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STUDIES ON INTESTINAL MOTILITY AND ADENOSINE 3' : 5' - CYCLIC MONOPHOSPHATE IN ANIMALS WITH EXPERIMENTALLY INDUCED ILEUS: EFFECT OF DRUGS ON INTESTINAL CYCLIC AMP UTILIZATION.

The Ohio State University, Ph.D., 1975
Pharmacology

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Andris Adamovics
1975
STUDIES ON INTESTINAL MOTILITY
AND ADENOSINE 3':5'-CYCLIC MONOPHOSPHATE
IN ANIMALS WITH EXPERIMENTALLY INDUCED ILEUS:
EFFECT OF DRUGS ON INTESTINAL CYCLIC AMP UTILIZATION

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Andris Adamovics, B.S., M.S.

*****
The Ohio State University
1975

Reading Committee: Approved by
Daniel Couri
John J. O'Neill
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Adviser
Department of Pharmacology
ACKNOWLEDGMENTS

Now that I have finally reached the end of my graduate work, I would like to express my gratitude to the many persons who have helped me in one way or another during the long years of study. There are a few persons, however, who deserve special mention:

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Publications


Presentation


Fields of Study

Biochemical Pharmacology, Dr. A. Horita

Drug Metabolism, Dr. D. Couri

Autonomic Pharmacology, Dr. B. Marks

Gastrointestinal Pharmacology and Physiology, Drs. D.F. Magee and K. Hanson

Experimental Animal Surgery, Dr. D.F. Magee

Toxicology and Drug Identification, Dr. D. Couri

Environmental Toxicology, Dr. F. Weir

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INTRODUCTION

The term "ileus" is used to describe a wide spectrum of intestinal complications which are characterized by the inability of the gastrointestinal tract to propel its contents in a normal progressive manner. Clinically, this term is not reserved only for the more serious cases which are characterized by the accumulation of gas and/or fluid within the intestinal lumen and are manifested by abdominal distention, but is also used to describe a condition such as an intestinal obstruction which can readily be diagnosed. In this sense ileus is not a disease but a symptom complex which becomes evident to some degree after most abdominal operations, childbirth, or any injury or trauma to the spinal column or the pelvic organs.

The basic cause for the disturbance in intestinal motility in ileus remains unknown. The contractility of the adynamic gut is unimpaired and responds in normal fashion to electrical stimulation. These observations suggest that ileus may be a normal local response to stress and may be due to a reflex mediated through the sympathetic nervous system.

A great deal has been accomplished in the prevention of this condition, but the treatment of established ileus
leaves much to be desired. Some medicinal agents have been used to modify ileus, but most of them have turned out to be ineffective.

No answer can be found in literature and no experimental studies have been done to investigate and study the cause of ileus at the cellular level.
REVIEW OF LITERATURE

A belief has existed for several hundred years that the well-being of man is associated with a proper functioning of the intestinal tract. Early civilizations knew that any malfunction of the gut can be precarious and therefore tried to use various natural medicines to "purge the belly of all evil spirits." (Holmstedt and Liljestrand, 1963). Writings left by these civilizations indicate that a condition similar to present day paralytic ileus was prevalent even at that time.

The Greek philosopher Hippocrates, born about 460 B.C., was familiar with such an intestinal malfunction. In his writings he described in great detail a condition similar to paralytic ileus and its treatment. He indicated that the only way to treat this condition is by surgery. The first surgical attempt to relieve this malady has been credited to Praxagoras who in 400 B.C. performed the first recorded enterostomy (Cantor, 1967).

The next written discourse concerning ileus is from the era of Tiberius (14-37 A.D.). Celsus, a distinguished Roman physician during the reign of Tiberius, considered ileus to be a malady of such a great suffering that the
attending physician should have the courage to put the patient out of his agony (Cantor, 1967). Strangely enough, during the last thousand years our understanding of this condition has advanced very little. We still describe ileus exactly in the same terms as did Celsus some twenty centuries ago.

Some 600 years later Thomas Sydenham described and differentiated between two distinct disease entities -- "true ileus" and "bastard ileus." He described the latter condition as being caused simply by mechanical obstruction of the intestinal tract, but the former as being "...caused by acid and malignant humors...lodged in the stomach and those parts of the intestinal canal nearest to it." (Sydenham, T., 1666). Two hundred years later, Sir Spencer Wells described paralytic ileus in great detail and pointed out that such a condition is frequently observed after childbirth and gynecological operations (Wells, 1874). A few years later Schroeder observed paralytic ileus as a complication following laparotomy and suggested that surgery may be the only treatment. It was not until Cannon and Murphy in 1906 and 1907 reported that any handling and exposure of the intestinal tract to air during surgery causes paralysis of the small intestine. They observed that this paralysis was preceded by a complete cessation of all normal intestinal peristaltic activity and thus concluded that
the cause for such a paralysis was probably physiological in origin.

The first attempt to describe normal intestinal activity was made by Wagner and Ludwig in the middle of the nineteenth century. They made their observations on animals killed just prior to experiments and described the intestinal movements as "like snakes in the nest." A more scientific approach to the study of normal intestinal activity was employed by Engelman in 1871. He used live animals and measured the intraluminal pressure by introducing an elastic rubber tube into the intestinal lumen and then recorded pressure changes on a Marey tambour. The next year B. v. Houckgeest (1872) reported about an experiment which he had performed on an "anesthetized" animal. The experiment consisted of placing the whole animal with its abdomen open in a bath of physiological saline and then observing the movements of the entire gastrointestinal tract.

Soon thereafter the first attempts to record the intestinal motility graphically were made by Openchowski (1889) Bunch (1898) and others. In 1899 and 1900 Bayliss and Starling in their now classical work on the small intestine of dogs and cats recorded the intestinal motility with the aid of a balloon placed in the lumen of the intestine which was then connected to a special "enterograph."
apparatus was supplied with a lever permitting them to record the changes in length as well as diameter of a small, immobilized intestinal segment.

A completely new method for the study of intestinal motility was introduced by Cannon in 1902. He gave bismuth subnitrate to cats and then used roentgen rays to examine and record the intestinal motility. By using this method Cannon described postoperative intestinal atony in laboratory animals (Cannon and Murphy, 1906 and 1907).

Subsequently this method has been used very extensively for the study of the intestinal motility in man.

A decade later Carlson (1912) used partially inflated balloons to record gastro-intestinal motility. This method was slow and did not provide sufficient information and therefore was modified by several subsequent investigators to permit better recording of intestinal motility (Gruber and Robinson, 1929; Menschan and Quigley, 1938). This balloon method, developed by Carlson, was later modified for recording intestinal motility in human volunteers. (Chapman and Stanbury, 1948; Posey, et al, 1948; Goodman, et al, 1952; Texter, et al, 1958; Connell, 1961). Valuable results concerning the motor activity of the gut in normal subjects were obtained with these experiments. To investigate intestinal motility in animals as well as in human
volunteers such techniques as open-ended tubes, electromagnetic pressure transducers, radio pill (Ross, et al, 1963) electropotential recording, or radioactive chromium (Connell, 1961; Hansky and Connell, 1962; Derblom, et al, 1966; Grevsten, et al, 1967; Purdor and Bass, 1973; Poulakos and Kent, 1973) were used. Later it was realized that all of these techniques had their limitations (Texter, 1963) and therefore could not be used very effectively to study the intestinal motility during postoperative paralysis in humans. Other experiments, intended to investigate normal as well as abnormal intestinal motility, also had their limitations. In these studies large laboratory animals, especially dogs, were surgically prepared with exteriorized intestinal loops (Douglas and Mann, 1939). The results from these experiments indicated that the propulsive motility of the small intestine is rhythmic and independent and is partially controlled by the parasympathetic and the sympathetic nervous systems.

All of the experiments mentioned were done on "normal" animals and provided information only about "normal intestinal activity", whereas information regarding "abnormal" intestinal activity was obtained mainly from human subjects. Several attempts were made to correlate the available animal data with human measurements but no definite conclusions
could be drawn.

Today the term ileus is used to designate a failure of the intestinal tract to move its contents in a normal progressive fashion (Finney, 1906; Olivecrona, 1926-27; Wagensteen, 1942; Devine, 1946-47; Magee, et al, 1957; Smith, et al, 1965; Harrower, 1968; Neely, Catchpole, 1971). Only during the last fifty years serious attempts have been made to investigate the cause of paralytic ileus. Different laboratory animals, such as: rats, cats, dogs and man himself, and numerous experimental techniques such as: rough and extensive gastrointestinal manipulation, handling and surgery (clamping or cutting) (Cannon and Murphy, 1906; Olivecrona, 1926; Markowitz and Campbell, 1927; Alvarez and Hosoi, 1929; Ross and Watson, 1963; Tinckler, 1965; Nylander and Wikstrom, 1967; Gutierrez, et al, 1971; Dubois, et al, 1973) cholecystectomy or splenectomy (Baker and Webster, 1968; Abe, et al, 1974) sympathectomy or vagotomy performed during abdominal surgery (Olivecrona, 1926; Ochsner, et al, 1930; Bingham, et al, 1950; Roth and Beams, 1959) resection or transposition of the small intestine, exteriorization of an intestinal loop and extension of a closed ileal loop with air (Frey, 1926; Ochsner, et al, 1930; Wakim and Mann, 1943; Frittelli, et al, 1963; Nylander and Wikstrom, 1968) induction of experimental peritonitis
or intraperitoneal infusion of gastric juice (Frey, 1926; Olivecrona, 1926; Markowitz and Campbell, 1927; Roden, 1937; Landaman and Longmire, 1967; Appert and Howard, 1972) retroperitoneal hematoma or irritation (Demel, 1926; Alvarez and Hosoi, 1929; Lindquist, 1968) cecectomy (Lynch, et al, 1963) stimulation of peripheral nerves or vagus (Lehman, 1912-13; Ochsner, et al, 1930; Roden, 1937; Smith, et al, 1965) traumatization of kidney (Roden, 1937) or squeezing of testes (Cannon and Murphy, 1907; Roden, 1937) have been employed but no definite conclusions have been obtained concerning the postoperative intestinal atony.

Treatment of this condition is just as varied as the theories concerning its origin. The most widely used therapy is simply the relief of symptoms by suction (Devine, 1946-47; Quan and Sterns, 1961; Gerber, 1963; Heimbach and Crout, 1971) or surgical intervention in order to restore the continuity of the gut (Wagensteen, 1942; Machella, 1960; Wojtalik, et al, 1973). Drug therapy was also tried but again with inconclusive results. The most commonly used drugs have been prostigmine (Schwartz, et al, 1942; Harrower, 1968; Heimbach, Crout, 1971; Neely, Catchpole, 1971; Petri, et al, 1971) choline (Frey, 1926; Hartman and Dock, 1926-27; Devine, 1946-47) urecholine (Ochsner, et al, 1930; LeQuense, 1957; Harrower, 1968; Gutierrez, et al,
1971; Neely, Catchpole, 1971) pilocarpine (Frey, 1926; Demel, 1926) and spinal or splanchnic anesthesia with pro-
caine (Wagner, 1919; Markowitz and Campbell, 1927; Ochsner, et al, 1930; Wagensteen, 1942; Helm, Ingelfinger, 1944; LeQuense, 1957). Other drugs which have been tried range
from sympathetic nerve blockers (Catchpole, 1966 and 1969; Heimbach and Crout, 1971; Neely and Catchpole, 1971; Dubois, et al, 1971; Milner and Hills, 1966; McNeill, 1966) to hor-
mones (Ochsner, et al, 1930; LeQuense, 1957) morphine alka-
loids, cathartics, vitamins (Devine, 1946-47; Harrower, 1968) and hypertonic saline administered intravenously
(Ochsner, et al, 1930; Wagensteen, 1942). Also such methods
as electrical nerve stimulation (Demel, 1926; de Villiers, et al, 1963) gastrointestinal pacing (Bilgutay, et al, 1963)
or acupuncture (Matsumoto and Hayes, 1973) have been tried
but again with disappointing results.

Recently performed biochemical studies on normal human
intestinal mucosa (Greene and Herman, 1972) have suggested
that mucosal adenyl cyclase responds to NaF stimulation and
that any change in the intracellular level of this enzyme
may be responsible for a great variety of gastrointestinal
diseases. These conclusions, however, were based on the re-
sults obtained from normal human mucosal biopsy samples.
Nevertheless, it has been suggested that the change in the
activity of this enzyme is important, and that it also may be involved in the regulation of intestinal motility. If such a control exists, it is only indirect because of the close proximity of the mucosa to the muscularis layers.

The results obtained in in vitro muscle-bath experiments nevertheless suggest that the intestinal contraction and relaxation is indeed regulated by the intracellular levels of cyclic AMP (Andersson and Mohme-Lundholm, 1970; Andersson, 1972a, 1973; Inatomi, et al, 1974; Phaffman and McFarland, 1973; Wilkenfeld and Levy, 1969). In these experiments it was found that in the regulation of intracellular levels of cyclic AMP not only the α-and β-adrenergic receptors were involved, but also drugs which stimulated or inhibited the levels of phosphodiesterase affected the intestinal activity.

With the advent of more sensitive biochemical methods it has become possible to obtain data from the tissues at the cellular level. In the light of this, it is therefore an appropriate time to reevaluate our approach for the study and understanding of the propulsive intestinal motility and to investigate possible changes which may take place at the cellular level in normal, as well as in cases of experimental, intestinal atony.
STATEMENT OF THE PROBLEM

A survey of literature indicates that the postoperative intestinal atony is a condition which usually develops during the first few days of postoperative period and is characterized by a temporary paralysis of the intestinal tract and the absence of all bowel sounds (Olivecrona, 1926-27; Streeten and Ward-McQuaid, 1952; Smith, et al, 1965; Harrower, 1968). In most cases recovery is prompt, but if it persists, it tends to be self-perpetuating.

In the past, progress has been slow in understanding the mechanism involved in the development of this self-perpetuating "paralytic ileus". The commonly used medicinal agents have been ineffective in these cases and the strain of "corrective" surgery has been too much for some patients. With the advent of specific and sensitive biochemical methods attention has been turned to the study of chemical changes taking place during propulsive intestinal motility at the subcellular level in normal as well as in diseased intestinal tract.

No experimental evidence exists, however, in which the propulsive intestinal motility of normal animals has been compared to that of experimentally induced intestinal atony. Therefore, it is the purpose of this study to investigate
in nonoperated as well as in cecectomized rats the propulsive intestinal motility by using the transport of charcoal meal as an index of intestinal activity; to investigate the effect of various medicinal agents on the transport of charcoal meal in nonoperated and operated animals; to determine the changes, if any, in the intracellular content or utilization of cAMP before and after cecectomy; and to investigate the effect of drugs on the turnover rate of cAMP and to correlate any changes occurring to the intestinal transport of charcoal meal.
METHODS

A. In Vivo Experiments

1. Intestinal motility at various time intervals after cecectomy.

For determining the effect of cecectomy on the transit of charcoal meal through the gastrointestinal tract of rats the method described by Lynch, et al (1963) was followed. In this study non-fasted male Wister rats (1950-250gm) were used. Under ether anesthesia the animal was shaved and the abdomen washed with 70% alcohol. The animal was placed dorsally on the "operating table" and fastened with strips of adhesive tape. A 3cm long incision was made through the wall of the lower abdomen along the linea alba. The cecum was identified, carefully lifted out and placed on a wet sponge. The mesentery connecting the ileum and the apex of the cecum was cut and the main part of the cecum was ligated close to the ileo-cecal valve with a 2-0 cotton thread. The cecum was then excised and the remaining stump cleaned with saline (Fig. 1). The incision was closed with 4-0 silk using continuous through-and-through sutures. The sham operation was identical
Fig. 1

SCHEMATIC REPRESENTATION OF CECECTOMY IN THE RAT

Cecum
Colon
Ileum
with the exception that the cecum was not ligated nor excised.

Gastrointestinal transit of charcoal meal was measured at 2 hour intervals throughout the first 24 hours post surgery. Two mililiters of charcoal meal, consisting of 12.5% powdered animal charcoal in 2% hydroxypropyl cellulose was administered orally to experimental animals. Forty-five minutes following the charcoal administration, the animal was killed by cervical dislocation. The entire gastrointestinal tract was removed and the length of the small intestine and the distance traversed by the charcoal meal measured and then expressed as a ratio of the entire length of the small intestine (transit time determination).

Non-operated, non-anesthetized control animals were treated in a similar fashion. At 45 min after an oral administration of 2ml of charcoal meal, control animals were sacrificed and the transit time determined as above.

2. Preparation of drugs.

Drugs used in this study were prepared in double distilled water and administered to rats
either orally or subcutaneously in the back of the neck. All control animals received an equivalent volume of distilled water. The following agents were used:

<table>
<thead>
<tr>
<th>AGENT</th>
<th>DOSE</th>
<th>ROUTE OF ADMINISTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>500mg/kg</td>
<td>p.o.</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>500mg/kg</td>
<td>p.o.</td>
</tr>
<tr>
<td>Imidazole</td>
<td>50mg/kg</td>
<td>p.o.</td>
</tr>
<tr>
<td>Theophylline</td>
<td>20mg/kg</td>
<td>s.c.</td>
</tr>
<tr>
<td>Atropine</td>
<td>5mg/kg</td>
<td>s.c.</td>
</tr>
<tr>
<td>Methacholine</td>
<td>20mg/kg</td>
<td>s.c.</td>
</tr>
</tbody>
</table>

3. The effect of drugs on intestinal motility in normal animals.

For studying the effect of various drugs on the gastrointestinal transit of charcoal meal in nonoperated animals, agents as indicated under A-2 were administered to experimental animals at zero time. One hour later all animals received 2ml of charcoal meal orally. After forty-five minutes the animals were sacrificed by cervical dislocation and the transit time determined (Fig. 2A).

Control animals were administered an equivalent volume of double-distilled water at zero time. One
Schedule for Oral Administration of Charcoal Meal in Transit Time Experiments. Drugs were administered orally and/or subcutaneously, as described in the Methods. All control animals received an equivalent dose of double distilled water orally.
SCHEDULE FOR ORAL ADMINISTRATION
OF CHARCOAL MEAL IN TRANSIT TIME EXPERIMENTS

A. NONOPERATED RATS

0  60 MIN  105 MIN
DRUG  CHARCOAL MEAL  SACRIFICE

B. CECECTOMIZED RATS

0  60 MIN  120 MIN  165 MIN
CECECTOMY  DRUG  CHARCOAL MEAL  SACRIFICE
hour later 2ml of charcoal meal was administered orally and the animals sacrificed 45 min later. Intestinal transit measurements were made as described above.

4. **Effect of drugs on experimentally induced intestinal atony.**

   The effect of drugs on the gastro-intestinal transit in cecectomized rats was determined two hours following cecectomy. For this study cecectomized animals were prepared as outlined under A-1.

   Agents as indicated under A-2 were administered to experimental animals one hour post-cecectomy. One hour later all animals received 2ml of charcoal meal orally (Fig. 2B). Transit times were measured after forty-five minutes.

B. **Cyclic AMP Content of Rat Intestine**

   The alterations in the intracellular concentration of cyclic 3',5'-adenosine monophosphate (cAMP) were studied in normal as well as in cecectomized control and drug treated rats. Animals were housed in standard laboratory cages and maintained under standard laboratory conditions and fed regular laboratory rat chow with water available *ad libitum*. 
1. Control studies
   a. Nonoperated animals.

   On the day of test, animals were killed by cervical dislocation, three 1cm wide intestinal segments quickly removed, placed in ice-cold Tris-HCl buffer, washed, weighed and placed in separate homogenizers each containing 2ml of ice-cold 0.1% perchloric acid. The three intestinal segments were from:
   
   a) 10cm from pyloris
   b) midportion of the small intestine
   c) 10cm from cecum

   The tissue segments were homogenized, poured into cellulose centrifuge tube and the homogenizer washed with 1ml of ice-cold 0.1% perchloric acid which was then added to the centrifuge tube. Homogenates were centrifuged at 5000xg, 15 min, 0°C. The supernatant was removed, placed in another tube and neutralized with six drops of 0.5M K-succinate. This neutralized supernatant was used for the determination of the intracellular cAMP levels. The pellet remaining in the centrifuge tube was dissolved in 10ml of 1N NaOH for the determination of total protein.
b. Cecectomized animals

Cecectomy was performed as outlined above under A-1. For in vitro studies, animals were killed by cervical dislocation 2 hours post-cecectomy and three 1cm wide intestinal segments removed and prepared as described above.

c. Determination of intestinal adenyl cyclase

For elucidating the mechanism of propulsive intestinal motility it was essential to establish whether the enzyme adenyl cyclase is involved in the regulation of this activity or whether the activity of this enzyme can be modified by various drug treatments.

The adenyl cyclase assay was done according to the method of Krishna, et al (1968). One cm segment of intestinal tissue was homogenized in 2ml of 0.32M sucrose in a glass homogenizer with a motor driven Teflon pestle. The crude homogenate was centrifuged at 5000xg, 0°C for 20 min. The supernatant was removed and incubated in duplicate with and without the presence of fluoride. The total volume of the incubation media was 0.4ml and consisted of $10^{-2}$M theophylline, $3.3 \times 10^{-3}$M MgSO$_4$, 1.2-1.5mM ATP and H$^3$-labelled
ATP: (Specific activity 20-40 Ci/m mole; New England Nuclear) in 0.04M Tris-HCl, pH 7.6. For fluoride stimulation, 10^{-2} NaF was added to the incubation medium. Incubation was carried out at 37° for 15 min and stopped by immersion into boiling water for 5 min. Separation and measurement of the cyclic AMP formed from the preparation was done according to the method of Gilman (1970).

2. Recovery experiment

This experiment was done in order to calculate the amount of radioactive label recovered during the processing of the tissue and the isolation of the intracellular cAMP.

On the day of test the animals were killed by cervical dislocation and a 5 cm wide intestinal segment removed from the midportion of the small intestine. The removed segment was washed in ice-cold 0.04M Tris-HCl buffer (pH 7.0) and placed in a homogenizer containing 3ml of ice-cold 0.1% perchloric acid. After homogenization, the homogenate was transferred to a centrifuge tube and the homogenizer washed with 1ml of ice-cold 0.1% perchloric acid which was then added to the centrifuge tube. To the homogenate was then added 100μl of ^3H-cAMP (5μCi/cc) (Schwarz/Mann, Adenosine 3':5'-cyclic Phosphate [8-^3H]).
Specific activity: 27Ci/m mole). The homogenate was mixed on a Vortex and centrifuged for 15 min at 5000xg and 0°C. The supernatant was removed by decantation, and neutralized with 6 drops of 0.5M K-Succinate (pH6.8). Then 100μl of this neutralized supernatant was placed into a scintillation vial containing 10ml of counting cocktail (PCS, Phase Complete System, Amersham/Searle). Each vial was counted for 10 min and the recovery of the added radioactive material determined.

To the neutralized supernatant was added 0.7cc of 0.4M ZnSO₄ and 0.8cc of 0.4M BaCl₂ (Chan, et al, 1973). The mixture was agitated for 20 sec and centrifuged at room temperature for 15 min at 1500xg. The pellet was discarded but to the supernatant was added 0.5ml of 16mM EDTA. Afterwards 100μl were removed and counted to determine the amount of label lost during the precipitation step.

The supernatant was transferred to a 15ml conical centrifuge tube and evaporated to dryness on a rotary evaporator (Buchler). The precipitate was dissolved in 200μl of 50% ethanol. Twenty μl containing 20μg of cold cAMP was added as a marker and the entire sample streaked on a thin layer plate (precoated TLC plate Silica Gel 60 F-254). The plate was developed for 1 hour in a solvent system containing:
chloroform  40 parts
methanol    20 parts
water       3 parts

After developing, the plate was air-dried and the carrier cAMP visualized under a short-wave U.V. light. A 4 x 5cm area was scraped off and eluted with 3ml of 50% ethanol. After centrifugation, 1.5ml of the supernatant was placed in a scintillation vial containing 13ml of PCS and counted for 10 min. From the remaining sample 0.3ml was pipetted into a cuvette, 0.7ml of 0.1N HCl and 0.1ml of 0.5M Tris-buffer (pH7.0) added and read at 258nm in a UV spectrophotometer (Varian Techtron, Model 635). Standard cAMP containing 10µg in a 1ml cuvette, 1cm light path, had an absorbancy of 0.390 at 258nm.

C. In Vivo Studies on Intestinal Cyclic AMP

1. Turnover of cyclic AMP

For studying the turnover rate of cyclic AMP in intestinal tissue samples from nonoperated and cecectomized rats, combined procedures were utilized from the methods described by Tague and Shanbour (1974) for the homogenation of tissue sample, Shimizer, et al(1969) for the thin layer chromatographic separation: Flouret and Hechter (1974) for the TLC solvent system. The combination of these methods proved adequate for the separation and isolation of cAMP from other nucleotides, adenine, and adenosine.
a. Studies on nonoperated animals

Wistar strain male rats, weighing between 150-250gm were used. Prior to the experiment, animals were maintained at standard laboratory conditions and fed Purina Laboratory Chow with water available ad libitum.

On the day of test the animals were injected I.P. with 0.1cc of labelled adenine containing 50μCi/cc (Amersham/Searle, Adenine-8-3H, Specific activity: 24 Ci/mmol or 179 mCi/mg)*. At 60 min after I.P. injection of labelled adenine, rats were sacrificed by cervical dislocation, the proximal part of the small intestine removed and a 5cm long segment excised 10cm distal from the ligament of Treitz (Fig. 3). The excised segment was placed in a dish containing ice-cold Tris-HCl buffer, cleaned, weighed and placed in a glass homogenizer containing 3ml of ice-cold 0.1% perchloric acid. After homogenization with a motor driven Teflon pestle, the homogenate was transferred to a cellulose centrifuge tube. The homogenizer was washed with 1ml of ice-cold 0.1% perchloric acid.

*Solution for injection was prepared by diluting the 3H-Adenine 1:20 with double distilled water containing cold adenine 40μM/100μl.
Figure 3

Schematic Presentation of the Upper Gastrointestinal Tract. The intestinal segment which was removed for cAMP determination is indicated.
Fig. 3

SCHEMATIC PRESENTATION OF THE UPPER GASTROINTESTINAL TRACT

Duodenum

Ligament of Treitz

Stomach

10 cm

Jejunum

5 cm

segment excised
which was then added to the homogenate. After centrifugation at 0°, 5000xg, the supernatant was removed and neutralized with six drops of 0.5M K-succinate; the pellet dissolved in 10cc of 1N NaOH and saved for total protein determination at a later time. To precipitate other nucleotides, 0.7cc of 0.4M ZnSO₄ and 0.8cc of 0.4M BaCl₂ were added to the neutralized supernatant. The resulting cloudy solution was mixed well for 10 seconds and then centrifuged at room temperature for 15 min. The precipitate was discarded but to the supernatant was added 0.5cc of 16mM EDTA. These supernatants were stored frozen and used for cAMP determination at a later date.

b. Studies on nonoperated, drug treated animals

(TREATMENT #1)

Commonly used medicinal agents were administered to male Wistar rats at zero time (Fig. 4A). Forty-five min later all animals received intraperitoneally 0.1ml of ³H-Adenine (5µCi). One hour later animals were sacrificed by cervical dislocation and a 5cm segment removed from the proximal jejunum (Fig. 3). This intestinal segment was processed as outlined above.
Treatment Schedule for Nonoperated and Operated Rats. Treatment #1 and cecectomy drug treatment schedule corresponds to the dosage schedule used in transit time experiments. In Treatment #2 drug and $^3$H-Adenine was administered at the same time. This was done to test the influence of drugs on the conversion of $^3$H-Adenine to $^3$H-cAMP.
TREATMENT SCHEDULE FOR NONOPERATED AND OPERATED RATS

**TREATMENT #1**

0 45 MIN 105 MIN

♦ DRUG  

♦ ³H-ADENINE  

♦ SACRIFICE

**TREATMENT #2**

0 60 MIN

♦ DRUG AND ³H-ADENINE  

♦ SACRIFICE

**CECECTOMY**

0 60 MIN 105 MIN 165 MIN

♦ CECECTOMY  

♦ DRUG  

♦ ³H-ADENINE  

♦ SACRIFICE

---

**Fig. 4**

TREATMENT SCHEDULE FOR NONOPERATED AND OPERATED RATS
c. Studies on nonoperated, drug treated animals
(TREATMENT #2).

This experiment was carried out in the same manner as outlined above, but the $^3$H-Adenine was administered to the experimental animals at the same time as the drug (Fig. 4B).

d. Studies on cecectomized animals

Rats were cecectomized as described in Methods, section A-1. Sixty minutes after the operation, drug was administered to rats as outlined in Fig. 4C. Fifteen min later animals received an I.P. injection of 0.1cc labelled adenine (50μCi/cc). Tissues were prepared for the cyclic AMP assay as outlined under B-2.

e. Studies on cecectomized drug treated animals

The cecum was removed as described in Methods, section A-1. Sixty min after the operation, drug was administered to rats as outlined in Fig. 4C. Fifteen min later animals received an I.P. injection of 0.1cc labelled adenine (50μCi/cc). Tissues were prepared for the cyclic AMP assay as outlined under B-2.
2. Cyclic AMP assay

Intracellular levels of cAMP in the gut segments of nonoperated and cecectomized and drug treated nonoperated and cecectomized rats were measured by using a radio-isotope dilution method developed by Gilman (1970) and made available as a diagnostic kit by Amersham/Searle Corp. (Arlington Heights, Illinois). This assay was carried out as follows:

a. Reconstitution of reagents

All reagents were reconstituted at room temperature by adding the required amount of distilled water to each vial. To maintain maximum stability, all reagents were stored frozen after reconstitution.

b. Preparation of standards

One ml of a standard solution of cAMP containing 16pM/ml was serially diluted with 0.05M Tris pH7.5 containing 4mM EDTA buffer to obtain the following concentrations:

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>16 picomoles</td>
</tr>
<tr>
<td>#2</td>
<td>8 picomoles</td>
</tr>
<tr>
<td>#3</td>
<td>4 picomoles</td>
</tr>
<tr>
<td>#4</td>
<td>2 picomoles</td>
</tr>
<tr>
<td>#5</td>
<td>1 picomole</td>
</tr>
</tbody>
</table>
c. Assay procedure

For determining the total intestinal cAMP content, the supernatant obtained after Zn-Ba precipitation was used. This assay was done at 0°C and performed as outlined in Table 1. All tubes in duplicate, were kept in ice-bath and solutions were added in the following order: Buffer solution, standard solution or the unknown, cAMP-H3, and the binding protein. The volume of each was as indicated in Table 1.

After the addition of the binding protein, all tubes were mixed for about 5 sec and then placed into a refrigerator for at least 1-1/2 hours and maintained at 2-4°C.

At the end of the incubation period 100μl of the charcoal suspension was added, the tubes vortexed for 10-12 sec and then centrifuged immediately at room temperature for about 5 min to sediment, 200μl of sample was removed, placed in scintillation vials containing 10ml of PCS liquid scintillation fluid, and then counted for 10 min.

d. Calculation of results

In this assay the blank tubes did not contain the binding protein and served to check the
<table>
<thead>
<tr>
<th>Tube Numbers</th>
<th>BLANK</th>
<th>ZERO</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA buffer</td>
<td>150</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0pM cAMP</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0pM cAMP</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0pM cAMP</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0pM cAMP</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.0pM cAMP</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>cAMP-H₃</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Binding protein</td>
<td>0</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All determinations were done in duplicate.
nonspecific binding. The counts per min (cpm) for the blank tubes were averaged and subtracted from the average values obtained with the standard or the unknown pairs. The value thus obtained indicated the amount bound in the absence of unlabeled cAMP (Co) or in the presence of standard or unknown unlabeled cAMP (Cx). The ratio of Co/Cx was calculated for each standard and plotted on ordinate against picomoles of unlabeled cAMP per tube on linear graph paper. A straight line was obtained with an intercept at 1.0 on the ordinate (Fig.5). Using the calculated Co/Cx value of the unknowns, the number of picomoles of unknown cAMP present in the 50μl sample was then read directly from the standard curve.

3. Isolation of labelled cyclic AMP

In order to isolate the labelled cAMP, the neutralized supernatant was transferred to a 15ml conical centrifuge tube and evaporated to dryness under vacuum on a rotary evaporator. The resulting precipitate was dissolved in 200μl of 50% ethanol to which 20μl, containing 20μg of cold cyclic AMP was added as a carrier. The entire sample was streaked on a thin layer plate and developed for one hour in a solvent system.
Figure 5

Cyclic AMP Standard. Standard plot obtained by plotting the Co/Cx ratio of standards. Each point represents the mean of eight observations.
Fig. 5

cAMP STANDARD

$C_0/C_r$ vs. pM cAMP
containing the following:

- chloroform  40 parts
- methanol    30 parts
- water       3 parts

After developing, the plate was air-dried for 15 min and the carrier cAMP visualized under short-wave U.V. light. The spot was marked and a 4 x 5 cm area scraped off. The scrapings were eluted with 3 ml of 50% ethanol. One half of the sample was transferred to a scintillation vial containing 13 ml of PCS counting fluid and the sample counted for 10 min. From the remaining sample 0.3 ml was pipetted into a cuvette, 0.7 ml of 0.1 N HCl and a 0.1 ml of 0.5 M Tris buffer added and the sample read at 258 nm in a U.V. spectrophotometer.

4. Protein determination

For determining the amount of protein present in the tissue homogenate, a modified method of Lowry, et al, 1951 was followed.

a. Standard curve

A stock solution containing 1 mg/ml of bovine serum albumin (BSA) was diluted to a concentration of 200 μg/ml. Using this freshly prepared solution, standard serial dilutions were made as follows:
<table>
<thead>
<tr>
<th>Tube #</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (ml of 200μg per ml)</td>
<td>0.9</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
<td>0.05</td>
<td>0.025</td>
<td>0.01</td>
</tr>
<tr>
<td>μg of BSA in each tube</td>
<td>180</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>10</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>1N NaOH (ml)</td>
<td>0.1</td>
<td>0.5</td>
<td>0.75</td>
<td>0.875</td>
<td>0.95</td>
<td>0.975</td>
<td>0.99</td>
</tr>
</tbody>
</table>

b. Unknown sample

After the centrifugation of the tissue homogenate, the supernatant was removed, neutralized with 0.5M K-succinate and saved for the cAMP assay. The pellet, which was obtained after the centrifugation of the homogenate, was dissolved in 10ml of 1N NaOH and used for the protein determination. The samples were prepared as follows:

<table>
<thead>
<tr>
<th>Tube #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of sample (ml)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.05</td>
<td>0.025</td>
<td>0.01</td>
</tr>
<tr>
<td>Volume of 1N NaOH (ml)</td>
<td>0.1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.45</td>
<td>0.475</td>
<td>0.49</td>
</tr>
<tr>
<td>Dilution factors</td>
<td>x25</td>
<td>x50</td>
<td>x100</td>
<td>x200</td>
<td>x400</td>
<td>x1000</td>
</tr>
</tbody>
</table>
c. Assay procedure

To each 0.5ml of sample, standard or unknown, 5ml of potassium-sodium tartrate and copper sulfate mixture was added. The tubes were vortexed for about 10 sec and then allowed to stand at room temperature for about 15 min. Thereafter 0.5ml of phenol reagent was added to each 5.5ml of sample. Immediately thereafter the solution was mixed on a Vortex mixer. After 30 min of standing at room temperature the tubes were read at 625nM in a spectrophotometer using water as a blank. Protein determinations were linear up to the concentration of 100μg/ml (Fig. 6). The amount of protein present in the sample was calculated from the standard curve obtained by using BSA as a protein standard.

5. Statistical calculations

All the results obtained were evaluated using one way analysis of variance and the Student's t-test. P values less than 0.05 were considered statistically significant. The mean value ± standard error of the mean (X ± S.E.) are presented in all figures and tables.
Figure 6

Standard Curve for Protein Determinations. Each point represents the mean of twenty-one observations.
Fig. 6

STANDARD CURVE FOR PROTEIN DETERMINATIONS

\[
\begin{align*}
\text{OD} & \quad \mu g/ml \\
0.3 & \quad 200 \\
0.2 & \quad 100 \\
0.1 & \quad 50 \\
0.08 & \quad 25 \\
0.06 & \quad 10 \\
0.04 & \quad 5 \\
0.02 & \quad 2 \\
\end{align*}
\]
RESULTS

A. In Vivo Experiments

1. Intestinal motility at various time intervals after cecectomy.

The results obtained in this study are presented in Fig. 7 and show that a very significant depression (p<.001) in intestinal propulsive motility exists at two hours post-cecectomy. Two hours later intestinal motility recovered slightly and reached a plateau at which it remained for about 12 hours. At 16 hours post-cecectomy the intestinal propulsive motility started to recover so that at 24 hours post-surgery it regained approximately 90% of its normal propulsive activity. Gross observations of the intestinal tract at the time of sacrifice revealed that the jejunum in the cecectomized animal was dilated and filled with gas and fluid. This was present up to 12 hours post-operation in all cecectomized but not in sham-operated or normal control animals.

In the sham-operated animals, on the other hand, the depression in the propulsive intestinal motility at two hours post-surgery, although significant
Figure 7

Effect of Cecectomy on Propulsive Intestinal Transit of Charcoal Meal. Each point represents the mean of five observations. The mean ± S.E. is represented.
Fig. 7

EFFECT OF CECECTOMY ON PROPULSIVE INTESTINAL TRANSPORT OF CHARCOAL MEAL

INTESTINAL TRANSIT (%)

TIME POST CECECTOMY (HRS)
was very slight and remained almost constant throughout the experimental period. This indicated that the laparotomy per se and the ether anesthesia had only a minimal effect on the intestinal transport. No distention of intestinal tract or other gross abnormalities were noted in any of the sham-operated animals at the time of sacrifice. The variation in propulsive intestinal activity observed in non-operated control animals was believed to be due to the interruption of their normal sleep-awake cycle.

2. **Effect of drugs on intestinal motility in normal animals.**

The effect of drugs on the gastrointestinal transit of charcoal meal in normal nonoperated animals are presented in Fig. 8. A significant stimulation of propulsive intestinal motility was induced by two of the agents - niacin and calcium chloride (p<.01). Other agents, such as imidazole, theophylline and methacholine produced only a slight depression (p<.01); whereas atropine, an agent which sometimes is used in the treatment of gastrointestinal disorders, caused the most pronounced inhibition of charcoal transit (p<.001). It is likely that this depression in intestinal transit was not due to an effect of this drug
The Effect of Drugs on the Transit of Charcoal Meal in Nonoperated Rats. Each vertical bar represents the mean ± standard error of the mean. Numbers in parenthesis represent the number of animals in each group.
Fig. 8

THE EFFECT OF DRUGS ON THE TRANSPORT
OF CHARCOAL MEAL IN NONOPERATED RATS

![Bar chart showing the effect of drugs on the transport of charcoal meal in nonoperated rats. The bars represent different drugs and their doses (in parentheses). The vertical axis represents intestinal transit (%), and the horizontal axis lists the drugs: Unoperated Controls (23), Niacin (5), Calcium Chloride (5), Imidazole (5), Theophylline (5), Atropine (9), Methacholine (6).]
on the gut motility, but rather due to the inhibition of gastric emptying. In all atropine treated animals greatly distended stomachs were noted at the time of sacrifice.

3. **Effect of drugs on experimentally induced intestinal atony.**

The effects of various drugs on the gastrointestinal transit of charcoal meal in 2 hour cecrectomized rats are presented in Fig. 9. As can be seen, a highly significant ($p<.001$) depression of the intestinal propulsive motility was obtained after cecectomy and sham operation. This depression, if left untreated, returned to normal in about 24 hours (Fig. 7); but if treated with certain agents, such as niacin, calcium chloride, or imidazole, the return to normal was accelerated. Also, no intestinal dilation with the concomitant presence of gas in the intestinal tract, as seen in the cecrectomized animals, was noted in drug treated animals during gross inspection of the intestinal tract at the time of sacrifice.

Other drugs, such as methacholine or atropine, had no effect on the intestinal transit of charcoal meal. At the time of sacrifice it was noted, however, that in these animals the jejunum and the
Figure 9

The Effect of Drugs on the Intestinal Transit of Charcoal Meal in 2-hour Cecectomy Animals. Each vertical bar represents the mean ± standard error of the mean. Number in parenthesis represents the number of animals in each group.
CHARGEOAT MEAL IN 2 HOUR CECECTOMIZED ANIMALS

EFFECT OF DRUGS ON THE INTESTINAL TRANSPIRATOF

FIG. 9
proximal ileum was dilated and filled with gas and fluid. The stomachs also were dilated and filled with charcoal meal, but only a small amount could be found in the intestinal tract. This thus indicated that these drugs depressed the intestinal transit of charcoal meal by affecting gastric emptying, but not the intestinal motility. On the other hand, theophylline further depressed (P<.001) the intestinal transit. In these animals no dilation of the intestinal tract nor the accumulation of gas and liquid was noted. Only a small amount of charcoal was seen to be present in the stomach. Thus it is assumed that the depression of intestinal motility induced by theophylline was due to its action on the intestinal tract.

No intestinal dilation or accumulation of gas or fluid in the intestinal lumen was seen in either nonoperated or sham-operated rats. Also, no charcoal meal was found in the stomach at the time of sacrifice.

B. Cyclic AMP Content of Rat Intestine

1. Control studies

a. Nonoperated animals

These studies were carried out in order to determine whether or not the total cAMP content
was relatively constant throughout the intestinal tract. The results obtained are presented in Table 2 and indicate that there was essentially no variation in the total cAMP content from the proximal to the distal segment.

b. Cecectomized animals.

For determining the effect of cecectomy on the intracellular content of cAMP, a group of rats were cecectomized and various regions of the intestinal tract obtained as indicated in Table 2. The results are presented in Table 3 and indicate that the intestinal content of cAMP remained relatively constant throughout the intestinal tract and was not influenced by surgical stress. These results also indicate that the cAMP content/100mg wet tissue was essentially the same in nonoperated as well as cecectomized rats.

c. Determination of intestinal adenyl cyclase

Adenyl cyclase activity was measured according to Krishna, et al (1968) in a crude membrane fraction prepared from the middle 1cm segment of the small intestine. Only minimal cyclase activity was apparent in these preparations. Intestinal enzyme proved to be uniquely unstable and difficult
### TABLE 2

**CYCLIC AMP CONTENT OF INTESTINAL SEGMENTS**

<table>
<thead>
<tr>
<th>Intestinal segment*</th>
<th>Number of Observations</th>
<th>Total cAMP pM/1cm**</th>
<th>cAMP pM/100mg wet wt.**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>10</td>
<td>139.4 ± 5.8</td>
<td>120.6 ± 6.8</td>
</tr>
<tr>
<td>Medial</td>
<td>8</td>
<td>160.1 ± 19.5</td>
<td>122.3 ± 13.0</td>
</tr>
<tr>
<td>Distal</td>
<td>7</td>
<td>146.8 ± 2.6</td>
<td>153.3 ± 17.1</td>
</tr>
</tbody>
</table>

*The three intestinal segments were taken from the following areas of the rat intestinal tract:

Proximal - 1cm wide segment from the area 10cm distal to the ligament of Treitz.

Medial - 1cm wide segment from the middle of the rat small intestine.

Distal - 1cm wide segment 10cm proximal to the ileocecal junction.

**Values represent the \( \bar{x} \pm S.E. \).

†These values obtained using Diagnostic Products (Culver City, California) cAMP Assay Kit and are not corrected for recovery.
### TABLE 3

**CYCLIC AMP CONTENT OF INTESTINAL SEGMENTS AFTER CECECTOMY†**

<table>
<thead>
<tr>
<th>Intestinal segment*</th>
<th>Number of Observations</th>
<th>Total cAMP pM/1cm**</th>
<th>cAMP pM/100mg wet wt.**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>6</td>
<td>105.3 ± 9.7</td>
<td>107.4 ± 10.0</td>
</tr>
<tr>
<td>Medial</td>
<td>6</td>
<td>97.3 ± 4.2</td>
<td>99.1 ± 7.8</td>
</tr>
<tr>
<td>Distal</td>
<td>6</td>
<td>91.7 ± 7.9</td>
<td>126.0 ± 9.1</td>
</tr>
</tbody>
</table>

*Two hours after cecectomy three intestinal segments were taken from the areas of rat intestinal tract as described in the legend of Table 2.

**Values represent $\bar{x} \pm$ S.E.

†These values obtained using Diagnostic Products (Culver City, California) cAMP Assay Kit and are not for recovery.
to measure. Also, activity was not stimulated in the presence of NaF. Attempts to determine cyclase activity were not pursued any further.

2. Cyclic AMP Recovery

In order to estimate any loss of the endogenous cyclic AMP during the processing of tissue sample, 100μl of $^3$H-cAMP* was added to a tissue homogenate prepared from 5cm long intestinal segments. The results presented in Table 4 show greater than 90% recovery after ZnSO$_4$ and BaCl$_2$ treatment. This removed all nucleotides or other interfering substances but left cAMP, adenine, and adenosine in the supernatant. Cyclic AMP was separated from adenosine and other nucleotides by thin layer chromatography (Fig. 10). Cyclic AMP was eluted from the silica gel with 3ml of 50% ethanol. One half of the eluate was counted in a scintillation counter, but the other half was used to measure absorption at 258nm. Recoveries of cAMP were calculated from standard cAMP absorptivity. Cyclic AMP recoveries ranged from 71.0% to 84.3% (Table 4 and 5).

*Labelled cAMP obtained from Schwarz/Mann. Adenosine 3':5' cyclic phosphate [8-$^3$H]: Specific activity: 27Ci/m mole; concentration: 0.5mCi/ml. Solution prepared containing 5μCi/ml.
### TABLE 4

**RECOVERY OF $^3\text{H}$-cAMP**

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>NUMBER OF OBSERVATIONS</th>
<th>$^3\text{H}$-cAMP cpm</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Homogenate</td>
<td>4</td>
<td>$2.30 \times 10^5$</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>$1.12 \times 10^5$</td>
<td>100</td>
</tr>
<tr>
<td>5000xg Supernatant</td>
<td>4</td>
<td>$2.10 \times 10^5$</td>
<td>92.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>$1.09 \times 10^5$</td>
<td>97.1</td>
</tr>
<tr>
<td>Supernatant after</td>
<td>4</td>
<td>$2.08 \times 10^5$</td>
<td>91.1</td>
</tr>
<tr>
<td>Zn-Ba precipitation</td>
<td>6</td>
<td>$1.05 \times 10^5$</td>
<td>93.6</td>
</tr>
</tbody>
</table>

### TABLE 5

**RECOVERY OF $^3\text{H}$-cAMP AFTER TLC**

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>NUMBER OF OBSERVATIONS</th>
<th>COUNTS RECOVERED</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>6</td>
<td>$8.6 \times 10^4$</td>
<td>76.4</td>
</tr>
</tbody>
</table>

Thin layer chromatography methods were as described in Methods.
Figure 10

Separation of cAMP from Other Nucleotides by Thin Layer Chromatography. Two hundred micrograms of each nucleotide was applied to TLC and cAMP was applied in varying amounts as indicated.
Fig. 10

SEPARATION OF cAMP FROM OTHER NUCLEOTIDES BY THIN LAYER CHROMATOGRAPHY

A = Adenine
AR = Adenosine
C. In Vivo Studies on Intestinal Cyclic AMP

1. Turnover of Cyclic AMP

a. Intestinal cyclic AMP levels in nonoperated animals

The possibility was investigated that very little of the isolated total cyclic AMP was contributed by mucosal layer. Results obtained are presented in Table 6 and 7. They show that essentially all of the cyclic AMP was from the muscle layers of the intestinal tract since the total cAMP content was not diminished by the removal of mucosa before the cAMP determination. This indicates that most of the cAMP was from the muscular layers.

b. Intestinal cyclic AMP levels in normal drug treated animals. TREATMENT #1

The effect of several drugs on the cyclic AMP content of intestinal tract was investigated. Results, as presented in Table 8 indicate that the treatment of animals with niacin, CaCl₂, imidazole, and theophylline significantly lowered the intestinal cAMP content, expressed on the basis of 100mg wet weight (p<.01). Atropine and methacholine had no appreciable effect when cAMP was expressed as pM/mg protein (Table 9). All drugs had a significant effect on the cAMP levels except methacholine.
TABLE 6

CAMP CONTENT IN INTESTINAL SEGMENTS OF NONTREATED RATS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF ANIMALS</th>
<th>CAMP pM/100mg (a)</th>
<th>$^3$H-cAMP Cts/100mg (b)</th>
<th>CAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>12</td>
<td>296.7 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Nontreated mucosa removed</td>
<td>6</td>
<td>316.4 ± 50.9</td>
<td>110.2 ± 15.6</td>
<td>0.36 ± 0.03</td>
</tr>
</tbody>
</table>

(a) Values represent picomoles cAMP/100mg wet weight expressed as the mean ± S.E.

(b) Values represent incorporation of $^3$H-Adenine into cAMP and are expressed as counts/100mg wet wt. The mean ± S.E. is indicated.
TABLE 7

CAMP CONTENT IN INTESTINAL SEGMENTS OF NONTREATED RATS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF ANIMALS</th>
<th>CAMP pM/mg protein (a)</th>
<th>$^3$H-CAMP Cts/mg protein (b)</th>
<th>CAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>12</td>
<td>21.8 ± 10.0</td>
<td>6.9 ± 0.8</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Nontreated mucosa removed</td>
<td>6</td>
<td>25.9 ± 5.5</td>
<td>8.7 ± 1.6</td>
<td>0.36 ± 0.02</td>
</tr>
</tbody>
</table>

(a) Values represent picomoles CAMP/mg protein expressed as the mean ± S.E.
(b) Values represent incorporation of $^3$H-Adenine into CAMP and are expressed as Cts/mg protein. The mean ± S.E. is indicated.
### TABLE 8

THE EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF $^{3}$H-ADENINE INTO cAMP OF AN INTESTINAL SEGMENT OF NONOPERATED ANIMALS (TREATMENT #1)

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP $^{(a)}$</th>
<th>$^{3}$H-cAMP $^{(b)}$</th>
<th>cAMP $^{(b)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated Controls</td>
<td>12</td>
<td>296.7 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>150.5 ± 16.3*</td>
<td>23.6 ± 5.2*</td>
<td>0.15 ± 0.02*</td>
</tr>
<tr>
<td>CaCl$_{2}$</td>
<td>6</td>
<td>153.9 ± 20.5*</td>
<td>55.3 ± 5.7*</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>192.9 ± 15.7*</td>
<td>52.3 ± 5.0*</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>166.6 ± 12.2*</td>
<td>122.5 ± 16.8</td>
<td>0.77 ± 0.12*</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>234.9 ± 25.4$^{(c)}$</td>
<td>320 ± 29.2*</td>
<td>1.39 ± 0.11*</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>203.5 ± 14.9$^{(c)}$</td>
<td>285.3 ± 51.4*</td>
<td>1.47 ± 0.31*</td>
</tr>
</tbody>
</table>

*Significant difference between drug treatment and nontreated controls ($p<0.01$).

(a) Values represent picomoles cAMP/100mg wet weight expressed as the mean ± S.E.

(b) Values represent incorporation of $^{3}$H-Adenine into cAMP and are expressed as counts/100mg wet weight. The mean ± S.E. is indicated.

(c) Values not significantly different from control (.05<$p$<.10).
TABLE 9

THE EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF $^3$H-ADENINE INTO cAMP OF AN INTESTINAL SEGMENT OF NONOPERATED ANIMALS (TREATMENT #1)

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/mg protein (a)</th>
<th>$^3$H-cAMP Cts/mg protein (b)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated Controls</td>
<td>12</td>
<td>21.8 + 2.9</td>
<td>6.9 + 0.8</td>
<td>0.34 + 0.03</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>10.5 + 1.1*</td>
<td>1.6 + 0.4*</td>
<td>0.15 + 0.03*</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>6</td>
<td>12.1 + 1.6*</td>
<td>4.4 + 0.5*</td>
<td>0.37 + 0.02</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>14.8 + 1.4*</td>
<td>4.0 + 0.4*</td>
<td>0.27 + 0.02</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>11.4 + 0.8*</td>
<td>8.3 + 1.1</td>
<td>0.77 + 0.12*</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>16.9 + 2.0*</td>
<td>23.2 + 2.5*</td>
<td>1.39 + 0.11*</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>19.4 + 1.4</td>
<td>27.1 + 4.7*</td>
<td>1.47 + 0.31*</td>
</tr>
</tbody>
</table>

*Significant difference between drug treatment and nontreated controls (p<.02).

(a) Values represent picomoles cAMP/mg protein expressed as the mean ± S.E.

(b) Values represent incorporation of $^3$H-Adenine into cAMP and are expressed as counts/mg protein. The mean ± S.E. is indicated.
Incorporation of labelled adenine into cAMP of the intestinal tract was significantly lowered (p<.01) by niacin, CaCl₂, or imidazole treatment; whereas treatment of animals with atropine or methacholine significantly (p<.001) increased the incorporation. Theophylline treatment, on the other hand, had no effect. Specific activity, which measures the utilization of cAMP, was decreased by niacin, but greatly increased after theophylline, atropine, or methacholine treatment. CaCl₂ or imidazole had no effect on the utilization of cAMP when compared to the controls (Table 8). A similar trend was observed when cAMP content was expressed as pM cAMP per mg protein (Table 9).

c. Intestinal cyclic AMP levels in nonoperated animals. TREATMENT #2.

The results, as presented in Table 10, show that there is a significant difference (p<.01) in the cyclic AMP content per 100mg wet tissue weight in Treatment Group #2 between the nonoperated controls and drug treated animals except after theophylline and methacholine. The conversion of
TABLE 10
THE EFFECT OF DRUG TREATMENT ON THE
INCORPORATION OF \( ^3\text{H}-\text{Adenine} \) INTO cAMP OF AN INTESTINAL
SEGMENT OF NONOPERATED ANIMALS (TREATMENT #2)

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/100mg (a)</th>
<th>( ^3\text{H}-\text{cAMP} ) Cts/100mg (b)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated controls</td>
<td>12</td>
<td>296.7 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>182.1 ± 19.6*</td>
<td>54.2 ± 3.7*</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>( \text{CaCl}_2 )</td>
<td>6</td>
<td>169.0 ± 6.8*</td>
<td>100.8 ± 9.3</td>
<td>0.59 ± 0.04*</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>186.0 ± 4.5*</td>
<td>28.6 ± 4.4*</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>209.7 ± 9.9</td>
<td>173.8 ± 6.8*</td>
<td>0.84 ± 0.06*</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>169.1 ± 8.9*</td>
<td>420.1 ± 13.9*</td>
<td>2.51 ± 0.13*</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>207.6 ± 17.8</td>
<td>210.6 ± 19.9*</td>
<td>1.56 ± 0.18*</td>
</tr>
</tbody>
</table>

*Significant difference between controls and drug treatment (p<.01).

(a) Values represent picomoles cAMP/100mg wet weight and expressed as the mean ± S.E.

(b) Values represent incorporation of \( ^3\text{H}-\text{Adenine} \) into cAMP and are expressed as counts/100mg wet weight. The mean ± S.E. is indicated.
$^{3}$H-Adenine to $^{3}$H-cAMP was significantly depressed by niacin and imidazole, but greatly elevated after theophylline, atropine and methacholine treatment; CaCl$_2$ was not different from the controls. Decreased specific activity was obtained only after imidazole treatment, whereas the treatment with other drugs significantly elevated the cAMP turnover. Niacin, however, showed no apparent effect on the turnover rate of cAMP. Since it decreased both the content and the incorporation to the same extent, thereby the specific activity was not changed.

When the cAMP content was expressed as pM per mg protein, changes in cAMP content were obtained only after imidazole and atropine treatment (Table 11). The observed difference in this case was not due to the change in total protein induced by the drug treatment, but rather to a slight drop and a great variability in the total cyclic AMP content. Other drugs showed no difference from controls. Incorporation of counts into cAMP was affected by all drugs except CaCl$_2$, but changes in the turnover rate of cAMP were induced by the treatment with all drugs except niacin, which had no effect on the specific activity of cAMP (Table 10.
<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/mg protein (a)</th>
<th>$^3$H-cAMP Cts/mg protein (b)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated controls</td>
<td>12</td>
<td>21.8 ± 2.9</td>
<td>6.9 ± 0.8</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>13.2 ± 1.5</td>
<td>3.9 ± 0.3*</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>6</td>
<td>16.1 ± 1.4</td>
<td>9.3 ± 0.7</td>
<td>0.59 ± 0.04*</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>12.9 ± 0.3*</td>
<td>2.0 ± 0.3*</td>
<td>0.15 ± 0.02*</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>16.4 ± 0.8</td>
<td>13.6 ± 0.8*</td>
<td>0.84 ± 0.06*</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>12.6 ± 0.9*</td>
<td>31.0 ± 1.2*</td>
<td>2.51 ± 0.13*</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>16.9 ± 1.5</td>
<td>24.7 ± 1.4*</td>
<td>1.53 ± 0.16*</td>
</tr>
</tbody>
</table>

*Significant difference between controls and drug treatment (p<.01).

(a) Values represent picomoles of cAMP/mg protein expressed as the mean ± S.E.

(b) Values represent incorporation of $^3$H-Adenine into cAMP and are expressed as counts/mg protein. The mean ± S.E. is indicated.
and 11).

d. **Intestinal cyclic AMP levels in 2 hour cecectomized animals**

When cAMP content and utilization were measured by the administration of $^3$H-Adenine to animals after cecectomy, there were no significant differences in cAMP content/100mg wet tissue weight or per mg protein between the two hour cecectomized and nonoperated animals (Table 12). A slight depression in total protein was noted in the cecectomized control group, but due to the great variability in protein of the nonoperated controls, the difference became insignificant.

The drug treatment of 2 hour cecectomized animals did not change the cAMP content per 100mg wet tissue from controls (Table 13). Significant changes in the incorporation of counts into cAMP were observed by the treatment with all the drugs except theophylline. In a similar manner the specific activity of cAMP was altered by all drug treatments except imidazole and theophylline.

A slightly different response was obtained when the cAMP content was expressed as pM/mg protein (Table 14). The treatment of rats with CaCl$_2$ and imidazole significantly depressed the cAMP content
### TABLE 12
CAMP CONTENT OF INTESTINAL SEGMENTS FROM NONOPERATED AND 2-HOUR CECECTOMIZED RATS

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of animals</th>
<th>CAMP pM/100mg (a)</th>
<th>CAMP pM/mg protein (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecectomized controls</td>
<td>6</td>
<td>231.6 ± 33.3</td>
<td>20.1 ± 3.1</td>
</tr>
<tr>
<td>Nonoperated controls</td>
<td>12</td>
<td>296.7 ± 33.7</td>
<td>21.8 ± 2.9</td>
</tr>
</tbody>
</table>

(a) Values represent picomoles of CAMP/100mg wet weight and expressed as the mean ± S.E.

(b) Values represent picomoles of CAMP/mg protein expressed as the mean ± S.E.
TABLE 13
THE EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF $^3$H-ADENINE INTO cAMP OF AN INTESTINAL SEGMENT FROM 2 HOUR CECECTOMIZED RATS

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/100mg (a)</th>
<th>$^3$H-cAMP Cts/100mg (b)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hour cecectomized</td>
<td>6</td>
<td>231.6 ± 33.3</td>
<td>114.0 ± 5.6</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>260.0 ± 23.1</td>
<td>68.8 ± 3.4*</td>
<td>0.27 ± 0.02*</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>6</td>
<td>197.9 ± 20.1</td>
<td>41.5 ± 7.0*</td>
<td>0.21 ± 0.02*</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>231.6 ± 24.1</td>
<td>83.8 ± 6.6*</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>247.6 ± 25.4</td>
<td>127.6 ± 19.1</td>
<td>0.56 ± 0.12</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>244.1 ± 40.7</td>
<td>24.3 ± 3.1*</td>
<td>0.11 ± 0.02*</td>
</tr>
<tr>
<td>Methacholine</td>
<td>5</td>
<td>279.5 ± 43.2</td>
<td>74.0 ± 4.5*</td>
<td>0.29 ± 0.05*</td>
</tr>
</tbody>
</table>

*Significant difference between controls and drug treatment (p<0.01).

(a) Values represent picomoles cAMP/100mg wet weight and are expressed as the mean ± S.E.

(b) Values represent incorporation of $^3$H-Adenine into cAMP and are expressed as Cts/100mg wet weight. The mean ± S.E. is indicated.
TABLE 14

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/mg protein (a)</th>
<th>³H-cAMP Cts/mg protein (b)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hour cecrectomized</td>
<td>6</td>
<td>20.1 ± 3.1</td>
<td>9.7 ± 0.5</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>21.4 ± 2.8</td>
<td>5.2 ± 0.3*</td>
<td>0.27 ± 0.02*</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>6</td>
<td>12.1 ± 1.3*</td>
<td>2.6 ± 0.5*</td>
<td>0.21 ± 0.02*</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>15.7 ± 2.1*</td>
<td>5.6 ± 0.6*</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>20.8 ± 2.5</td>
<td>10.4 ± 1.3</td>
<td>0.55 ± 0.12</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>17.9 ± 3.3</td>
<td>1.8 ± 0.2*</td>
<td>0.11 ± 0.02*</td>
</tr>
<tr>
<td>Methacholine</td>
<td>5</td>
<td>21.7 ± 2.6</td>
<td>5.9 ± 0.4*</td>
<td>0.29 ± 0.05*</td>
</tr>
</tbody>
</table>

*Significant difference between controls and drug treatment (p<.025).

(a) Values represent picomoles cAMP/mg protein and are expressed as the mean ± S.E.

(b) Values represent incorporation of ³H-Adenine into cAMP and are expressed as Cts/mg protein. The mean ± S.E. is indicated.
whereas the other drugs had no effect when compared to controls. This effect was probably due to the changes in total protein observed after CaCl₂ and imidazole treatments. Incorporation of counts into cAMP was significantly depressed by all drugs except theophylline. Also, the specific activity of cAMP was significantly depressed by the treatment with all drugs except imidazole and theophylline.

2. Comparison of results
   a. Comparison of Treatment #1 with Treatment #2.

   Comparing the effect of drugs in the two treatment groups, it is evident that the changes noted were qualitatively in the same direction after both treatment schedules (see cAMP/100mg or mg protein Table 15 and 16). In both treatment groups niacin significantly decreased the incorporation of labelled adenine into ^3H-cAMP. In Treatment #1 and #2 niacin treatment diminished incorporation to approximately 25 and 50% respectively to control. CaCl₂ treatment decreased the incorporation of counts in Treatment #1 but had no effect in Treatment #2; whereas imidazole decreased the incorporation by 50% in
Table 15

Effect of Drug Treatment on the Incorporation of \(^3\text{H}\)-Adenine into cAMP of Intestinal segment from Nonoperated Rats\(a\).

a) Treatment schedules or as described in Methods section, Fig. 4.

b) Values represent picomoles cAMP/100wet weight and are expressed as the mean ± S.E.

c) Values represent incorporation of \(^3\text{H}\)-Adenine into cAMP and are expressed as Cts/100mg wet weight. The mean ± S.E. is indicated.

Significant differences exist in cAMP/100mg after theophylline and atropine treatments between Treatment #1 and Treatment #2. Significance: (p<.025) and (p<.05) respectively. Significant differences also exist in the incorporation of \(^3\text{H}\)-Adenine into cAMP and between specific activities of Treatment #1 and Treatment #2 after:

<table>
<thead>
<tr>
<th>Drugs</th>
<th>(^3\text{H})-cAMP Cts/100mg</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>(p&lt;.001)</td>
<td>(p&lt;.005)</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>(p&lt;.005)</td>
<td>(p&lt;.001)</td>
</tr>
<tr>
<td>Imidazole</td>
<td>(p=.005)</td>
<td>(p&lt;.005)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>(p&lt;.02)</td>
<td>-</td>
</tr>
<tr>
<td>Atropine</td>
<td>(p&lt;.02)</td>
<td>(p&lt;.001)</td>
</tr>
</tbody>
</table>
Table 15

EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF $^3$H-ADENINE INTO cAMP OF INTESTINAL SEGMENTS OF NONOPERATED RATS (a)

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/100mg (b)</th>
<th>$^3$H-cAMP Cts/100mg (c)</th>
<th>cAMP Cts/pM</th>
<th>Number of animals</th>
<th>cAMP pM/100mg (b)</th>
<th>$^3$H-cAMP Cts/100mg (c)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>2967 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
<td>12</td>
<td>2967 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>*150.5 ± 16.3</td>
<td>*23.6 ± 5.2</td>
<td>*0.15 ± 0.02</td>
<td>6</td>
<td>**182.1 ± 19.6</td>
<td>**54.2 ± 3.7</td>
<td>**0.31 ± 0.02</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>*192.9 ± 15.7</td>
<td>*52.3 ± 50</td>
<td>*0.27 ± 0.02</td>
<td>6</td>
<td>**186 ± 4.5</td>
<td>**28.6 ± 4.4</td>
<td>**0.15 ± 0.02</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>6</td>
<td>*153.9 ± 20.5</td>
<td>*55.3 ± 5.7</td>
<td>*0.37 ± 0.02</td>
<td>6</td>
<td>**169 ± 6.8</td>
<td>100.8 ± 9.3</td>
<td>**0.59 ± 0.04</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>234.9 ± 25.4</td>
<td>320.0 ± 29.2</td>
<td>1.39 ± 0.11</td>
<td>6</td>
<td>**169 ± 8.9</td>
<td>**420.1 ± 13.9</td>
<td>**2.51 ± 0.13</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>203.5 ± 14.9</td>
<td>285.3 ± 51.4</td>
<td>1.47 ± 0.31</td>
<td>6</td>
<td>2076 ± 17.8</td>
<td>**310.6 ± 19.9</td>
<td>**1.56 ± 0.18</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>*166.6 ± 12.2</td>
<td>122.5 ± 16.8</td>
<td>*0.77 ± 0.12</td>
<td>6</td>
<td>2097 ± 9.9</td>
<td>**173.8 ± 6.8</td>
<td>**0.84 ± 0.06</td>
</tr>
</tbody>
</table>

* Significant difference between controls and drug treatment (p < 0.05)

** Significant difference between controls and drug treatment (p < 0.01)
Table 16

Effect of Drug Treatment on the Incorporation of $^3$H-Adenine into cAMP of Intestinal Segment from Nonoperated Rats (a).

**a)** Treatment schedules or as described in Methods section, Fig. 4.

**b)** Values represent picomoles cAMP/mg protein and are expressed as the mean ± S.E.

**c)** Values represent incorporation of $^3$H-Adenine into cAMP and are expressed as Cts/mg protein. The mean ± S.E. is indicated.

Significant difference exists in cAMP/mg protein after theophylline treatment between Treatment #1 and Treatment #2. Significance ($p<.005$). Significant differences are also found to exist in incorporation of $^3$H-Adenine into cAMP and specific activities between Treatment #1 and Treatment #2 after various drug treatments.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$^3$H-cAMP Cts/mg cAMP protein</th>
<th>Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>(p&lt;.001)</td>
<td>(p&lt;.005)</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>(p&lt;.001)</td>
<td>(p&lt;.001)</td>
</tr>
<tr>
<td>Imidazole</td>
<td>(p&lt;.005)</td>
<td>(p&lt;.005)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>(p&lt;.005)</td>
<td>-</td>
</tr>
<tr>
<td>Atropine</td>
<td>(p&lt;.02)</td>
<td>(p&lt;.001)</td>
</tr>
</tbody>
</table>
## Table 15

**EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF $^3$H-ADENINE INTO cAMP OF INTESTINAL SEGMENTS OF NONOPERATED RATS**

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/100mg</th>
<th>$^3$H-cAMP Cts/100mg</th>
<th>cAMP Cts/pM</th>
<th>Number of animals</th>
<th>cAMP pM/100mg</th>
<th>$^3$H-cAMP Cts/100mg</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>12</td>
<td>2967 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
<td>12</td>
<td>2967 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>150.5 ± 16.3</td>
<td>23.6 ± 5.2</td>
<td>0.15 ± 0.02</td>
<td>6</td>
<td>182.1 ± 19.6</td>
<td>54.2 ± 3.7</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>192.9 ± 15.7</td>
<td>52.3 ± 50</td>
<td>0.27 ± 0.02</td>
<td>6</td>
<td>186 ± 4.5</td>
<td>28.6 ± 4.4</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>6</td>
<td>153.9 ± 20.5</td>
<td>55.3 ± 5.7</td>
<td>0.37 ± 0.02</td>
<td>6</td>
<td>169 ± 6.8</td>
<td>100.8 ± 9.3</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>234.9 ± 25.4</td>
<td>320.0 ± 29.2</td>
<td>1.39 ± 0.11</td>
<td>6</td>
<td>169 ± 8.9</td>
<td>420.1 ± 13.9</td>
<td>2.51 ± 0.13</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>203.5 ± 14.9</td>
<td>285.3 ± 51.4</td>
<td>1.47 ± 0.31</td>
<td>6</td>
<td>2076 ± 17.8</td>
<td>310.6 ± 19.9</td>
<td>1.56 ± 0.18</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>166.6 ± 12.2</td>
<td>122.5 ± 16.8</td>
<td>0.77 ± 0.12</td>
<td>6</td>
<td>2097 ± 9.9</td>
<td>173.8 ± 6.8</td>
<td>0.84 ± 0.06</td>
</tr>
</tbody>
</table>

* * Significant difference between controls and drug treatment ($p < 0.05$)

** Significant difference between controls and drug treatment ($p < 0.01$)
Table 16

EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF \(^{3}\text{H}\)-ADENINE INTO \(\text{cAMP}\) OF INTESTINAL SEGMENTS OF NONOPERATED RATS\(^{(a)}\)

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>(\text{cAMP} , \text{pM/mg protein}) (^{(b)})</th>
<th>(\text{(^{3}\text{H-cAMP} , \text{cts/mg protein})}) (^{(c)})</th>
<th>(\text{(^{3}\text{H-cAMP} , \text{cts/pM})})</th>
<th>Number of animals</th>
<th>(\text{cAMP} , \text{pM/mg protein}) (^{(b)})</th>
<th>(\text{(^{3}\text{H-cAMP} , \text{cts/mg protein})}) (^{(c)})</th>
<th>(\text{(^{3}\text{H-cAMP} , \text{cts/pM})})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>12</td>
<td>(21.8 \pm 2.9) (6.9 \pm 0.8)</td>
<td>(0.34 \pm 0.03)</td>
<td>12</td>
<td>(21.8 \pm 2.9) (6.9 \pm 0.8)</td>
<td>(0.34 \pm 0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>(10.5 \pm 1.1) (1.6 \pm 0.4)</td>
<td>(0.15 \pm 0.03)</td>
<td>6</td>
<td>(13.2 \pm 1.5) (3.9 \pm 0.3)</td>
<td>(0.31 \pm 0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>(14.8 \pm 1.4) (4.0 \pm 0.4)</td>
<td>(0.27 \pm 0.02)</td>
<td>6</td>
<td>(12.9 \pm 0.3) (2.0 \pm 0.3)</td>
<td>(0.15 \pm 0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{CaCl}_2)</td>
<td>6</td>
<td>(12.1 \pm 1.6) (4.4 \pm 0.5)</td>
<td>(0.37 \pm 0.02)</td>
<td>6</td>
<td>(16.1 \pm 1.4) (9.3 \pm 0.7)</td>
<td>(0.59 \pm 0.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>(16.9 \pm 2.0) (23.2 \pm 2.5)</td>
<td>(1.39 \pm 0.11)</td>
<td>6</td>
<td>(12.6 \pm 0.9) (31.0 \pm 1.2)</td>
<td>(2.51 \pm 0.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>(19.4 \pm 1.4) (27.1 \pm 4.7)</td>
<td>(1.47 \pm 0.31)</td>
<td>6</td>
<td>(16.9 \pm 1.5) (24.7 \pm 1.4)</td>
<td>(1.53 \pm 0.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>(11.4 \pm 0.8) (8.3 \pm 1.1)</td>
<td>(0.77 \pm 0.1)</td>
<td>6</td>
<td>(16.4 \pm 0.8) (13.6 \pm 0.8)</td>
<td>(0.84 \pm 0.06)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* \text{SIGNIFICANT DIFFERENCE BETWEEN CONTROLS AND DRUG TREATMENT (p < 0.05)}

\** \text{SIGNIFICANT DIFFERENCE BETWEEN CONTROLS AND DRUG TREATMENT (p < 0.01)}
Treatment #1 but by 70% in Treatment #2. Treatment of animals with theophylline, atropine, or methacholine increased the incorporation of counts in both treatment groups but in Treatment #2 the increase was approximately 1.5 fold greater than in Treatment #1.

Exposure of animals to drugs also affected the turnover rate of cAMP in both treatment groups as reflected in the specific activity of cAMP. Niacin significantly decreased the specific activity of cAMP in Treatment #1 but it had no effect in Treatment #2. Other agents, such as CaCl$_2$ and imidazole, had no effect in Treatment #1 but in Treatment #2 CaCl$_2$ significantly elevated, whereas imidazole significantly lowered, the cAMP specific activity. Drugs such as theophylline, atropine and methacholine, significantly increased the specific activity of cAMP in both treatment groups. The increase with atropine was approximately 2 fold greater in Treatment #2 than in Treatment #1.

Similar results were obtained when cAMP was expressed per mg protein (Table 16).

b. Comparison of Treatment #1 to 2 hour cecectomy.

No obvious differences were noted in the cAMP content or the incorporation of labelled adenine
into cAMP of nonoperated controls of Treatment #1 and 2 hour cecectomy; but the specific activity was approximately 1.5 fold higher in the latter treatment. Drug treatment, on the other hand, had a very pronounced effect on the cAMP content in Treatment #1 but had no such effect in cecectomy-mized animals. In these animals drug treatment affected the incorporation of labelled adenine as well as the utilization of cAMP (Table 17). A comparison of data from Treatment #1 and 2 hour cecectomy showed that niacin and imidazole treatment increased the incorporation of labelled adenine in the cecectomized animals about 3 and 1.5 fold respectively, but CaCl₂ and theophylline had essentially no effect. Atropine and methacholine treatment markedly decreased the incorporation in the cecectomized animals. The turnover rate of cAMP was affected to the same extent. Niacin and imidazole treatment increased the turnover of cAMP about 30 to 50% but CaCl₂ decreased it by 50%. On the other hand the treatment with theophylline, atropine, or methacholine decreased the specific activity by 1.5, 14 and 5 fold respectively. Similar results were obtained when cAMP was expressed as pM per mg protein (Table 18).
Table 17

Effect of Drug Treatment on the Incorporation of $^3$H-Adenine into cAMP of Intestinal Segments from Nonoperated and Cecectomy Rats (a).

a) Treatment schedule as described in Methods section, Fig. 4.

b) Values represent picomoles MP/100mg wet weight and are expressed as the mean ± S.E.

c) Values represent incorporation of $^3$H-Adenine into cAMP and are expressed as Cts/100mg wet weight. The mean ± S.E. is indicated.

Significant differences exist in cAMP/100mg wet weight after niacin and theophylline treatment between Treatment #1 and cecectomy. Significance: (p<.005) and (p<.02) respectively. Significant differences were also found to exist in incorporation of $^3$H-Adenine into cAMP and specific activities between Treatment #1 and Cecectomy after various drug treatments.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$^3$H-cAMP Cts/100mg</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>(p&lt;.001)</td>
<td>(p&lt;.005)</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>-</td>
<td>(p&lt;.001)</td>
</tr>
<tr>
<td>Imidazole</td>
<td>(p&lt;.005)</td>
<td>-</td>
</tr>
<tr>
<td>Atropine</td>
<td>(p&lt;.001)</td>
<td>(p&lt;.001)</td>
</tr>
<tr>
<td>Methacholine</td>
<td>(p&lt;.005)</td>
<td>(p&lt;.01)</td>
</tr>
</tbody>
</table>
Table 17

EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF $^3$H-ADENINE INTO cAMP OF INTESTINAL SEGMENTS OF NONOPERATED AND CECECTOMIZED RATS(a)

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/100mg(b)</th>
<th>$^3$H-cAMP Cts/100mg(c)</th>
<th>cAMP Cts/pM</th>
<th>Number of animals</th>
<th>cAMP pM/100mg(b)</th>
<th>$^3$H-cAMP Cts/100mg(c)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>12</td>
<td>296.7 ± 33.7</td>
<td>94.7 ± 9.4</td>
<td>0.34 ± 0.03</td>
<td>6</td>
<td>231.6 ± 33.3</td>
<td>114.0 ± 5.6</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>*150.5 ± 16.3</td>
<td>*23.6 ± 5.2</td>
<td>*0.15 ± 0.02</td>
<td>6</td>
<td>260.0 ± 23.1</td>
<td>**68.8 ± 3.4</td>
<td>**0.27 ± 0.02</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>*192.9 ± 15.7</td>
<td>*52.3 ± 5.0</td>
<td>0.27 ± 0.02</td>
<td>6</td>
<td>231.6 ± 24.1</td>
<td>**83.8 ± 6.6</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>6</td>
<td>*153.9 ± 20.5</td>
<td>*55.3 ± 5.7</td>
<td>0.37 ± 0.02</td>
<td>6</td>
<td>197.9 ± 20.1</td>
<td>**41.5 ± 7.0</td>
<td>**0.21 ± 0.02</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>234.9 ± 25.4</td>
<td>*320.0 ± 29.2</td>
<td>*1.39 ± 0.1</td>
<td>6</td>
<td>244.1 ± 40.7</td>
<td>**24.3 ± 3.1</td>
<td>**0.11 ± 0.02</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>203.5 ± 14.9</td>
<td>*285.3 ± 51.4</td>
<td>*1.47 ± 0.3</td>
<td>5</td>
<td>279.5 ± 43.2</td>
<td>**74.0 ± 4.5</td>
<td>**0.29 ± 0.05</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>*166.6 ± 12.2</td>
<td>122.5 ± 16.8</td>
<td>*0.77 ± 0.1</td>
<td>6</td>
<td>247.6 ± 25.4</td>
<td>127.6 ± 19.1</td>
<td>0.56 ± 0.12</td>
</tr>
</tbody>
</table>

* Significant difference between controls and drug treatment (p<.01)
** Significant difference between controls and drug treatment (p<.001)
Table 18

Effect of Drug Treatment on the Incorporation of $^3$H-Adenine into cAMP of Intestinal Segments from Nonoperated and Cecrectomized Rats(a).

- Treatment schedule or as described in Methods section, Fig. 4.
- Values represent picomoles cAMP/mg protein and are expressed as the mean ± S.E.
- Values represent incorporation of $^3$H-Adenine into cAMP and are expressed as Cts/mg protein. The mean ± S.E. is indicated.

Significant differences were found to exist in cAMP/mg protein after niacin and theophylline treatment between Treatment #1 and Cecectomy. Significance: (p<.005) and (p<.01) respectively. Significant differences were also found to exist in incorporation of $^3$H-Adenine into cAMP and specific activities between Treatment #1 and Cecectomy after various drug treatments.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$^3$H-cAMP Cts/mg protein</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>(p&lt;.001)</td>
<td>(p&lt;.005)</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>(p&lt;.02)</td>
<td>(p&lt;.001)</td>
</tr>
<tr>
<td>Imidazole</td>
<td>(p&lt;.05)</td>
<td>-</td>
</tr>
<tr>
<td>Atropine</td>
<td>(p&lt;.001)</td>
<td>(p&lt;.001)</td>
</tr>
<tr>
<td>Methacholine</td>
<td>(p&lt;.005)</td>
<td>(p&lt;.01)</td>
</tr>
</tbody>
</table>
Table 18

EFFECT OF DRUG TREATMENT ON THE INCORPORATION OF $^3$H-ADENINE INTO cAMP OF INTESTINAL SEGMENTS OF NONOPERATED AND CECECTOMIZED RATS

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Number of animals</th>
<th>cAMP pM/mg protein (b)</th>
<th>$^3$H-cAMP Cts/mg protein (c)</th>
<th>cAMP Cts/pM</th>
<th>Number of animals</th>
<th>cAMP pM/mg protein (b)</th>
<th>cAMP Cts/mg protein (c)</th>
<th>cAMP Cts/pM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td>12</td>
<td>21.8 ± 29</td>
<td>6.9 ± 0.8</td>
<td>0.34 ± 0.03</td>
<td>6</td>
<td>20.1 ± 3.1</td>
<td>9.7 ± 0.5</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>Niacin</td>
<td>6</td>
<td>* 10.5 ± 1.1</td>
<td>* 1.6 ± 0.4</td>
<td>* 0.15 ± 0.03</td>
<td>6</td>
<td>21.4 ± 2.8</td>
<td>** 5.2 ± 0.3</td>
<td>** 0.27 ± 0.02</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6</td>
<td>* 14.8 ± 1.4</td>
<td>* 4.0 ± 0.4</td>
<td>0.27 ± 0.02</td>
<td>6</td>
<td>** 15.7 ± 2.1</td>
<td>** 5.6 ± 0.6</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>6</td>
<td>* 12.1 ± 1.6</td>
<td>* 4.4 ± 0.5</td>
<td>0.37 ± 0.02</td>
<td>6</td>
<td>** 12.1 ± 1.3</td>
<td>** 2.6 ± 0.5</td>
<td>** 0.21 ± 0.02</td>
</tr>
<tr>
<td>Atropine</td>
<td>6</td>
<td>* 16.9 ± 2.0</td>
<td>* 23.2 ± 2.5</td>
<td>1.39 ± 0.11</td>
<td>6</td>
<td>17.9 ± 3.3</td>
<td>** 1.8 ± 0.2</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Methacholine</td>
<td>6</td>
<td>19.4 ± 1.4</td>
<td>* 27.1 ± 4.7</td>
<td>* 1.47 ± 0.31</td>
<td>5</td>
<td>21.7 ± 2.6</td>
<td>** 5.9 ± 0.4</td>
<td>** 0.29 ± 0.05</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6</td>
<td>* 11.4 ± 0.8</td>
<td>8.3 ± 1.1</td>
<td>* 0.77 ± 0.11</td>
<td>6</td>
<td>20.8 ± 2.5</td>
<td>10.4 ± 1.3</td>
<td>0.55 ± 0.12</td>
</tr>
</tbody>
</table>

* Significant difference between controls and drug treatment (p<0.05)
** Significant difference between controls and drug treatment (p<0.01)
DISCUSSION

Ileus: General Considerations

Ileus, by definition, is the failure of the intestinal tract to transport its content to the point of elimination. This may be the result of an inefficiency or a failure of the intestinal musculature. Most commonly such a failure is associated with a surgical operation; but it may also arise after childbirth, mechanical trauma to the pelvic organs, or derangement in intestinal physiology without mechanical obstruction being present. In cases of surgical operations, several factors may be involved which might have an adverse effect on the gastro-intestinal tract. These factors are:

- Pre-operative medication
- Anesthetic agent used
- Operative trauma
- Postoperative medication

The cause for this post-surgical diminution in gut motility is not known, but is believed to be a symptom-complex brought on by a multiplicity of causes. It is believed, however, that this condition is not due to an
actual paralysis of the intestinal musculature, but rather to a reflex inhibition of its activity mediated via the sympathetic nerves. No matter what the cause of this dreaded condition, patients in whom this condition is observed are in grave danger unless it is corrected by drugs or by surgical intervention.

It was already well appreciated by Bayliss and Starling (1899) that the intestinal smooth muscle exists in a dynamic state and that it contracts not only in response to a nervous impulse, but that it can also contract spontaneously and rhythmically. Since this observation at the turn of the century, our progress in understanding even the normal intestinal motility has been very slow. This progress has partially been delayed by a confused nomenclature, by methodology, and the interpretation of the obtained results. A good example in this respect is the use of terms such as "motility", "peristalsis", and "tone" which have frequently been used indiscriminately and without precise definition. In respect to methodology, it has been finally accepted that there is no ideal method for the study of intestinal motility. No matter which method is used in the study, it can only provide information about a limited aspect so that in any given study the method must be selected with consideration to the particular aspect of motility about which information is desired.
To a certain extent such a confusion has also existed in the study of pathophysiological conditions of gastrointestinal tract. Such a condition as ileus has vaguely been defined and our understanding and treatment of it has not changed during the last seventy years. At the turn of the century it was explained that ileus is caused by many factors but primarily either by the overactivity of the sympathetic nervous system or the accumulation of various toxic substances into the distended intestinal tract. Only within the last few years our attention has been directed towards the cellular level. Some attempts have been made to correlate the various intracellular reactions with the changes observed in the whole animal. These correlations have only been hypothetical, but nevertheless our understanding of some basic responses have become more extensive.

After examining more closely the results obtained in this study, it became evident that some reactions at the cellular level - not only the hyperactivity of the sympathetic nervous system - are involved in the induction of ileus. It was also obvious that the anesthetic agent may have had some effect on the etiology of ileus but not as great as was proposed by some investigators in the past (Alvarez, 1818; Bisgard and Johnson, 1939; Helm and Ingelfinger, 1944; Wells, et al, 1961; Tinckler, 1965). Although
ether was used as an anesthetic agent. In this study, it is not believed that the pronounced inhibition of the propulsive intestinal motility was caused by this agent. If ether would have been the causative agent, then much greater inhibition should have been noted in sham operated animals. Although there was a significant difference (p<.001) between the sham operated and nonoperated control animals at two hours, no such difference was observed at 24 hours. Neither was there any significant difference noted in non-operated animals or sham-operated animals between 2 hours and 24 hours. This only indicated that any effect on propulsive intestinal motility contributed by the anesthetic agent had been minimal. Therefore the inhibition of propulsive intestinal motility observed in cecrectomized rats was believed to be the result of the operative procedure per se. Whether this inhibition was due to an overstimulation or a reflex inhibition of the sympathetic nervous system could not be determined in this experiment. The question, however, arose whether true ileus can be induced in experimental animals using this particular surgical procedure. Following the symptom complex described by Wagensteen in 1942 there was no doubt that a true paralytic ileus was induced in these experimental animals. Gross inspection of the intestinal tract at the time of sacrifice revealed that the small intestine had greatly dilated segments; that these dilated segments were filled
with fluid and gas; and that the dilated intestinal wall appeared dusky in appearance.

Treatment of Paralytic Ileus

Investigators in the past have suggested that the treatments of choice for paralytic ileus are cholinesterase inhibitors, especially neostigmine (Wagensteen, 1942; Devine, 1946-47; Bilboa, et al, 1959; Goodman and Gilman, 1966; Modell, 1972) or parasympathomimetic agents, such as urecholine and methacholine (Goodman and Gilman, 1966; Modell, 1972). Rationale for this type of treatment in ileus has been derived primarily from the results obtained in vitro experiments which lately have been questioned. (Alvarez, 1918; Bernheim, 1931; Bernheim, 1934; Ginsberg and Miller, 1953; Munro, 1953; Sharma, et al, 1964, Cox and Lomas, 1971). Some investigators have shown, however, that these drugs increased intestinal motility in some experimental animals as well as in man (Quigley et al, 1934; Ingelfinger, 1943; McMahon, et al, 1949; Kewenter and Kock, 1960; Connell, 1961; Texter, 1963; Neely and Catchpole, 1967; Dubois and Bremer, 1972) but it did not necessarily mean that propulsive intestinal motility was increased. The results obtained in this study would indicate the opposite. As can be seen in Fig. 2 and 3, administration of methacholine to nonoperated animals actually decreased the propulsive
Intestinal motility (p<.02). In cecectomized animals, contrary to the present day treatment of ileus, this drug had virtually no effect (p<.10). The only anticholinergic drug tested, which was atropine, produced the anticipated results in both cases.

**In Vitro Studies**

It was proposed by Burnstock and others (Burnstock, et al, 1970; Satchell and Burnstock, 1971; Burnstock, 1972; Sneddon, et al, 1973; Hulme, Weston, 1974) that some intestinal functions in animals may be controlled by "purigenic nerves." Similar conclusions were arrived at by Andersson and others after in vitro experiments. Their results clearly indicated that cAMP was involved in the regulation of intestinal motility (Levine, 1970; Andersson, Nilsson, 1972) and that any drug which is capable of regulating intracellular levels of this compound will stimulate or inhibit intestinal motility. (Kim and Levine, 1967; Kim, et al, 1968; Kawasaki, et al, 1969; Wilkenfeld and Levy, 1969; Andersson and Mohme-Lundholm, 1970; Takagi, et al, 1971; Andersson, 1972a; Andersson, 1972b; Takayanagi, et al, 1972; Andersson, 1973; Inatomi, et al, 1974). These and other findings indicated that the control of propulsive intestinal motility can be achieved by stimulating adenylate cyclase (Kupiecki and Marshall, 1968; Peterson, et al, 1968; Nakano,
Transit Time Studies

When compounds possessing stimulatory or inhibitory activities (in vitro) were administered to nonoperated animals, it was found that a significant stimulation (p<.005) of propulsive intestinal motility was obtained with niacin and calcium chloride; whereas imidazole, theophylline, and methacholine only slightly depressed the transit of the charcoal meal. The only drug which significantly depressed the intestinal transit was atropine (p<.001). In two hour cecectomized animals the responses observed were more dramatic. Although the stimulation of propulsive intestinal motility was lower than seen in non-operated controls, nevertheless, the treatment of 2 hour cecectomized animals with niacin, calcium chloride and imidazole, significantly stimulated the intestinal transit (p<.001). According to the conclusions of in vitro experiments, these three drugs affected the intestinal transit either by inhibiting the enzyme adenylate cyclase (niacin, CaCl₂) increasing the metabolism of cyclic AMP by stimulating the enzyme phosphodiesterase (imidazole) or supplying
excess of calcium ions in order to reduce the effectiveness of cAMP (CaCl₂). The other three drugs tested had either no effect on the propulsive intestinal motility (methacholine, atropine) or further depressed it by blocking phosphodiesterase (theophylline). The possibility exists, however, that any of the drugs used in this study may have a different systemic effect than has been shown in vitro experiments and that the final response may not be as a result of stimulation or inhibition of adenylate cyclase or phosphodiesterase, but as a result of an unrelated systemic reaction.

Cyclic AMP turnover Studies

After preliminary experiments it was found that the interpretation of results based only on the specific activity of cAMP was rather difficult. In order to obtain any meaningful conclusions the total cAMP pool, Km of adenyl cyclase (K₁) and phosphodiesterase (K₂) were taken into consideration.

It was concluded that any changes in the specific activity were either due to relative rates of K₁ which can vary as follows:

\[
\begin{align*}
K_1 & \quad \text{CAMP*} \quad K_2 \\
\text{CAMP} & \quad \text{CAMP}
\end{align*}
\]
\[ K_1 < K_2 \]
\[ \Leftrightarrow K_1 = K_2 \]
\[ K_1 > K_2 \]

This then indicated that any drug which changed the specific activity of cAMP \textit{in vivo} might not have acted on adenyl cyclase or phosphodiesterase alone, but rather on both enzymes at the same time. Therefore, in all further interpretations of the results, the total response was considered, not any particular effect on any one enzyme.

From the results obtained in the propulsive intestinal motility experiments it is difficult to conclude whether the final response obtained was due to an inhibition, stimulation of enzymes at the subcellular level, or simply due to a direct effect of the drug on the sympathetic nervous system. In an attempt to clarify some of these questions, tritium labelled adenine was administered to experimental animals and the incorporation of this compound into cAMP determined in nonoperated and drug treated or cecrectomized and drug treated rats. For the sake of emphasis, the effect of only two drugs on the total cyclic AMP content and the incorporation of $^3$H-Adenine into cAMP will be considered. Also, in order to maintain all experimental conditions as constant as possible, all animals in Treatment #1 and in the cecrectomized groups were exposed to drug for one hour and 45 min. In
transit time studies it was found that this was the time when the most optimal response was obtained. To a certain extent, this also corresponded to the time when the blood levels of the drugs tested reached the maximal concentrations.

Preliminary experiments were done in order to investigate the effect of the above mentioned agents on the enzyme adenyl cyclase. The results obtained indicated that this enzyme was either present in intestinal tract in very low concentrations, had a very low Km, or behaved in a different manner from that found in other tissues. It was also found that intestinal adenyl cyclase did not respond to a commonly used stimulatory agent such as NaF. The review of literature revealed that the enzyme adenyl cyclase in some organs was inhibited by the addition of theophylline to the incubation media (Sheppard, 1970) and that the activity was influenced by microenvironment, ions, and nucleotides (Rodbell, 1971). Thereafter it was concluded that some other method was necessary in order to measure the changes in the total intestinal cAMP pool. Therefore, to obtain meaningful results, it was decided to measure the turnover rate of cAMP rather than the activity of specific enzymes. In preliminary experiments it was found that the best incorporation of $^3\text{H}$-adenine into cAMP in vivo took place when the label was allowed to remain in the animal's body for 60 min. Obtained
results indicated that at the end of 60 min about 2.5x more label was incorporated into cyclic AMP than at 30 min or 90 min.

**Effect of Drugs on \(^3\text{H}-\text{cAMP}\) Turnover in Nonoperated Rats**

Determination of the total intracellular cAMP content in a 5cm jejunal segment indicated that the levels were quite variable. However, when the total cAMP content was expressed as pM/100mg wet tissue or per mg protein, the levels were more uniform and drug influences became more apparent. The treatment of nonoperated rats with niacin significantly (p<.01) lowered the intracellular cAMP content. It is believed that this response was the result of the drug treatment because tissue weight and total protein contents were rather constant.

The incorporation of \(^3\text{H}-\text{Adenine}\) into cAMP provided an additional interpretation of the results. With niacin treatment about 25% and 50% incorporation occurred when expressed per 100mg wet tissue or per mg protein respectively, relative to controls. The specific activity calculated from the mg of wet tissue and total protein were identical and 50% lower than obtained with nonoperated controls. This indicated that measurements of total cAMP, total protein, and the incorporation of \(^3\text{H}-\text{Adenine}\) into cAMP were correct, confirmed by independent measurements, and suggested that niacin greatly
affected the turnover and utilization of cAMP.

The lowering in total cAMP content after the administration of theophylline was similar to that obtained after niacin treatment. The incorporation of $^3$H-Adenine into cAMP, however, differed significantly from that seen after the niacin treatment. The incorporation of $^3$H-Adenine into cAMP and expressed as Cts/100mg wet tissue or per mg protein remained essentially the same as seen in nonoperated controls, but the specific activity was about 2x higher than controls. This suggested that although theophylline treatment lowered the total cAMP, it had no influence on the formation of labelled cAMP, but because of the high specific activity, theophylline treatment most likely affected the metabolism of cAMP and thus increased the turnover and the utilization of cAMP.

Comparing these results with the ones obtained in transit time experiments on nonoperated rats, the hypothesis was confirmed that low utilization of cAMP stimulated the intestinal transit, but that the presence of high amounts of cAMP or high turnover rate of cAMP inhibits intestinal transit of charcoal meal.

Similar results - low incorporation of label and low specific activity - were obtained with the other two chemical compounds (CaCl$_2$ and Imidazole), which stimulated the
intestinal transit of charcoal meal. A trend similar to that of theophylline was obtained with the other two drugs (atropine and methacholine) which inhibited the intestinal transport of charcoal meal.

**Effect of Drugs on \(^3\)H-cAMP Turnover in 2-hour Cecectomized Rats.**

In 2-hour cecectomized control animals the total cAMP content and the incorporation of labelled adenine into cAMP was essentially the same as observed in nonoperated control rats. The specific activity, however, was about 1.5x higher (p<.005). This indicated that the operation per se had no significant effect on the total cAMP pool, but that the turnover or utilization of cAMP was much greater in operated than in nonoperated controls. The administration of niacin to these animals did not alter the total cAMP pool but significantly decreased the incorporation of labelled cAMP/100mg wet tissue or per mg protein as well as the specific activity. Comparing these results with the ones obtained after niacin treatment of nonoperated animals, it became evident that the niacin treatment of operated rats significantly increased the total cAMP content as well as the incorporation of labelled cAMP and the specific activity. Nevertheless, in both cases the utilization or turnover of cAMP was significantly decreased. This decrease in the utilization of cAMP was
reflected in the increased propulsive intestinal motility observed in nonoperated as well as in operated rats (with niacin treatment).

The treatment of two hour cecectomized animals with theophylline had very little effect on total cAMP pool, incorporation of labelled precursor into cAMP, or the specific activity. In no case did these values differ from those obtained with operated control animals. This indicated that the turnover of cAMP in theophylline treated, cecectomized rats were essentially the same as in cecectomized control animals. However, comparing these results with the ones obtained after theophylline treatment of nonoperated animals, significant changes were evident only in the total cAMP pool, but not in the incorporation of labelled Adenine into cAMP or the specific activity. Comparing these results with the ones obtained in the propulsive intestinal motility experiments, it became evident that theophylline had a similar effect on transit in nonoperated as well as in cecectomized animals. It is believed that this depression in intestinal transit was related to the utilization or turnover rate of cAMP.

A similar trend was also observed with atropine and methacholine in nonoperated animals in the utilization of cAMP and the inhibition of intestinal transport of charcoal meal. However, the inhibited transport was not associated
with an increased utilization of cAMP in 2 hour cecectomized rats. In this case not only the specific activity was significantly decreased, but also incorporation of counts into cAMP was lowered. It appears that these two drugs in some other way might have affected the metabolism of cAMP.

Drug metabolism studies have indicated that the time period during which the animal is exposed to a particular agent is important for obtaining the maximum response. Keeping this in mind, in the transit time experiments it was determined that the optimum response was obtained when the dye meal was administered to the experimental animal one hour post-drug and the animal sacrificed forty-five minutes later. On the other hand, in the experiments where the incorporation of labelled adenine into cAMP was determined, it was found that the best incorporation was obtained one hour after the administration of the $^3$H-Adenine to the animals.

**Effect of Drugs on cAMP after Treatment #1 and #2**

In the next group of experiments it was decided to maintain a constant time period during which the animal was exposed to the labelled adenine while decreasing the time span during which the animal was exposed to the drug. In all the subsequent experiments of Treatment #2 schedule the animals received the drug and labelled adenine at the same time and were sacrificed one hour later.
Under Treatment #2 the effect of niacin on the cAMP content per 100mg wet tissue or per mg protein was essentially the same as observed in nonoperated, niacin treated animals. However, the incorporation of $^3$H-Adenine into cAMP and the specific activity in both cases was increased about 2 fold. This indicated that the shorter time exposure of the animals to the drug had a pronounced effect on the utilization of cAMP. Comparing these results to nonoperated controls, (Treatment #1), a significant difference ($p<.02$) was obtained only between cAMP content and incorporation of $^3$H-Adenine but not between specific activities. This indicated that the exposure of animals to niacin for a shorter period of time had a pronounced effect on the total cAMP content and the incorporation of $^3$H-Adenine but not on the utilization of cAMP. This suggested that niacin may affect adenyl cyclase and in this manner affect the intestinal propulsive motility. The response to CaCl$_2$ was essentially the same as to niacin. A slightly different response was obtained after imidazole treatment. The incorporation of $^3$H-Adenine into cAMP and the specific activity was about 2 fold higher in Treatment #1 than in Treatment #2. This suggested that the effect of imidazole was more prolonged than observed with other agents.

On the other hand, the treatment of rats with theophylline under Treatment #2 showed a significant ($p<.005$) effect
on the total cAMP content and the incorporation of $^3$H-Adenine but not on the specific activity. This suggested that the time period during which animals were exposed to theophylline had very little effect on the utilization of cAMP, but had a significant effect on the incorporation of $^3$H-Adenine as well as on the total cAMP content. Comparing these results to the ones obtained under Treatment #1, significant effects were seen only in the incorporation of $^3$H-Adenine and specific activity, but not in the total cAMP content. This indicated that the time period during which animals were exposed to theophylline had no effect on the utilization of cAMP, but only on the total content and the incorporation of $^3$H-Adenine. There was an indication that theophylline treatment may block the phosphodiesterase (high specific activity) but the low total cAMP content suggested that it may also have some other activity which in turn influenced the cAMP content. The response of rats to the other two drugs (atropine and methacholine) was similar to that observed with theophylline.

Conclusions

There is sufficient evidence in literature to indicate that cyclic AMP is involved in the contractile process of intestinal smooth muscle. This evidence has been obtained only from in vitro preparations and do not exclude the possibility that such an involvement of cAMP in the contraction
may also take place in vivo. From these conclusions it can be postulated that if such a relationship has been found in intestinal preparations obtained from normal laboratory animals, then in an experimentally induced intestinal pathophysiologic state this relationship should also exist and should be altered to a certain extent. The results of the experiments reported in this study, nevertheless, suggested that there exists an intimate relationship between the intestinal propulsive motility and the utilization of cAMP. Such a relationship has been observed but it has not been as clear cut as expected. Taking into account that these experiments were performed on the whole animal, some unexpected responses were anticipated. The drug treatment of operated as well as nonoperated animals indicated that the medicinal agents which were known to relax intestinal preparation in vitro also appeared to decrease intestinal propulsive motility in vivo. According to Andersson (1972b), these agents induced relaxation by increasing the total cAMP content. In vivo, however, such an increase in total cAMP was not observed, but the specific activity, which indicated the utilization, was increased more than 5 fold (e.g. theophylline).

From the results of this study it was concluded that changes in intestinal propulsive motility, as seen in nonoperated control as well as in drug treated animals, were related
to decreased total cAMP content. It was also seen that increased intestinal motility was related to decreased incorporation of $^3\text{H}$-Adenine into cAMP and decreased specific activity. Decrease or no change in intestinal transit, on the other hand, was related to increased incorporation of $^3\text{H}$-Adenine into cAMP or specific activity.

Similar conclusions were obtained when the results of 2 hour cecectomyized rats were analyzed. In this group it was noted that changes in intestinal propulsive motility were related to a constant intracellular cAMP level. If the intestinal transit was increased, there was a concomitant decrease in the incorporation of $^3\text{H}$-Adenine into cAMP and the specific activity. Decrease or no change in intestinal transit, on the other hand, was related to a decrease in the incorporation of the label or the specific activity.

In conclusion it can be stated that a relationship between a decreased utilization of cAMP and an augmented propulsive intestinal motility has been found to exist in the experiments described here. The involvement of cAMP in the contraction of intestinal smooth muscle is more complex and probably involves more than just a decrease in the intracellular levels of cAMP. Nevertheless, the results obtained indicate that the compounds which stimulate or augment the smooth muscle contraction in vitro by lowering the cAMP
content also evoked a similar response in vivo by decreasing the utilization of cAMP. Thus it is concluded that increased propulsive intestinal motility is related to a decreased turnover of cAMP, whereas drugs which depressed intestinal transport caused an increased utilization of cAMP.
SUMMARY

1. Propulsive intestinal motility, using transport of charcoal meal as an index of motility, was studied in nonoperated and cecectomized rats. Results obtained indicated that:

   a. the intestinal motility was significantly depressed in 100% of the operated animals
   b. that this depression in motility was not caused by the anesthetic agent
   c. that the intestinal motility of the operated animals remained depressed throughout 16 hours and then gradually returned to normal in approximately 24 hours.

2. Treatment of nonoperated animals with niacin, CaCl₂, imidazole, theophylline, atropine, or methacholine showed that:

   a. niacin and CaCl₂ slightly increased the intestinal transport of charcoal meal
   b. that imidazole had very little effect
   c. that theophylline, atropine and methacholine significantly depressed this intestinal transit.
3. Treatment of cecectomized animals with the same agents revealed that:
   a. niacin, CaCl₂, and imidazole significantly increased intestinal transit of charcoal meal 2 hours post-cecectomy.
   b. theophylline, atropine and methacholine further depressed charcoal meal transit.

4. Determination of total cAMP present in 1 cm segments excised from three different areas of nonoperated and 2 hour cecectomized animal intestinal tracts revealed that the content was relatively constant throughout the entire length of the small intestine and that it was essentially the same in both groups of animals.

5. Attempts to determine the changes in total cAMP content by measuring the activity of intestinal adenyl cyclase were unsuccessful. The inability to determine the activity of this enzyme was possibly either due to the presence of low levels or an inhibition caused by some intracellular constituent or some component of the incubation media, e.g. theophylline or NaF.

6. The effect of cecectomy or drugs on the utilization of cAMP in the intestinal tract was measured by determining the incorporation of intraperitoneally administered
$^{3}\text{H}$-Adenine into cAMP. The results obtained indicated that:

A. Nonoperated animals
   a. in nonoperated animals niacin significantly decreased the specific activity of cAMP as well as the incorporation of $^{3}\text{H}$-Adenine.
   b. CaCl$_2$ and imidazole had very little effect on the specific activity of cAMP, but affected the incorporation of $^{3}\text{H}$-Adenine not only per 100mg wet tissue but also per mg protein.
   c. Theophylline, atropine, and methacholine significantly elevated not only the utilization of cAMP, but also the incorporation of $^{3}\text{H}$-Adenine.

B. Cecectomized animals
   a. in cecectomized animals niacin and CaCl$_2$ treatment significantly decreased the specific activity as well as the incorporation of $^{3}\text{H}$-Adenine.
   b. imidazole treatment had very little effect on the specific activity but affected the incorporation of $^{3}\text{H}$-Adenine.
   c. theophylline had no effect on the specific activity or the incorporation of $^{3}\text{H}$-Adenine either per 100mg wet tissue or per mg protein.
d. atropine and methacholine treatment significantly decreased the specific activity as well as the incorporation of $^3$H-Adenine. This was seen either per 100mg wet tissue or mg protein.

7. To determine what part of the total cAMP was contributed by the mucosa, experiments were run in which the mucosa was removed from the excised intestinal segment. These results showed that cAMP content in intestinal segments stripped of mucosa was not different from levels obtained in the presence of mucosa.

8. Other experiments where the drug and $^3$H-Adenine were given simultaneously the results showed that:
   a. niacin and imidazole treatment significantly decreased the utilization of cAMP as well as the incorporation of $^3$H-Adenine
   b. the treatment with CaCl$_2$ significantly elevated the specific activity of cAMP, but had very little effect on the incorporation of $^3$H-Adenine.
   c. treatment with theophylline, atropine, or methacholine significantly increased not only the turnover rate of cAMP but also the incorporation of $^3$H-Adenine.
9. The results obtained indicated that a positive correlation exists between the increased propulsive intestinal motility and the agents which decreased the turnover rate of cAMP, and between decreased intestinal motility and the agents which significantly elevated the utilization of cAMP.
BIBLIOGRAPHY


