1972

Repression of β -galactosidase synthesis in Escherichia coli by salicylates

Joan Carlyn Olson
Portland State University

Follow this and additional works at: https://pdxscholar.library.pdx.edu/open_access_etds

Part of the [Bacteriology Commons](#), and the [Microbial Physiology Commons](#)

Let us know how access to this document benefits you.

Recommended Citation

Olson, Joan Carlyn, "Repression of β -galactosidase synthesis in Escherichia coli by salicylates" (1972).
Dissertations and Theses. Paper 1125.
<https://doi.org/10.15760/etd.1125>

This Thesis is brought to you for free and open access. It has been accepted for inclusion in Dissertations and Theses by an authorized administrator of PDXScholar. Please contact us if we can make this document more accessible: pdxscholar@pdx.edu.

AN ABSTRACT OF THE THESIS OF Joan Carlyn Olson for the Master of Science in Biology presented July 26, 1972.

Title: Repression of β -Galactosidase Synthesis in Escherichia coli by Salicylates

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

[REDACTED]

John W. Myers, Chairman

[REDACTED]

Earl Fisher

[REDACTED]

Gordon L. Kilgour

Salicylic acid, and to a lesser extent aspirin, have been shown to repress β -galactosidase synthesis in Escherichia coli. The repression is not due to decreased inducer uptake, nor does it result from competition with inducer for repressor. Dinitrophenol does not exert similar effects on β -galactosidase synthesis. Cyclic adenosine 3',5'-monophosphate partially relieves the repression. The extent of the relief by cyclic adenosine monophosphate seems to depend on the concentration of salicylic acid. This indicates there may be direct interaction between the actions of cyclic adenosine monophosphate and salicylic acid in E. coli cells.

REPRESSION OF β -GALACTOSIDASE SYNTHESIS
IN ESCHERICHIA COLI BY SALICYLATES

by

JOAN CARLYN OLSON

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGY

Portland State University
1972

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of
Joan Carlyn Olson presented July 28, 1972.

John W. Myers, Chairman

Earl Fisher

Gordon L. Kilgour

APPROVED:

Earl Fisher, Head, Department of Biology

David T. Clark, Dean of Graduate Studies

July 28, 1972

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	iv
LIST OF FIGURES	v
 CHAPTER	
I INTRODUCTION	1
II MATERIALS AND METHODS	
Bacterial Strains	4
Growth Conditions	4
β -Galactosidase Assay	5
Materials	5
III RESULTS	
The Effects of Salicylates on β -Galactosidase Synthesis	6
The Effect of Cyclic AMP on Repression by Salicylic Acid	9
The Effect of Salicylates on Constitutive Enzyme Synthesis	16
The Effect of 2,4-Dinitrophenol on β -Galactosidase Synthesis	
IV DISCUSSION	23
BIBLIOGRAPHY	26
APPENDIX	28

LIST OF FIGURES

FIGURE	PAGE
1. Effect of Salicylic Acid on Differential Rate of β -Galactosidase Synthesis	11
2. Effect of Cyclic AMP and Salicylic Acid on Differential β -Galactosidase Synthesis	13
3. Effect of Cyclic AMP and Salicylic Acid on β -Galactosidase Synthesis	15
4. Effect of Cyclic AMP and Salicylic Acid on β -Galactosidase Synthesis	17
5. Effect of Salicylic Acid on Differential β -Galactosidase Synthesis in Constitutive Strain 3300	19
6. Effect of DNP on Differential β -Galactosidase Synthesis	22

LIST OF TABLES

TABLE		PAGE
I	Effects of Aspirin on Differential Rate of Synthesis of β -Galactosidase	7
II	Effect of Salicylic Acid on Differential Rate of Synthesis of β -Galactosidase	8

INTRODUCTION

Although aspirin is one of the most commonly used drugs, its actual mechanism of action within the body still remains unclear. Acetylsalicylic acid belongs to the group of drugs referred to as salicylates, all of which have similar pharmacological properties. Their activity is believed to be dependent on the drug's salicylic acid content, and substitutions on the carboxyl or hydroxyl groups alter only their toxicity or potency (1).

Aspirin is most commonly recognized for its analgesic, antipyretic, and antiinflammatory effects. Recently Vane (2) demonstrated antiinflammatory acids, such as aspirin, salicylic acid and indomethacin, inhibit enzymes which catalyze prostaglandin (PGE_2 and $\text{PGF}_{2\alpha}$ *) synthesis in cell free extracts of guinea pig lungs. PGE_1 and PGE_2 , when injected into the third ventricle of cats, also proves to be a potent inducer of fevers (2). Thus, Vane postulated aspirin produces its antipyretic and antiinflammatory responses by inhibiting synthesis of prostaglandins. Although prostaglandins when administered intradermally in man do not produce pain (2), some of aspirin's analgesic activity may result from reduction of inflammation (3). Smith and Willis (4) supported this evidence when they independently observed aspirin to inhibit conversion of arachidonic acid into prostaglandins in human platelets.

* All abbreviations used in text are defined in appendix.

Aspirin has many other diverse actions within the body. Included among these is its ability to prevent collagen and epinephrine induced aggregation of platelets. Platelet aggregation occurs in two phases. The first is characterized by a rapid change in the shape of platelets, the second, or actual aggregation of cells, follows the release of ADP from platelets (10). Aspirin blocks the second phase of aggregation (7)(24). This, again, relates the actions of aspirin to prostaglandins for PGE_1 and PGE_2 are also known to have effects on platelet aggregation.

Other actions of aspirin are its ability to lower blood sugar in certain diabetic patients (1), to alter ion transport and membrane potential in varied cell types (1)(16), to reduce lipogenesis and depress free fatty acid release in muscle tissue (1), and to uncouple oxidative phosphorylation (5).

Another compound, cyclic adenosine 3',5'-monophosphate (cAMP), has also been shown to have a broad spectrum of effects at sites similar to those acted upon by salicylates. These include: 1) the mediation by cAMP of prostaglandin's inhibition of platelet aggregation (7)(10), 2) the mediation by cAMP of insulin's induction of glucose uptake in hepatic cells (11), 3) its complex effects on ion transport and membrane potentials in various cell types (18)(25)(17), and 4) its stimulation of lipolysis in human and rat fat cells (27).

Because cyclic AMP initiates the action and release of many hormones, it has become known as a ubiquitous "second messenger". Adenyl cyclase is activated when an external stimulus, such as a hormone comes in contact with the cell surface. The increase in intracellular levels of cyclic AMP activate other specific enzymes. ACTH's stimulation of

adenyl cyclase in the adrenal cortex, followed by the activation of enzymes synthesizing cortisol exemplifies this (12). Also, thyroid hormone secretion is induced when TSH activates thyroid adenyyl cyclase (13).

There is some evidence cAMP may, in a similar manner, mediate the synthesis of prostaglandins (10). Epinephrine and norepinephrine, both known to stimulate adenyyl cyclase in certain cells, stimulate release of prostaglandins from the dog spleen (14). In addition it has been shown prostaglandins stimulate adenyyl cyclase in human platelets (7).

The question arose whether some of the effects of aspirin might be mediated by its effects on the cAMP systems of various target cells. In order to determine whether a relationship exists between the action of aspirin and cyclic AMP, the effect of salicylates on β -galactosidase synthesis in the bacterial cell was observed. Synthesis of β -galactosidase in Escherichia coli is known to require cyclic AMP (15). In E. coli, cAMP acts at the transcriptional level of enzyme synthesis by promoting initiation of β -galactosidase mRNA synthesis. The nucleotide is believed to activate a protein, called cAMP receptor protein, which in turn allows the protein to bind to the promoter region of the lac operon. Its presence enhances the binding of RNA polymerase to the proper initiation site of lac DNA (6). Consequently, increased synthesis of β -galactosidase occurs with optimum concentrations of cAMP and repression occurs when the cAMP concentration is lowered.

This paper reports the effects salicylates have on β -galactosidase synthesis in E. coli and the relationship between cAMP's and salicylic acid's mechanism of action within the bacterial cell.

MATERIALS AND METHODS

Bacterial Strains

For the initial experiments, Escherichia coli strain 3000 ($\text{lac } i^+$) was used. To determine salicylates' effect on inducer uptake, a constitutive strain 3300 ($\text{lac } i^-$) was used. Both strains were provided by Dr. John W. Myers.

Growth Conditions

Bacteria were grown in double strength Minimal Broth Davis medium (Bacto) supplemented with .0002% vitamin B₁ and either 0.2% glucose or 0.5% glycerol. Double strength media was used since this has essentially the same constituents as the minimal medium used in earlier experiments observing effects of cyclic AMP on E. coli (7).

Before each experiment, fresh stationary cultures were prepared from bacterial slants. Stationary cultures were inoculated into Klett Flasks to obtain an initial cell density of 10^{-8} /ml., and grown on a shaker at 37° C. until log phase was reached. To induce β -galactosidase synthesis, isopropyl β -D-thiogalactopyranoside (IPTG) was added to the cells in log phase, producing a final concentration of 5×10^{-4} M. Further additions, depending on the experiment, were made to log phase cultures.

All cell density measurements were taken on a Klett-Summerson Photoelectric Colorimeter (Model 8000-3), using a 660 m μ filter.

β -Galactosidase Assay

β -galactosidase was assayed according to the procedure of Pardee, Jacob, and Monod (8), with slight modification. At specific times 0.9 ml. of sample was withdrawn from the cultures and added to tubes containing 2 drops of 0.25% sodium dodecyl sulfate (SDS) and 2 drops chloroform (9). Both toluene treatment and SDS - chloroform treatment were preliminarily tested to determine the best method of releasing enzyme from the cell. The latter did not alter enzyme stability and proved to increase the sensitivity of the assay.

The amount of enzyme present was measured spectrophotometrically using a Klett-Summerson Photoelectric Colorimeter (Model 800-3), with a 420 m μ filter. One unit of enzyme is defined as the amount producing 1 m μ mole of o-nitrophenol per minute at 28° C, pH 7.0. The units of enzyme in the sample can be calculated from the fact 1 m μ mole per ml. of o-nitrophenol has a Klett reading of 0.45 under experimental conditions used here.

To verify the assay procedure with E. coli strain 3000, enzyme activity was confirmed to be linear in relation to time and concentration.

Materials

All chemicals were commercially obtained. Cyclic adenosine 3', 5'-monophosphate was obtained from Sigma, and its purity was confirmed chromatographically and spectrophotometrically. Based on readings by a Cary Spectrophotometer, the cyclic AMP proved to be 99% pure by weight. Cyclic AMP's biological activity was confirmed by its ability to reverse glucose repression.

RESULTS

The Effects of Salicylates on β -Galactosidase Synthesis

Various concentrations of aspirin were added to E. coli strain 3000 cultures growing on glycerol medium. At a 5×10^{-3} M concentration of acetylsalicylic acid, the differential rate of β -galactosidase synthesis decreased (Table I). This was accompanied by a slight decrease in bacterial growth rate. At 1×10^{-3} M and 2.5×10^{-3} M, aspirin had no significant effect on β -galactosidase synthesis.

Since aspirin's pharmacological action depends on its salicylic acid content (1), the effect of salicylic acid on β -galactosidase synthesis was determined. At all concentrations, salicylic acid produced a greater effect on the differential rate of enzyme synthesis than aspirin (Table II). Salicylic acid also inhibited growth to a considerable extent, but in all cases the effect on enzyme synthesis was greater.

Control experiments showed both aspirin and salicylic acid had no effect on the enzyme assay system.

Since the utilization of glycerol requires the inducible enzyme glycerol kinase, known to be sensitive to catabolite repression (6), it was postulated the effect of salicylic acid on growth rate resulted from an inhibition of glycerol kinase synthesis. Such an inhibition would have the effect of masking decreases in the differential rate of β -galactosidase synthesis. For this reason, it was decided to use a carbon source not subject to catabolite repression, and presumably

TABLE I

EFFECTS OF ASPIRIN ON

DIFFERENTIAL RATE OF SYNTHESIS OF β -GALACTOSIDASE

Exp. No.	Aspirin (M)	Diff. Rate Enz. Synth. (EU/ml/ΔKR)	% of Control Rate	Growth Rate (1/gen. time)	% of Control Rate
1.	-(cont.)	36.1	100	.72/hr	100
	1×10^{-3}	36.1	100	.63/hr	87
	2.5×10^{-3}	36.1	100	.67/hr	93
	5×10^{-3}	25.4	70	.60/hr	82.5
2.	-(cont.)	31.3	100	.65/hr	100
	5×10^{-3}	21.0	67	.54/hr	83
3.	-(cont.)	33.3	100	.67/hr	100
	5×10^{-3}	25.9	78	.58/hr	87

E. coli strain 3000 was grown in glycerol medium until log phase was obtained. At this time the culture was divided and transferred to flasks containing 5×10^{-4} M. IPTG and the indicated amount of dissolved aspirin. The control flask contained water in place of the dissolved aspirin solution. To insure dilutions would not limit the media, flasks were also initially supplemented with 4x Minimal Broth Davis.

At various cell densities 0.9 ml samples were taken and assayed for β -Galactosidase activity.

TABLE II
EFFECT OF SALICYLIC ACID ON
DIFFERENTIAL RATE OF SYNTHESIS OF β -GALACTOSIDASE

Exp. No.	Salicylic Acid (M)	Diff. Rate Enz. Synth.		Growth Rate	
		(EU/ml/ Δ KR)	% of control rate	(1/gen. time)	% of Control
1.	-(control)	37.0	100	.60/hr	100
	5×10^{-3}	6.2	17	.33/hr	55
2.	-(control)	28.3	100	.71/hr	100
	3.87×10^{-3}	6.5	23	.44/hr	62
3.	-(control)	31.5	100	.60/hr	100
	1×10^{-3}	20.6	65	.52/hr	87

Cells were grown and sampled as in Table I with the exception that dissolved salicylic acid was added to cells in log phase in place of aspirin.

not subject to salicylic acid inhibition. In all subsequent experiments glucose was used as carbon source.

Upon the addition of $5 \times 10^{-3}M$ salicylic acid to cells grown in glucose media, there was an immediate cessation of β -galactosidase synthesis, lasting over a generation of growth. This was followed by a recovery of synthesis, but at a decreased rate relative to the control (Fig. 1). At $1 \times 10^{-3}M$, salicylic acid decreased the differential rate of enzyme synthesis, but there was apparently no lag in synthesis after its addition.

From this data it appears salicylic acid more effectively represses β -galactosidase synthesis in glucose grown cells than in cells grown in glycerol media. It should be noted, however, the inhibitory effect of salicylic acid on growth rate is the same whether the carbon source is glucose or glycerol. The reason for this inhibition remains obscure.

The Effect of Cyclic AMP on Repression by Salicylic Acid

If salicylic acid acts by decreasing cyclic AMP levels then cAMP should reverse the effects of salicylates. Figure 2 shows the results of an experiment where $5 \times 10^{-3}M$ cyclic AMP and $2.5 \times 10^{-3}M$ salicylic acid were added simultaneously to glucose grown cells. It can be seen that cAMP reverses the repression caused by salicylic acid. However, the reversal is only partial since the differential rate is not restored to the level of the control plus cAMP cells.

Salicylic acid in these experiments also caused a decrease in growth rate, and it was further decreased when cAMP was added to the cells. Because of this, the data from Figure 2 was replotted with time units on the abscissa in place of cell density (Fig. 3). This allows

FIGURE 1

EFFECT OF SALICYLIC ACID ON THE DIFFERENTIAL RATE
OF β -GALACTOSIDASE SYNTHESIS

Strain 3000 was grown in glucose medium until log phase was reached. Approximately 1 hour following induction with IPTG, the culture was separated and transferred to flasks containing the indicated additions. Samples were taken and assayed for β -galactosidase as in Table I. Since dissolved salicylic acid was added, a dilution in cell density occurred. The first point on the graph represents samples taken immediately after the culture was separated and diluted.

Flask		Growth Rate	Differential Enz. Rate
A.	Control	.75/hr	30.8 EU/ml/ Δ KR
B.	Salicylic Acid (1×10^{-3} M)	.68/hr	18.0 EU/ml/ Δ KR
C.	Salicylic Acid (5×10^{-3} M)	.35/hr	9.5 EU/ml/ Δ KR

FIGURE 1

FIGURE 2

EFFECT OF CYCLIC AMP AND SALICYLIC ACID
ON DIFFERENTIAL β -GALACTOSIDASE SYNTHESIS

IPTG was added to a log phase culture of strain 3000 grown in glucose medium. Approximately 45 minutes after induction the culture was divided equally into flasks containing the indicated additions. Samples were taken and β -galactosidase was assayed as previously indicated. The arrow at 30 Klett units represents the time the culture was divided and transferred.

Flask		Growth Rate	Differential Rate Enzyme Synthesis
A.	Control	.67/hr	23 EU/ml/ KR
B.	Cyclic AMP (5×10^{-3} M)	.57/hr	31.5 EU/ml/ Δ KR
C.	Salicylic acid (2.5×10^{-3} M)	.41/hr	13.5 EU/ml/ Δ KR
D.	Salicylic acid 2.5×10^{-3} M + cAMP (5×10^{-3} M)	.35/hr	25 EU/ml/ Δ KR

FIGURE 2

FIGURE 3

EFFECT OF CYCLIC AMP AND SALICYLIC ACID
ON β -GALACTOSIDASE SYNTHESIS

This represents the same experimental data as in Figure 1. In the graph enzyme units are plotted in relation to time rather than in relation to cell density. The arrow indicates the time the culture was divided and all additions were made.

FIGURE 3

the effect of salicylic acid to be noted independently of its effect on generation time. Even on this graph cAMP is shown to partially relieve repression caused by salicylates.

Previously Pastan and Perlman (21)(22) emphasized cAMP had no effect on growth rate of E. coli strain 3000, which was in contrast to these results. Their data, however, refer to using 1×10^{-3} M cAMP. Since 5×10^{-3} M cyclic AMP is believed to overcome both transient and catabolite repression caused by glucose in strain 3000 (23), the effect of this concentration on salicylic acid's repression was observed.

Similar results were obtained in a second experiment using 5×10^{-3} M salicylic acid and 5×10^{-3} M cAMP (Fig. 4). The degree of reversal was again only partial. Because growth inhibition at this concentration of salicylic acid with cAMP was so great, the curve is not extended very far.

From these data it appears there is an interaction between the effects of salicylic acid and those of cAMP. Since repression produced by the higher concentration of salicylic acid seems to be less effectively reversed by 5×10^{-3} M cAMP, there is a possibility the degree of interaction between the two may be concentration dependent.

The Effect of Salicylates on Constitutive Enzyme Synthesis

To eliminate the possibility salicylic acid may repress β galactosidase synthesis by preventing inducer uptake or by competing with inducer for repressor, an experiment was carried out using strain 3300, constitutive for β -galactosidase synthesis. From Figure 5 it is evident that 2.5×10^{-3} M salicylic acid had the same effect on strain 3300 as it had previously on strain 3000. Therefore, salicylates act independently

EFFECT OF CYCLIC AMP AND SALICYLIC ACID ON DIFFERENTIAL
 β -GALACTOSIDASE SYNTHESIS

Figure 4. Strain 3000 culture was grown sampled and assayed for β -galactosidase as in Figure 2, with the exception a higher concentration of salicylic acid was added after separation. Since salicylic acid was in dissolved form, a dilution occurred upon its addition. This was compensated for in other flasks by an equal dilution.

Flask		Growth Rate	Diff. Enz. Rate
A.	Control	.71/hr	22 EU/mL/ΔKR
B.	Control + cAMP (5×10^{-3} M)	.52/hr	31 EU/mL/ΔKR
C.	Salicylic acid (5×10^{-3} M)	.22/hr	12 EU/mL/ΔKR
D.	Salicylic acid (5×10^{-3} M) + cAMP (5×10^{-3} M)	.15/hr	16 EU/mL/ΔKR

FIGURE 5

EFFECT OF SALICYLIC ACID ON DIFFERENTIAL β -GALACTOSIDASE
SYNTHESIS IN CONSTITUTIVE STRAIN 3300

Strain 3300 was grown in glucose medium. When the cells reached log phase, 2.5×10^{-3} M salicylic acid was added. Cells were sampled and assayed as in earlier experiments. IPTG (5×10^{-4} M) was added to control flasks to note the degree of constitutiveness of the strain. The first points, again, represent samples taken immediately after the culture was divided and additions were made.

Flask		Growth Rate	Diff. Enz. Synth.
A.	Control	.89/hr	6.5 EU/ml Δ KR
B.	Control + IPTG (5×10^{-4} M)	.89/hr	6.5 EU/ml Δ KR
C.	Salicylic acid (2.5×10^{-3} M)	.54/hr	4.6 EU/ml Δ KR
D.	Salicylic acid (2.5×10^{-3} M) + IPTG (5×10^{-4} M)	.54/hr	3.0 EU/ml Δ KR

FIGURE 5

of inducer uptake and of inducer's derepression of lac mRNA synthesis.

The Effect of 2,4-Dinitrophenol on β -Galactosidase Synthesis

Since salicylates are known to be uncouplers of oxidative phosphorylation (5), it has been suggested some of their pharmacological effects result from their uncoupling action. This would imply that other uncouplers should preferentially inhibit β -galactosidase synthesis. For this reason the effect of 2,4-dinitrophenol (DNP) on β -galactosidase synthesis was observed. Because DNP is more effective in inhibiting oxidative phosphorylation than salicylic acid, and is consequently more toxic, a concentration of the drug was found which would inhibit growth to approximately the same degree as $2.5 \times 10^{-3}M$ salicylic acid. This was found to be $2.5 \times 10^{-4}M$. Figure 6 illustrates DNP does not repress differential synthesis of β -galactosidase. This implies that although DNP lowers ATP levels, there is no apparent effect of the drug on levels of cAMP. Also, it appears salicylic acid is not exerting its repression of enzyme synthesis by inhibiting oxidative phosphorylation.

FIGURE 6

EFFECT OF DNP ON DIFFERENTIAL β -GALACTOSIDASE SYNTHESIS

When strain 3000 cells reached log phase, they were induced with IPTG. Approximately one hour later the culture was divided and transferred to flasks containing the indicated additions. The cells, as in previous experiments, were sampled at various times and assayed for B-galactosidase activity. Since 2,4-dinitrophenol was in solution, its addition resulted in dilution of cultures. This dilution was equally compensated for in control flasks. The first points on the graph represent samples taken immediately after separation of the culture.

Flask		Growth Rate	Differential Enz. Rate
A.	Control	.75/hr	22 EU/ml/ Δ KR
B.	Control + cAMP (5×10^{-3} M)	.64/hr	50 EU/ml/ Δ KR
C.	DNP (2.5×10^{-4} M)	.42/hr	22 EU/ml/ Δ KR
D.	DNP (2.5×10^{-4} M) + cAMP (5×10^{-3} M)	.29/hr	42 EU/ml/ Δ KR

FIGURE 6

DISCUSSION

Salicylic acid, and to a lesser extent aspirin, cause repression of β -galactosidase synthesis in Escherichia coli. This effect is not due to an inhibition of inducer uptake, nor is it due to interference with inducer's inactivation of lac repressor, since strains constitutive for β -galactosidase synthesis are also repressed by salicylic acid. In addition, because dinitrophenol is without effect on differential β -galactosidase synthesis, it appears salicylates do not repress enzyme synthesis by inhibiting oxidative phosphorylation.

At these concentrations, however, salicylic acid does decrease growth rate, and direct or indirect effects of this on the observed repression of enzyme synthesis still remain. Since the differential rate of enzyme synthesis is decreased and also since 2.5×10^{-4} M DNP decreases growth rate approximately the same degree without having effects on differential synthesis, salicylates' inhibition of growth is not the sole factor responsible for its effects on enzyme synthesis.

When 5×10^{-3} M cyclic AMP was added to cells treated with 2.5×10^{-3} and 5×10^{-3} M salicylic acid, repression of differential enzyme synthesis was partially relieved. This suggests salicylic acid represses β -galactosidase synthesis by directly lowering intracellular levels of cyclic AMP or by indirectly acting on a site which in turn releases cAMP from the cell. Since cAMP only partially relieves repression caused by salicylic acid and because there appears to be an inverse relationship between

the concentration of salicylic acid and the effectiveness in which cAMP relieves its repression, it is possible cAMP and salicylic acid may indirectly compete for their actions on β -galactosidase synthesis.

There are several possible sites where salicylic acid could interfere with cAMP's mechanism of action. One would be at the level of cyclic AMP receptor protein. Since interference here usually implies a type of allosteric interaction, in this case between salicylic acid versus cAMP for the protein, it is not likely to occur. Another possible site of action would include salicylic acid's effect at the membrane level. Since both compounds are known to influence ion flow in various cells, there is a chance this may, in turn, cause a number of effects. However, because ion flow systems in the bacterial cell have not been clarified, it is difficult to predict direct or indirect interactions between salicylic acid and cAMP at this level. In addition, because cAMP is known to activate many enzymes catalyzing de novo synthesis (12)(13)(26), salicylic acid may exert its effects by interfering with this step.

These represent sites salicylates may act upon to either release cAMP from the cell, inhibit adenyl cyclase activity, stimulate phosphodiesterase activity, or interfere with activation of CRP. All would interfere with cAMP's action within the cell, and would consequently serve to explain salicylic acid's repression of β -galactosidase synthesis.

In conclusion the repression of β -galactosidase synthesis by salicylic acid in E. coli appears to be partially relieved by cAMP. This raises the possibility salicylates may act by decreasing intracellular cAMP activities. This would occur if the two competed

for a common ion or reaction site, or if salicylic acid stimulated the release of cAMP from the cell.

These studies imply salicylates may also have a direct effect on lowering intracellular levels of cAMP in eukaryotic cells. This would help to explain aspirin's diverse and complex effects within the body.

BIBLIOGRAPHY

1. L. Goodman, A. Gilman. 1970. The Pharmacological Basis of Therapeutics. 4th Edition. Macmillan Co. :314-329.
2. J. Vane. 1971. Nature New Biol. 231: 232.
3. A. Hammond. 1971. Science. 174: 48.
4. J. Smith, A. Willis. 1971. Nature New Biol. 231: 235.
5. J. Miyahara, R. Karler. 1965. Biochem. J. 97: 197.
6. I. Pastan, R. Perlman. 1970. Science. 169: 339.
7. H. Shio, P. Ramwell. 1972. Nature New Biol. 236: 45.
8. A. Pardee, F. Jacob, J. Monod. 1959. J. Mol. Biol. 1: 165.
9. M. Abesnik, C. Levinthal. 1970. Cold Spring Harbor Symp. Quant. Biol. 35: 456.
10. H. Shio, J. Shaw, P. Ramwell. 1971. Ann. N.Y. Acad. Sci. 185: 327.
11. J. Exton, S. Lewis, R. Ho, G. Robinson, C. Park. ibid. 85.
12. L. Garren, G. Gill, G. Walton. ibid. 210.
13. J. Dumont, C. Willems, J. Van Sande, P. Neve. ibid. 291.
14. S. Ferreira, S. Moncada, J. Vane. 1971. Nature New Biol. 231: 237.
15. I. Pastan, R. Perlman. 1968. J. Biol. Chem. 243: 5420.
16. H. Levitan, J. Barker. 1972. Science. 176: 1423.
17. R. Levine, D. Wilson. 1971. Ann N.Y. Acad. Sci. 185: 363.
18. F. More , S. Jard. ibid. 351.
19. C. Wilson, O. Gisvold, R. Doerge. 1966 Textbook of Organic Medicinal and Pharmaceutical Chemistry. 5th Edition. Lippincott Co. : 673-684.
20. R. Lefkowitz, J. Roth, I. Pastan 1971 Ann.N.Y. Acad. Sci. 185: 195.

21. R. Perlman, I. Pastan. 1968. Biochem. Biophys. Res. Comm. 30: 656.
22. R. Perlman, I. Pastan. 1968. Proc. Nat. Acad. Sci. 61: 1336.
23. I. Pastan, R. Perlman. 1969. Nature 223: 811.
24. M. Sahud, P. Aggler. 1969. New Eng. J. Med. 280: 453.
25. D. Schafer, W. Lust, J. Polson, J. Hedtke, B. Sirca., A. Thakur, N. Goldberg. 1971. Ann N.Y. Acad. Sci. 185: 376.
26. C. Villar-Palasi, L. Shen. L.C. Shen. ibid 74.
27. J. Beavo, N. Rogers, O. Crofford, C. Baird, J. Hardman, E. Sutherland, E. Newman. ibid. 129.

APPENDIX

ABBREVIATIONS

PGE ₁	Prostaglandin E ₁ , C ₂₀ H ₃₄ O ₅ .
PGE ₂	Prostaglandin E ₂ , C ₂₀ H ₃₂ O ₅ .
PGF ₂	Prostaglandin F _{2α} , PGE ₂ with a reduced keto group forming an αhydroxy
cAMP	Cyclic adenosine 3',5'-monophosphate.
ATP	Adenosine 5'-triphosphate
ADP	Adenosine 5'-diphosphate
ACTH	Adrenocorticotropic hormone
TSH	Thyroid stimulating hormone
IPTG	Isopropyl β-D-thiogalactopyranoside
mRNA	Messenger riboneucloic acid
DNP	2,4-Dinitrophenol
CRP	Cyclic AMP receptor protein