on the intestinal absorptive ion-transporting mechanisms.

## METHODS

All experiments were performed on the terminal ileum of 3-kg, male, New Zealand white rabbits which were not fasted. Studies were performed using a Ringer solution of the following composition, in millimoles per liter: Na, 140; K, 5.2; Ca, 1.2; Mg, 1.2; Cl, 119.8;  $\text{HCO}_3$ , 25;  $\text{H}_2\text{PO}_4$ , 0.4; and  $\text{HPO}_4$ , 2.4. Solution pH was 7.4 when bubbled with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ . Either mannitol or ASA was present in the solutions in 0.1-20 mM concentrations. To adjust the pH of ASA-containing solution to 7.4, they were titrated with 10 N NaOH. This added 2-4 meq/liter Na to the 10-mM ASA solutions. In cAMP experiments, the Na salt of the nucleotide was added to solutions with reduced NaCl concentration (135 mM Na, 114.8 Cl), so that the final Na and Cl concentration differed little from the basic Ringer solution. In the experiments reported here, *n* equals the number of animals. The significance of differences in means was tested by the paired and unpaired Student's *t* test, and by an analysis of variance (8).

**Electrolyte transport measurements.** The methods of removing the muscle layers from the intestine, and measuring electrolyte fluxes, short-circuit current ( $I_{sc}$ ), potential difference (PD), and conductance (G) have been previously described (9). In brief, after stripping the muscularis, four sections of terminal ileum were mounted as a flat sheet in Ussing-type chambers with apertures of 1.13 cm<sup>2</sup>. Circulating 37°C Ringer reservoirs on the mucosal (m) and serosal (s) sides of the intestine were connected to an automatic voltage clamp via Ringer-agar bridges and calomel and Ag-AgCl electrodes. Tissues were continuously short-circuited except for 15-s intervals every 20 min when the open-circuit PD was read. G was calculated from the PD and  $I_{sc}$  using Ohm's law. Radioisotopes and cholera toxin were added to the bathing solutions upon stabilization of the electrical parameters (usually 10-30 min after mounting the tissues). Active cholera toxin<sup>2</sup> was added to the mucosal solution (2 µg/ml) of one-half the mounted tissues and heat-inactivated cholera toxin to the other tissues which served as controls. Na and Cl fluxes were measured in separate pieces of intestine. Simultaneous, bidirectional Na flux determinations were performed with <sup>22</sup>Na and <sup>24</sup>Na, while oppositely directed unidirectional flux rates were obtained with <sup>36</sup>Cl on adjacent pieces of intestine. Electrolyte flux measurements were performed in four conditions: 10 mM mannitol-Ringer without and with cholera toxin, and 10 mM ASA-Ringer without and with cholera toxin. These four conditions are henceforth known, respectively, as the control, cholera toxin, ASA, and ASA-cholera toxin tissues. Na flux determinations were made in all four conditions at the same time on intestine from the same animals. Chloride flux

rates were measured in control and cholera toxin-treated tissues in one group of animals and, in separate animals, in ASA and ASA-cholera toxin tissues. In separate studies, oppositely directed Na and Cl fluxes were measured in adjacent pieces of ileum mounted in mannitol or ASA-Ringer solution containing 7.5 mM cAMP (Sigma Chemical Co., St. Louis, Mo.) in the serosal bath and 7.5 mM mannitol in the mucosal bath. Seven 20-min flux periods were measured beginning 20 min after addition of isotopes and/or cholera toxin. Since 45-60 min are required for any cholera toxin effect, the value for each animal was derived by averaging the last three flux periods (100-160 min after cholera toxin), and the mean±SE for each group calculated from these values. In the cAMP experiments, the fluxes were derived by averaging all seven periods. The ability of α-adrenergic blocking agents to inhibit ASA action was explored by determining the effect of ASA on the electrical parameters of rabbit ileum after prior incubation of the tissue with either 10 µM phentolamine (Regitine mesylate; Ciba Pharmaceutical Co., Summit, N. J.) or 10 µM phenoxymethazine (Dibenzylamine; Smith, Kline & French, Philadelphia, Pa.).

**Cyclic nucleotide measurements.** Rabbit ileum was quickly removed and rinsed with ice-cold Ringer solution saturated with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ . The mucosa was separated from the underlying muscle by scraping with the edge of a glass slide, minced into small pieces, and kept cold until it was placed in the incubation solutions. Incubation solutions were the same as those used in flux studies; 10 mM mannitol-Ringer or ASA-Ringer, with or without cholera toxin (2-5 µg/ml). Approximately 300-500 mg of tissues was placed in each Erlenmeyer flask, gassed with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ , and continuously shaken at 100 rpm at 37°C in a Dubnoff metabolic shaker. Samples of tissue for cyclic nucleotide assay were taken immediately before incubation and after 30 min, 120 min, and 180 min of incubation. Each sample was rapidly frozen in liquid nitrogen and kept at -80°C until processing. Tissue samples were then homogenized in 6% trichloroacetic acid and cyclic nucleotide content measured in triplicate by the radioimmunoassay of Steiner et al. (10). Results were expressed as picomoles cyclic nucleotide per milligram Lowry protein (11).

Specificity of the assay was determined in three ways. 50 pmol of cAMP and 5 pmol cyclic guanosine 5'-monophosphate (GMP) (both from Sigma Chemical Co.) were added for recovery. The recovery (*n* = 10) was 98.7±2.88% for cAMP and 104.2±7.1% for cGMP. Treatment of tissue extracts with beef phosphodiesterase (Sigma Chemical Co., lot 120C-T, 7401) at 37°C for 45 min resulted in destruction of 80-100% of cGMP and 84-100% of cAMP. In addition, graded amounts of tissue extracts added to the radioimmunoassay yielded linear measurements of both cyclic nucleotides. When possible, all samples for either cyclic nucleotide assay were assayed together, thereby giving very small interassay variability. The radioimmunoassay for cyclic nucleotides gave a binding capacity for cAMP of 43% and for cGMP of 45%.

## RESULTS

### Aspirin-cholera toxin studies

**Electrical parameters.** The effect of ASA (0.1-10 mM) on the electrical parameters (PD,  $I_{sc}$ , and G) of rabbit ileum is shown in Fig. 1. The PD was significantly reduced by 5 mM ASA, but a consistent effect on all the electrical parameters was not evident until 10 mM

<sup>2</sup> Prepared under contract for the National Institute of Allergy and Infectious Diseases by R. A. Finkelstein, Ph.D., The University of Texas Southwestern Medical School, Dallas, Tex.

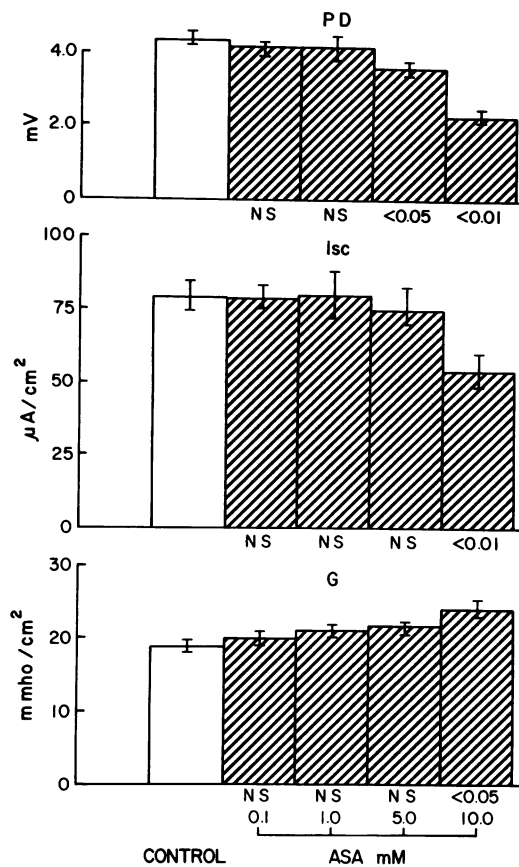


FIGURE 1 The effect of ASA, 0.1–10 mM, on the PD, I<sub>sc</sub>, and G of in vitro rabbit ileum. The means ± SE are compared to 10 mM mannitol (control) in paired tissues from the same animals (*n* = 6). The *P* values below the bars refer to the significance of difference between that mean ± SE and the control. NS = not significant.

ASA was employed. In separate experiments (*n* = 6), the effect of 20 mM ASA was examined. This high concentration often reduced the PD and the I<sub>sc</sub> to zero and greatly increased G, making it difficult to tell if the tissues were viable. Because of this, and since ASA is of limited solubility at a neutral pH, 10 mM ASA was chosen as the concentration for the remainder of the studies.

We next investigated whether ASA has its effect on, or via entry through, either the m or s cell membranes. Fig. 2 demonstrates the effect of 10 mM ASA on the electrical parameters when the drug was in only the m or s bathing solutions. The effect of ASA in the s solution is far greater than when ASA is only in the m solution and does not differ, importantly, from the effects noted with ASA in both the m and s solutions. For convenience, subsequent studies were performed with ASA in both the m and s solutions. Replacement of ASA-Ringer with mannitol-Ringer solutions led to a transient

(45 min) increase in PD and I<sub>sc</sub> that was approximately 75% of control values, indicating at least partial reversibility of the ASA effect.

The time-course of the PD, I<sub>sc</sub>, and G of rabbit ileum mounted in either 10 mM mannitol-Ringer or ASA-Ringer, and the effect of cholera toxin on these electrical parameters in both solutions, is shown in Fig. 3. As previously reported (12, 13), the response of the electrical parameters to cholera toxin was a sustained increase in PD, a relatively short-lived increase in I<sub>sc</sub> and a significant reduction in G. ASA markedly depressed the PD and I<sub>sc</sub>, and increased G of rabbit ileum, but the response to cholera toxin was similar to that noted in mannitol-Ringer solution. The only demonstrable difference in the effect of cholera toxin was that a sustained increase in the I<sub>sc</sub> was elicited in ASA-Ringer. Table I summarizes the mean electrical parameters for the four conditions for the 100–160-min time period. ASA decreased the PD and I<sub>sc</sub> of rabbit ileum by 57 and 46%,

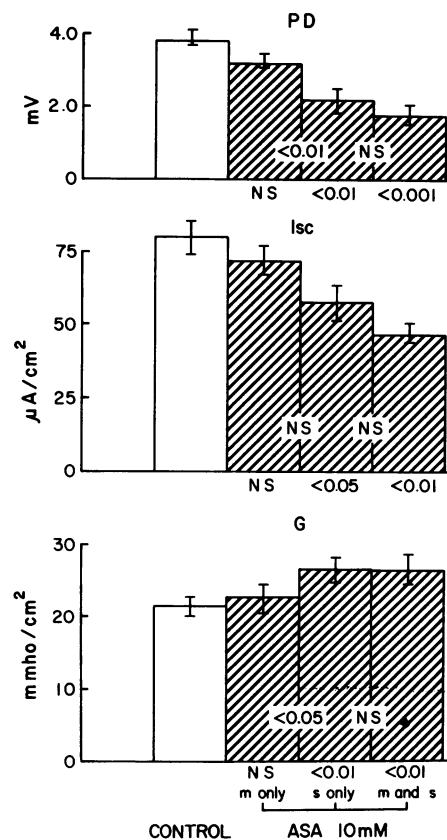


FIGURE 2 The effect of 10 mM ASA on the PD, I<sub>sc</sub>, and G of rabbit ileum when the drug is present in only the mucosal (m), serosal (s), or both (m and s) bathing solutions. The values are compared to 10 mM mannitol in paired tissues from the same animals (*n* = 7). The *P* values below the bars refer to the significance of differences between that mean ± SE and the control, and the *P* values between bars refers to those two means. NS = not significant.

respectively, and at the same time increased  $G$  (28%). Cholera toxin had an opposite effect; it significantly increased the PD by 20% and decreased  $G$  by 17%. However, in ASA solution, the response to cholera toxin was qualitatively similar to that in mannitol solution; the PD was increased (80%),  $G$  was reduced (14%), and now there was a sustained increase in  $I_{sc}$  (55%). The differences between the electrical parameters of cholera toxin-treated and ASA-cholera toxin-treated intestine were all significant ( $P < 0.001$ ).

**Electrolyte fluxes.** Table II indicates the unidirectional and net electrolyte fluxes in paired tissues in the four conditions (control, ASA, cholera toxin, and ASA-cholera toxin). ASA significantly increased both the m to s flux (m to s;  $J_{ms}^{Na}$ ) and s to m flux (s to m;  $J_{sm}^{Na}$ ) of Na. The effect on  $J_{ms}^{Na}$  is proportionately greater, thus net Na flux ( $J_{net}^{Na}$ ) was increased from  $1.1 \pm 0.3$  to  $3.0 \pm 0.50 \mu\text{eq}/\text{h} \cdot \text{cm}^2$ . Cholera toxin had an opposite effect, in that it significantly inhibited  $J_{ms}^{Na}$ , resulting in net Na secretion of  $-0.4 \pm 0.4$ . In the presence of ASA, cholera toxin had no effect on  $J_{ms}^{Na}$ , as compared to the control, and net Na transport appears to be reversed from se-

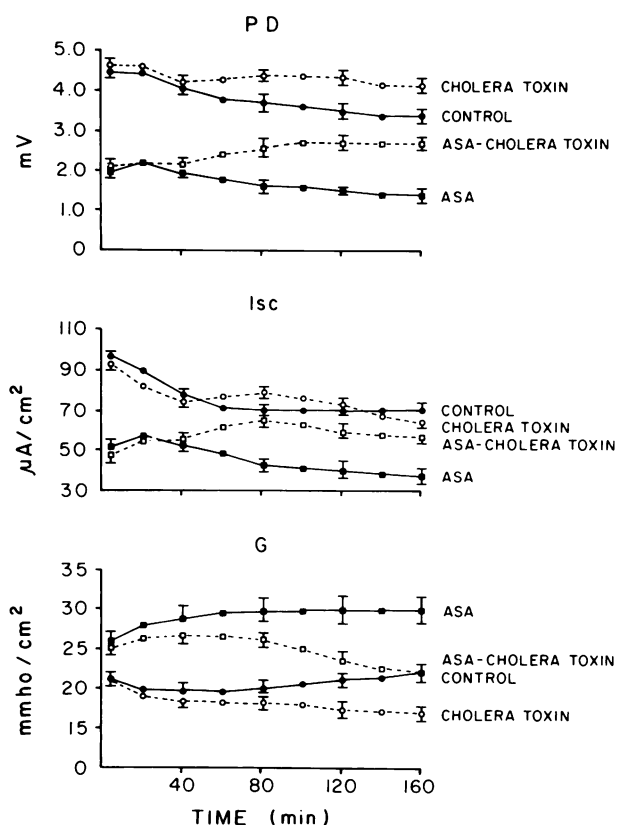


FIGURE 3 The time-course of the PD,  $I_{sc}$ , and  $G$  of rabbit ileum in 10 mM mannitol-Ringer solution, without (control, ●,  $n = 63$ ) and with cholera toxin (○,  $n = 63$ ); and in 10 mM ASA-Ringer solution, without (■,  $n = 47$ ) and with cholera toxin (□,  $n = 47$ ). Means  $\pm$  SE are given.

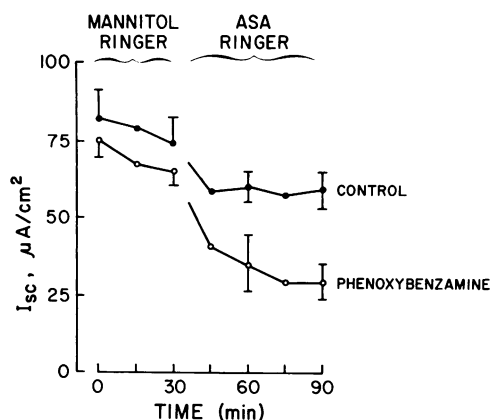


FIGURE 4 The effect of  $10 \mu\text{M}$  phenoxybenzamine on the ASA-induced inhibition of the  $I_{sc}$  of rabbit ileum (mean  $\pm$  SE). Paired tissues ( $n = 5$ ) were incubated in mannitol-Ringer solution with or without phenoxybenzamine for 30 min and then the solution was changed to ASA-Ringer, with or without phenoxybenzamine.

cretion (as seen in cholera toxin-treated tissues) to absorption of  $0.6 \pm 0.3 \mu\text{eq}/\text{h} \cdot \text{cm}^2$ . This value was not significantly different from the control.

The effect of ASA on Cl fluxes in control and cholera toxin-stimulated ileum is very similar to that on Na flux. ASA increased both unidirectional fluxes,  $J_{ms}^{Cl}$  greater than  $J_{sm}^{Cl}$ , such that  $J_{net}^{Cl}$  was increased from  $0.3 \pm 0.3$  to  $1.6 \pm 0.5 \mu\text{eq}/\text{h} \cdot \text{cm}^2$ . In control tissues, cholera toxin caused Cl secretion,  $-1.2 \pm 0.2 \mu\text{eq}/\text{h} \cdot \text{cm}^2$ , primarily through inhibition of  $J_{ms}^{Cl}$ . In the presence of ASA, cholera toxin-stimulated Cl secretion appears to be inhibited by 58% ( $-1.2$  to  $-0.5 \pm 0.4 \mu\text{eq}/\text{h} \cdot \text{cm}^2$ ). This difference in  $J_{net}^{Cl}$  was significant at  $P < 0.05$ .

The residual flux,  $J_{net}^R$ , which represents the net ionic transport unaccounted for by the measured net Na and Cl fluxes, can be calculated by combining the  $I_{sc}$  from the Na and Cl experiments as follows:  $J_{net}^R = I_{sc} - (J_{net}^{Na} - J_{net}^{Cl})$ . Although  $J_{net}^R$  could represent net cation absorption or anion secretion of any charged species, there is compelling evidence in rabbit ileum that  $J_{net}^R$  represents net  $\text{HCO}_3^-$  secretion (14).<sup>3</sup> ASA reduced  $J_{net}^R$  to zero. Cholera toxin had no effect on  $J_{net}^R$  in mannitol-Ringer solution. ASA appeared to inhibit  $J_{net}^R$  in the presence of cholera toxin by 39%, but this decrease in  $J_{net}^R$  from  $1.85 \pm 0.33$  to  $1.1 \pm 0.20 \mu\text{eq}/\text{h} \cdot \text{cm}^2$  was not statistically significant by an analysis of variance.<sup>4</sup>

<sup>3</sup> By  $\text{HCO}_3^-$  secretion, we do not necessarily imply that the ion species transported is  $\text{HCO}_3^-$ .  $\text{H}^+$  or  $\text{OH}^-$  transport could give similar results (15).

<sup>4</sup> As determined in the present experiments,  $J_{net}^R$  is the algebraic sum of five values:  $I_{sc}$ ,  $J_{ms}^{Na}$ ,  $J_{sm}^{Na}$ ,  $J_{ms}^{Cl}$ , and  $J_{sm}^{Cl}$ . The proper statistical test for the significance of differences in  $J_{net}^R$  is, therefore, an analysis of variance which taken into account the errors inherent in each of these five values.

TABLE I  
PD,  $I_{sc}$ , and G of Rabbit Ileum Mounted in 10 mM Mannitol-Ringer (Control) or 10 mM ASA-Ringer Solution, with and without Cholera Toxin, 2.0  $\mu\text{g/ml}$ \*

	Control (63)‡	ASA (47)	Cholera toxin (63)	P§	ASA-cholera toxin (47)
PD, mV	3.5±0.1	1.5±0.1	4.2±0.1	<0.001	2.7±0.1
$I_{sc}$ , $\mu\text{A}/\text{cm}^2$	71±1.7	38±2.5	71±1.7	<0.001	59±2.7
G, mmho/cm <sup>2</sup>	21.3±0.7	27.3±1.2	17.7±0.5	<0.001	23.4±0.8¶

\* Means±SE for the 100–160-min period after mounting the tissues and addition of cholera toxin.

‡ Number of tissues, in parentheses.

§ Significance of difference between cholera toxin- and ASA-cholera toxin-treated tissues.

|| and ¶ Significance of difference between this value and the control,  $P < 0.001$  and  $P < 0.05$ , respectively.

**Adrenergic blocker studies.** The effects of ASA on the electrical parameters and electrolyte fluxes in the *in vitro* rabbit ileum are similar to those of epinephrine as reported by Field and McColl (16). These investigators also showed that the inhibitory effect of epinephrine on the  $I_{sc}$  was prevented by  $\alpha$ -adrenergic blockers, but not by  $\beta$ -adrenergic blockers. Since ASA's effect could be due to release of catecholamines from tissue stores, we determined the ability of  $\alpha$ -blockers to prevent the inhibitory effect of ASA on the electrical parameters in rabbit ileum. Paired tissues were incubated for 30 min in mannitol-Ringer solution in the presence or absence of 10  $\mu\text{M}$  phenoxybenzamine or phentolamine. The mannitol-Ringer was then replaced by ASA-Ringer (with or without the blocker) and the electrical parameters followed for an additional hour. As shown in Fig. 4, phenoxybenzamine did not prevent the inhibitory effect of ASA on the  $I_{sc}$  of rabbit ileum. In fact, as was also true with phentolamine ( $n = 3$ ), the ASA inhibition of  $I_{sc}$  (and PD) was greater in the tissue-pair incubated with the  $\alpha$ -blocker.

**Cyclic nucleotide studies.** Since ASA's action could involve cyclic nucleotides, tissue cAMP and cGMP con-

centrations were measured after incubation under the same four conditions as electrolyte fluxes were measured: control, ASA, cholera toxin, and ASA-cholera toxin (Fig. 5). Cholera toxin significantly increased cAMP concentrations at 120 and 180 min of incubation as compared to controls ( $P < 0.001$ ). ASA reduced cAMP levels in both unstimulated ( $P < 0.05$ ) and cholera toxin-stimulated ( $P < 0.01$ ) tissues. Neither ASA nor cholera toxin had any effect on tissue cGMP concentrations.

The ability of ASA to inhibit cAMP-stimulated electrolyte secretion was further studied by the simultaneous measurement of Na and Cl fluxes in paired tissues secreting in response to exogenously added cAMP (Table III). The secretory rate in these tissues was several-fold greater than that induced by cholera toxin (Table II).<sup>5</sup> Nevertheless, ASA reduced the secretory rates of Na, Cl, and  $\text{HCO}_3^-$  ( $J_{net}^R$ ) by approximately 50%. In absolute terms, the ASA-induced change in net electrolyte flux

<sup>5</sup> The secretory response to cAMP reported here was somewhat greater than that reported previously from this laboratory (13) or by others (17, 18), and probably represents biological variability among different animal populations.

TABLE II  
Sodium, Chloride, and Bicarbonate ( $J_{net}^R$ ) Fluxes in Rabbit Ileum Mounted in 10 mM Mannitol-Ringer (Control) or 10 mM ASA-Ringer Solution, with and without Cholera Toxin, 2.0  $\mu\text{g/ml}$ \*

Solution (n)‡	Sodium			Chloride			$J_{net}^R$ §	$I_{sc}$
	$J_{ms}$	$J_{sm}$	$J_{net}$	$J_{ms}$	$J_{sm}$	$J_{net}$		
Control (13, 25)	10.5±0.7	9.4±0.6	1.1±0.3	6.3±0.3	6.0±0.2	0.3±0.3	1.9±0.1	2.65±0.04
ASA (13, 17)	14.4±0.7	11.4±0.6¶	3.0±0.5	9.2±0.4**	7.6±0.4**	1.6±0.5¶	0±0.2¶	1.42±0.09**
Cholera toxin (13, 25)	8.5±0.4¶	8.9±0.6	-0.4±0.4	4.3±0.2**	5.5±0.2¶	-1.2±0.2	1.9±0.3	2.65±0.04
P††	<0.01	NS	<0.01	<0.001	<0.001	<0.05	NS	<0.001
ASA-cholera toxin (13, 17)	10.7±0.7	10.1±0.6	0.6±0.3	7.7±0.6¶	8.2±0.4**	-0.5±0.4	1.1±0.2	2.22±0.10

\* Means±SE for the 100–160-min period after mounting the tissues and addition of cholera toxin; microequivalents per hour per square centimeter.

‡ Number of tissues in Na experiments and number of tissue pairs in Cl experiments.

§  $J_{net}^R = I_{sc} - (J_{Na_{net}} - J_{Cl_{net}})$  and could represent net cation absorption or anion secretion. There is evidence in rabbit ileum that  $J_{net}^R$  is  $\text{HCO}_3^-$  secretion (see text).

||, ¶, \*\* Significance of difference between this value and the control,  $P < 0.01$ ,  $< 0.05$ , and  $< 0.001$ , respectively.

†† Significance of difference between cholera toxin- and ASA-cholera toxin-treated tissues. NS = not significant.

was greater in the cAMP studies than in the cholera toxin studies. For example, the ASA-induced change in  $J_{net}^{Na}$  was  $(-3.5) - (-6.5) = 3.0$  in the cAMP group and only  $0.6 - (-0.4) = 1.0 \mu\text{eq}/\text{h}\cdot\text{cm}^2$  in the cholera toxin group.

## DISCUSSION

Although the previously cited studies (4-7) essentially rule out a role for prostaglandins as a link between cholera toxin and the resulting diarrhea, it is clear from experiments in vivo (2, 3), and the present experiments in vitro, that ASA inhibits cholera toxin-stimulated intestinal secretion and stimulates electrolyte absorption in normal intestine. An inhibitory effect of ASA on intestinal secretion of other types has been noted previously in studies of spontaneous secretion from canine duodenal pouches (19). In addition, other anti-inflammatory

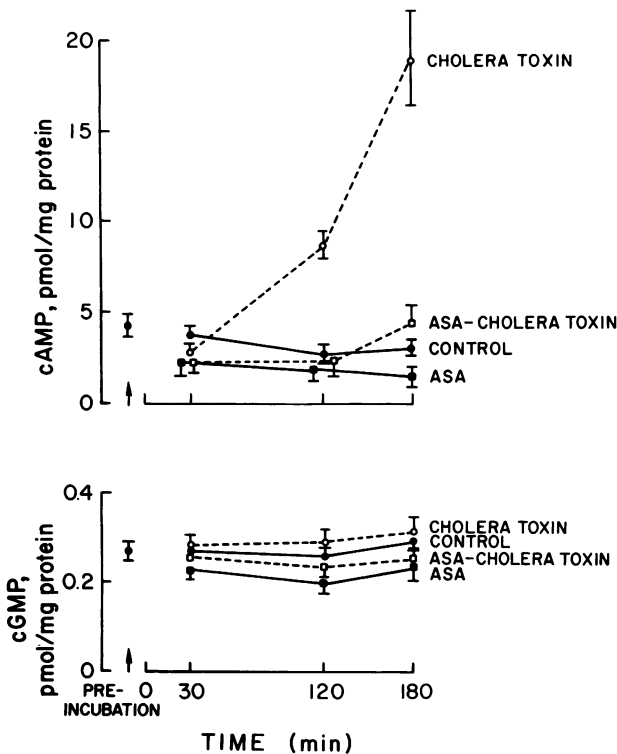


FIGURE 5 Rabbit ileal cAMP and cGMP concentrations (mean  $\pm$  SE) in paired tissues ( $n=5$ ) incubated in mannitol-Ringer solution; without (control, ●), and with cholera toxin (○); and in ASA-Ringer solution, without (■), and with cholera toxin (□). cAMP concentrations were significantly greater than control values after 120-180-min incubation with cholera toxin ( $P < 0.001$ ). ASA is significantly different from control ( $P < 0.05$ ), and ASA-cholera toxin is significantly different from cholera toxin ( $P < 0.01$ ) at 120 and 180 min also. There was no significant difference in cGMP concentrations between the tissues incubated in any of the four conditions.

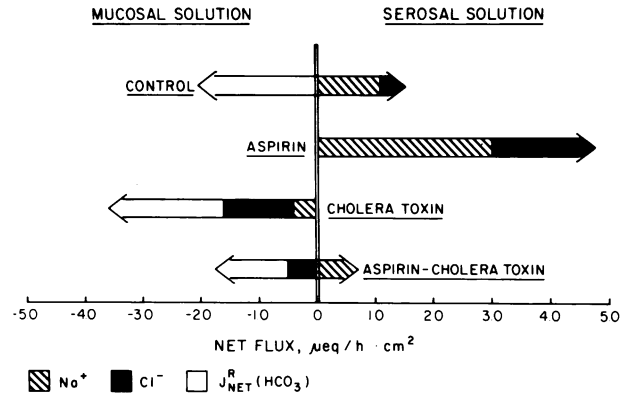


FIGURE 6 Summary of the effects of ASA, cholera toxin, and ASA-cholera toxin on net ion transport in the short-circuited rabbit ileum. Net transport rates of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $J_{net}^B$  ( $\text{HCO}_3^-$ ) are given. The length of the bars refer to the magnitude of transport. Bars to the left of zero infer net s to m transport (secretion), while bars to the right of zero infer net m to s transport (absorption).

drugs, such as indomethacin, have been shown to inhibit cholera-, salmonella-, or shigella-mediated intestinal secretion (20, 21). Our investigation gives some insight into the possible mechanism of action of these anti-inflammatory drugs. In Fig. 6 are summarized the directions and magnitudes of net transport of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $J_{net}^B$  ( $\text{HCO}_3^-$ ) in control, ASA, cholera toxin, and ASA-cholera toxin-treated intestine. ASA promotes solute (and presumably water) absorption in rabbit intestine by markedly stimulating net  $\text{Na}^+$  and  $\text{Cl}^-$  absorption and inhibiting  $\text{HCO}_3^-$  secretion. Cholera toxin's effect is essentially the opposite—it stimulates  $\text{Na}^+$  and  $\text{Cl}^-$  secretion both in vitro and in vivo, and in vivo it stimulates  $\text{HCO}_3^-$  secretion as well. When cholera toxin is given together with ASA, the resulting pattern of transport appears to be the algebraic sum of the opposing absorptive and secretory action of these two agents. In addition, although the cholera toxin-induced changes in the electrical parameters of the in vitro rabbit ileum were somewhat different in ASA-Ringer as compared to mannitol-Ringer (Fig. 3), the PD and  $I_{sc}$  were stimulated by cholera toxin in both instances. Together, these points suggest that the inhibitory effect of ASA on cholera toxin-stimulated secretion is simply one of stimulated absorption superimposed on continued secretion. In this respect it would be similar to the effect of intraluminal glucose on cholera toxin-stimulated secretion. However, as discussed below, it is also possible that ASA has its effect through inhibition of cAMP-mediated secretory mechanisms.

ASA has a significant effect on intestinal transport in normal tissues. First, ASA significantly decreased the PD and  $I_{sc}$  of in vitro rabbit ileum by approximately 50%, and increased G by 28%. This effect of ASA is

TABLE III  
Sodium, Chloride, and Bicarbonate ( $J_{net}^R$ ) Fluxes in Rabbit Ileum Mounted in Ringer Solution Containing 7.5 mM cAMP and either 10 mM Mannitol or 10 mM Aspirin\*

Solution (n)‡	Sodium			Chloride			$J_{net}^R$ §	$I_{sc}$
	$J_{ms}$	$J_{sm}$	$J_{net}$	$J_{ms}$	$J_{sm}$	$J_{net}$		
cAMP (11)	4.6±0.2	11.1±0.5	-6.5±0.5	4.0±0.3	5.5±0.3	-1.4±0.3	7.5±0.6	2.5±0.1
P	<0.001	NS	<0.001	<0.05	NS	<0.05	<0.02	<0.001
cAMP-ASA (11)	6.7±0.5	10.2±0.5	-3.5±0.5	5.0±0.4	5.7±0.6	-0.7±0.3	4.4±0.8	1.8±0.1

\* Means±SE for the period 30–160 min after mounting the tissues,  $\mu\text{eq}/\text{h}\cdot\text{cm}^2$ .

‡ Number of tissue pairs, in parentheses.

§  $J_{net}^R = I_{sc} - (J_{net}^{Na} - J_{net}^{Cl})$  and could represent net cation absorption or anion secretion. There is evidence in rabbit ileum that  $J_{net}^R$  is  $\text{HCO}_3^-$  secretion (see text).

|| Significance of difference between cAMP and cAMP-ASA treated tissues. NS = not significant.

similar to that noted by Kasbekar in the in vitro gastric mucosa (21, 22). However, in intestine, in spite of this seemingly noxious effect, the active m to s transport of both Na and Cl were significantly stimulated. The reduction in PD and  $I_{sc}$  might be secondary to an inhibition of electrogenic  $\text{HCO}_3^-$  secretion, since the residual flux, which probably represents  $\text{HCO}_3^-$  secretion, was reduced to zero by ASA. However, there is controversy regarding the rheogenicity of  $\text{HCO}_3^-$  secretion in the rabbit ileum (17, 23, 24). Therefore, the ASA effect on PD and  $I_{sc}$  may be due to inhibition of other rheogenic ion transport processes. Another significant difference between the gastric and intestinal response to ASA in vitro is that ASA is significantly more effective and its action irreversible when added to the m bath of the gastric preparation. In intestine, ASA is more effective when added to the s bath and the effect on intestine also seems to be partially reversible by changing the bathing solution to ASA-free Ringer.

One of the most intriguing aspects regarding the present results is the striking resemblance between the effect of ASA and the effect of catecholamines on the normal, in vitro rabbit ileum. As reported by Field et al. (16, 18) in studies with techniques essentially identical to those used here, epinephrine and norepinephrine inhibited the PD and  $I_{sc}$ , increased G, stimulated Na and Cl absorption, and reduced  $J_{net}^R$  ( $\text{HCO}_3^-$  secretion) to zero. The mechanism of the effect of these  $\alpha$ -adrenergic drugs is unclear at present (18). An obvious explanation for the mechanism of ASA's action could be that ASA has adrenergic activity or that it releases catecholamines from terminal sites in the intestinal mucosa. There is no precedent for the first explanation, in that ASA does not have adrenergic activity when tested in other systems (25). In addition, we have been unable to block the ASA effect on the electrical parameter with  $\alpha$ -adrenergic blockers which do block these catecholamine effects in rabbit ileum (16, 18). This suggests that the effect of

ASA on ion transport is distal to the site where  $\alpha$ -adrenergic drugs enter this chain of events. ASA may work directly on the cell membranes or the ion-transporting pumps. Such an action of salicylate has been reported in the erythrocyte (26, 27) and nervous tissues (28). Alternatively, as discussed below, ASA's effect could involve cAMP-mediated mechanisms.

In spite of evidence suggesting that ASA's absorptive effects are independent from cholera toxin's secretory action, it is possible that the secretory and absorptive processes of rabbit ileum are closely interrelated. Nellans et al. (29) and Schultz and Curran (30) have suggested that the brush border-located, coupled NaCl influx mechanism of rabbit ileum (31) responds to changes in intracellular cAMP concentrations by either NaCl ( $\text{HCO}_3^-$ ) absorption or NaCl ( $\text{HCO}_3^-$ ) secretion. If this were true then the ASA (and catecholamines) effects could still involve cyclic nucleotides. Indeed, ASA reduced cAMP levels in normal tissue and inhibited the increase in tissue cAMP concentrations brought about by cholera toxin. These data, alone, would suggest that an inhibition of adenylate cyclase or a stimulation of phosphodiesterase by ASA reduced cellular cAMP concentrations which, in turn, inhibited secretion and/or stimulated absorption. Although this might be the case, our additional experiments with exogenous cAMP suggest that this alone cannot be the explanation of ASA's mechanism of action. Thus, ASA inhibited the Na and anion secretion induced by exogenously added cAMP, a condition where tissue production or degradation of cyclic nucleotides are less likely to be a limiting factor. Therefore, if ASA's effect involves a cAMP-mediated system, ASA might well operate at a step distal to cAMP production or degradation. The cAMP-protein kinase or protein kinase-electrolyte pump steps could be the locus of ASA's action. The effect of ASA on cholera toxin-induced intestinal secretion then becomes similar, from the point of view of the possible mechanisms involved,



to the ASA inhibition of cAMP-induced lipolysis in fat cells (32-34). In this model system. Schönhöfer et al. (33) and Dinnendahl et al. (34) have shown that salicylates decrease cAMP levels in fat cells but the more important effect seems to be inhibition of a cAMP-dependent protein kinase. These issues will need to be clarified in the case of ASA inhibition of cholera toxin action of the intestine.

Whether ASA is effective in the treatment of the diarrhea of cholera will have to be determined by trials in man. A tolerable blood level of ASA in man is 2-3 mM, which is somewhat lower than the effective dose in our in vitro studies. The data in Fig. 2 suggest that the blood concentration, rather than luminal concentration, would be important for the intestinal effect. Whether useful in the treatment of cholera or not, investigation of the mechanism of action of this drug might give important insight into the mechanism of electrolyte transport in normal intestine and into other possible therapeutic modalities for the treatment of diarrheas due to intestinal secretion.

#### ACKNOWLEDGMENTS

The authors gratefully acknowledge the guidance and facilities of Dr. Alton L. Steiner for the measurements of cyclic nucleotides.

This investigation was supported by research grant AM 15350 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

#### REFERENCES

- Bennett, A. 1971. Cholera and prostaglandins. *Nature (Lond.)* 231: 536.
- Jacoby, H. I., and C. H. Marshall. 1972. Antagonism of cholera enterotoxin by anti-inflammatory agents in the rat. *Nature (Lond.)* 235: 163-165.
- Finck, A. D., and R. L. Katz. 1972. Prevention of cholera-induced intestinal secretion in the cat by aspirin. *Nature (Lond.)* 238: 273-274.
- Kimberg, D. V., M. Field, J. Johnson, A. Henderson, and E. Gershon. 1971. Stimulation of intestinal mucosal adenylyl cyclase by cholera enterotoxin and prostaglandins. *J. Clin. Invest.* 50: 1218-1230.
- Bourne, H. R. 1973. Cholera enterotoxin: Failure of anti-inflammatory agents to prevent cyclic AMP accumulation. *Nature (Lond.)* 241: 399.
- Kimberg, D. V., M. Field, E. Gershon, and A. Henderson. 1974. Effects of prostaglandins and cholera enterotoxin on intestinal mucosal cyclic AMP accumulation. Evidence against an essential role for prostaglandins in the action of toxin. *J. Clin. Invest.* 53: 941-949.
- Hindi, S., N. Hudson, and D. E. Wilson. 1975. Prostaglandin E (PGE) in cholera toxin (CT) induced intestinal secretion. *Gastroenterology* 68: 1013. (Abstr.)
- Snedecor, G. W., and W. F. Cochran. 1967. *Statistical Methods*. Iowa State University Press, Ames, Iowa. 6th edition. 593 pp.
- Powell, D. W., H. J. Binder, and P. F. Curran. 1972. Electrolyte secretion by the guinea pig ileum in vitro. *Am. J. Physiol.* 223: 531-537.
- Steiner, A. L., A. S. Pagliara, L. R. Chase, and D. M. Kipnis. 1972. Radioimmunoassay for cyclic nucleotides. II. Adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in mammalian tissues and body fluids. *J. Biol. Chem.* 247: 1114-1120.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- Powell, D. W., H. J. Binder, and P. F. Curran. 1973. Active electrolyte secretion stimulated by cholera toxin in rabbit ileum in vitro. *Am. J. Physiol.* 225: 781-787.
- Powell, D. W., R. K. Farris, and S. T. Carbonetto. 1974. Theophylline, cyclic AMP, cholera toxin and electrolyte transport by rabbit ileum. *Am. J. Physiol.* 227: 1428-1435.
- Dietz, J., and M. Field. 1973. Ion transport in rabbit ileal mucosa. IV. Bicarbonate secretion. *Am. J. Physiol.* 225: 858-861.
- Hubel, K. A. 1974. The mechanism of bicarbonate secretion in rabbit ileum exposed to cholera toxin. *J. Clin. Invest.* 53: 964-970.
- Field, M., and I. McColl. 1973. Ion transport in rabbit ileal mucosa. III. Effect of catecholamines. *Am. J. Physiol.* 225: 852-857.
- Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic 3',5'-AMP. *Am. J. Physiol.* 221: 992-997.
- Field, M., H. E. Sheerin, A. Henderson, and P. L. Smitt. 1975. Catecholamine effects on cyclic AMP levels and ion secretion in rabbit ileal mucosa. *Am. J. Physiol.* 229: 86-92.
- Russell, T. R., and R. S. Jones. 1974. The effect of aspirin on duodenal secretion. *Proc. Soc. Exp. Biol. Med.* 145: 967-969.
- Gots, R. E., S. B. Formal, and R. A. Giannella. 1974. Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri*, and cholera-mediated rabbit ileal secretion. *J. Infect. Dis.* 130: 280-284.
- Wald, A., G. S. Gotterer, G. R. Rajendra, and T. R. Hendrix. 1975. Effect of indomethacin on cholera induced intestinal cAMP and fluid movement. *Clin. Res.* 23: 396A. (Abstr.)
- Kasbekar, D. K. 1973. Effect of salicylate and related compounds on gastric HCl secretion. *Am. J. Physiol.* 225: 521-527.
- Powell, D. W. 1974. Intestinal conductance and permselectivity changes with theophylline and cholera toxin. *Am. J. Physiol.* 227: 1436-1443.
- Field, M., D. Fromm, and I. McColl. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* 220: 1388-1396.
- Collier, H. O. J. 1969. A pharmacological analysis of aspirin. *Adv. Pharmacol. Chemother.* 7: 333-405.
- Wieth, J. O. 1970. Paradoxical temperature dependence of sodium and potassium fluxes in human red cells. *J. Physiol. (Lond.)* 207: 563-580.
- Wieth, J. O. 1970. Effect of some monovalent anions on chloride and sulfate permeability of human red cells. *J. Physiol. (Lond.)* 207: 581-609.
- Levitan, H., and J. L. Barker. 1972. Membrane permeability: cation selectivity reversibly altered by salicylate. *Science (Wash. D. C.)* 178: 63-64.
- Nellans, H. N., R. A. Frizzell, and S. G. Schultz. 1974. Brush border processes and transepithelial Na and Cl transport by rabbit ileum. *Am. J. Physiol.* 226: 1131-1141.

30. Schultz, S. G., and P. F. Curran. 1974. Sodium and chloride transport across isolated rabbit ileum. *Curr. Top. Membranes Transp.* 5: 225-281.
31. Nellans, H. N., R. A. Frizzell, and S. G. Schultz. 1973. Coupled sodium-chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* 225: 467-475.
32. Stone, D. B., J. D. Brown, and A. A. Steele. 1969. Effect of sodium salicylate on induced lipolysis in fat cells of the rat. *Metab. Clin. Exp.* 18: 620-624.
33. Schönhöfer, P. S., J. Sohn, H. D. Peters, and V. Dinnendahl. 1973. Effects of sodium salicylate and acetylsalicylic acid on the lipolytic system of fat cells. *Biochem. Pharmacol.* 22: 629-637.
34. Dinnendahl, V., H. D. Peters, and P. S. Schönhöfer. 1973. Effect of sodium salicylate and acetylsalicylic acid on cyclic 3',5'-AMP-dependent protein kinase. *Biochem. Pharmacol.* 22: 2223-2228.