THE MODE OF ACTION OF THE ANTHRAQUINONE PURGATIVES IN THE CAT.

FRANK C. MACINTOSH.

Dept. of Pharmacology, Dalhousie Univ., Halifax, N. S.

(Received January 23, 1933).

ABSTRACT.

Slightly alkaline extracts of aloes stimulate movement of the small and large intestine. Increased alkalinity increases the action of the drug. Absorption from the intestine is diminished by the anthraquinone purgatives. Neutral solutions are without effect.

The X-ray researches of Magnus[1], Stierlin[2], Meyer-Betz and Gebhardt[3] and others, have demonstrated that the purgative action of the anthraquinone derivatives is due entirely or almost entirely to their effect on the large intestine. The barium meal mixed with the purgative passes through the stomach and small intestine at the normal rate, but immediately on its entrance into the colon causes in that organ a marked activity which results after about half an hour in defaecation. Lenz[4] observed the movements of the gastrointestinal tract in cats provided with abdominal windows, and also found that senna and related purgatives acted on the large intestine. These experiments disproved the earlier theory of Hans Meyer[5], who believed that the delayed action of aloes was due to a gradual liberation of the active principle in the intestine. It is, however, possible that the drug is stable in the small intestine, and that conditions favouring the liberation of the active principle are met with only in the colon.

Several investigators[6,7] have found that aloin, senna, etc., strongly excite the isolated wall of both the large and small intestine. Such experiments show nothing of the normal

1 Magnus, *Arch. ges. Physiol.* **122,** 251 (1908).
2 Stierlin, *Münch. med. Wochschr.* **1910,** 434.
3 Meyer-Betz and Gebhardt, *Münch. med. Wochschr.* **1912,** 1773.
4 Lenz, *Arch. inter. pharmacodynamie.* **28,** 75 (1923).
5 H. Meyer, *Arch. exp. Path. Pharmakol.* **27,** 186 (1891).
6 Hagena, *Diss.* Giessen. **1911.**
7 MacCallum, *Univ. of Calif. Pub.* **1906,** p. 86.

mode of action of the purgatives, since the action of the drug when applied to the serous side of the wall may be quite different from its action when applied to the mucosal side. The majority of experiments on the intact intestine of animals with opened abdominal wall are likewise open to objection. For example, most of the observers (e. g. Nasse[8], Baeumker[9], Ott and Scott[10]) report pronounced action on the small as well as on the large intestine.

It has been generally assumed that the anthraquinone derivatives, in contrast to the saline purgatives, have no direct action of the absorptive mechanism of the intestinal wall. The fluid character of the stood is explained as due to the shortened stay of the faeces in the colon and the consequent diminished absorption of water. Direct experiments to prove this point appear to have been carried out only on the small intestine, on which the drugs have normally no action. Baeumker[9] and Brieger[11] reported normal absorption in tied-off intestinal loops into which senna, aloes, etc. had been injected. Carnot and Glenard[12] on the other hand, perfused the surviving intestine with senna infusion and found increased transudation into the lumen. Increased secretion has also been described by MacCallum[7].

While it may be accepted as proved that the anthraquinone purgatives normally act mainly or entirely by stimulating the movements of the large intestine, there are some points of interest which can only be settled by acute experiments. Such questions are these: Is the restricted action on the large intestine due to the greater irritability of the large intestine wall, or to some change undergone by the drug in the colon? Is the increased peristalsis due to a direct action of the purgative on the intestinal musculature, or is it reflexly produced by irritation of the mucosa? Finally, is the fluid character of the stool due entirely to the decreased time given for absorption,

8 Nasse, *Beitrage zur Physiologie der Darmbewegung.* Leipzig **1866.**
9 Baeumker, *Diss.* Gottingen **1880.**
10 Ott and Scott, Ott's *Contrib. to Physiol.* 18, no. 7, (**1909**).
11 Brieger, *Arch. exp. Path. Pharmakol.* **8,** 355 (1878).
12 Carnot and Glenard, *Compt. rend. soc. biol.* **74,** 120 (1913).

or is there also some interference with the absorptive mechanism of the gut? In the experiments to be described it was endeavoured to secure at least partial answers to these questions.

METHODS.

The drugs used were aloin, aloes, rhubarb, and cascara. As aloin alone of these is completely soluble in water, it was used in most of the experiments. The other drugs gave similar results in all cases in which they were used, and indeed no investigator has described qualitative differences in their action.

Cats were used in all the experiments, as resembling man in their digestive movements generally and in their response to the anthraquinone purgatives in particular[13]. Their diet contained considerable meat (aloin acts on cats only on protein feeding).

After some experimenting, the method of opening the abdomen in a warm saline bath, was used throughout. Besides preventing cooling and drying of the intestine, both of which cause abnormal motility, this method has the following advantages: (a) the movements can be observed directly ad oculos without disturbance; (b) the graphical record is not distorted by movements of the diaphragm and the underlying gut; (c) a nearly straight loop of intestine can be used, minimizing the excitation produced when the fluid within the loop is changed. Ringer's solution was used in most cases for the bath, but 0.9 per cent NaCl appeared to be equally satisfactory.

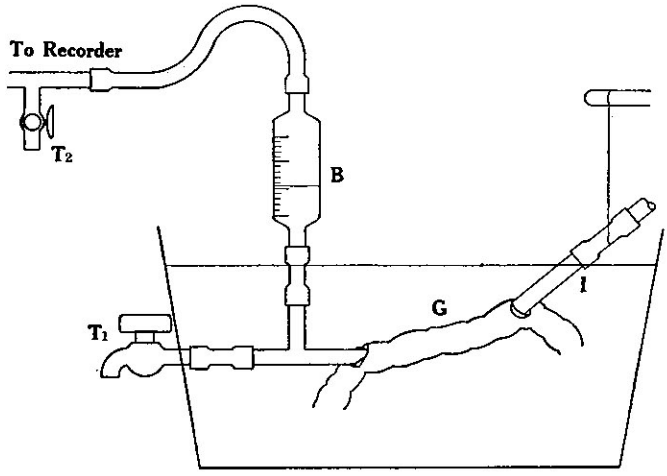
The animals were anaesthetized with chloralose (0.1 gm. per Kg.) given intravenously, and a tracheal cannula was inserted to prevent obstruction of the respiratory passages by mucus. Intestinal movements were recorded by the method of Babkin[14]. The abdomen was opened in the mid-line and a loop of intestine (duodenum, ileum; or ascending colon) 5-10 cm. long was selected. At each end of this loop a longitudinal

13 Magnus, *Anthrachinonderivate*, Heffter's *Handbuch der experimentelle Pharmakologie*, 2er Band, 2 Halfte. Springer, Berlin, **1924**.

14 Babkin, *Die äussere Sekretion der Verdauungsdrusen*. Springer, Berlin. **1928**. 2nd ed., p. 845.

incision 1-2 cm. long was made through both layers of muscle along a line opposite to the mesentery, and the muscle was carefully separated by means of a blunt instrument from the submucosa. Two ligatures were then passed around the loop at each incision between the muscle and the mucosa. One of these was used to tie off the intestine beyond the loop selected; the other was used to secure a glass tube whose end was inserted into the lumen through an incision in the mucosa. A T-tube was used at the lower end of the loop, a straight tube at the upper end. (See diagram). The T-tube was connected by means of rubber tubing to an outlet Tap T_2 set into the side of the bath, and also to a graduated glass bulb which was in turn connected to a recording tambour. In this way there was no interference with the blood and nerve supply to the loop. In the colon the procedure of separating the muscle from the mucosa is more difficult, and in most of the experiments the less desirable method was adopted of securing the tubes by means of ligatures passed around the undissected gut.

The loop was gently washed through with warm saline from a syringe, and the excess saline removed by gentle blowing of air through the loop. Fluid of the desired composition, carefully adjusted to body temperature, was then introduced from the syringe, the outlet tap being closed, until the fluid reached a suitable height in the bulb (4-5 cm. above the level of the bath). The quantity injected was noted, and the same volume used when the fluid was replaced. Precautions were taken to keep the temperature of the bath constant to within 0.1°C., to keep the loop at a constant depth below the surface of the bath, and to avoid the introduction of air bubbles into the loop. The adjustment tap T_2 was closed when the fluid reached a definite height in the bulb B; in this way it was possible to observe variations in the tone of the loop after changing the fluid. To withdraw the fluid, taps T_1 and T_2 were opened and the loop was gently blown through with air from a syringe. Much care was necessary in this operation to avoid extraneous stimulation caused by stretching of the gut. The graphic record was continually checked by naked eye inspection.



B, bulb; G, loop of gut; I, inlet tube; T_1, outlet tap; T_2, adjustment tap.
DIAGRAM SHOWING ARRANGEMENT OF APPARATUS.

Absorption was measured directly by introducing a known volume of fluid, permitting it to remain in the loop for a fixed period (e. g. 30 minutes), and measuring the volume withdrawn. This gives values or the volume absorbed, accurate to about 10 percent, which is sufficient. No attempt was made to distinguish between absorption and secretion.

Experimental Results.

A considerable number of experiments were performed to see if aloin could act on the small intestine. A loop of duodenum or ileum was filled with a 0.1% or 1% solution of aloin in 0.9% NaCl, and movements and absorption compared with a period of equal length during which the loop was filled with saline alone. Such preparations of aloin were uniformly found to be without effect on the motility of the loop (Fig. 1). There was generally a slight decrease of absorption, especially with the stronger solutions; this decrease is probably to be ascribed to the somewhat increased osmotic pressure of the fluid; it

was never very great, and sometimes was entirely absent. One experiment may be quoted to illustrate the results obtained.

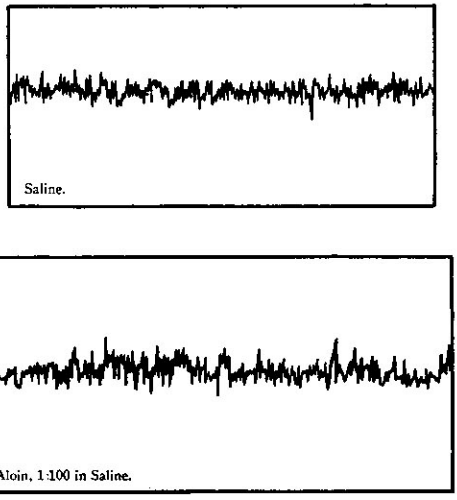


Fig. I. Duodenum.

Loop of Duodenum, length 8 cm.

Fluid	Volume introduced	Volume withdrawn	Length of period	Absorption
Saline	18 cc.	16.0cc.	20 min.	2.0 cc.
Saline	18	15.9	do	2.1
Aloin 1:1,000	18	16.2	do	1.8
Aloin 1:100	18	16.6	do	1.4
Saline	18	16.3	do	1.7

When Ringer's solution containing 0.05 percent $NaHCO_3$ was used instead of 0.9 percent NaCl, varying results were obtained. In one or two experiments there was a definite stimulation of peristalsis, but in others this was either doubtful or absent. As before, there was no constant effect on absorption.

Curiously enough, neutral solutions of aloin were found to act on loops of colon in exactly the same way. Such solutions neither excited the movements of the large intestine nor caused any appreciable diminution in the amount of fluid absorbed (Fig. II). In one experiment in which the faeca

matter had been completely washed out of the loop, there did appear to be a considerable stimulation of peristalsis, accompanied by transudation into the lumen. In twelve other experiments, however, no such action was seen.

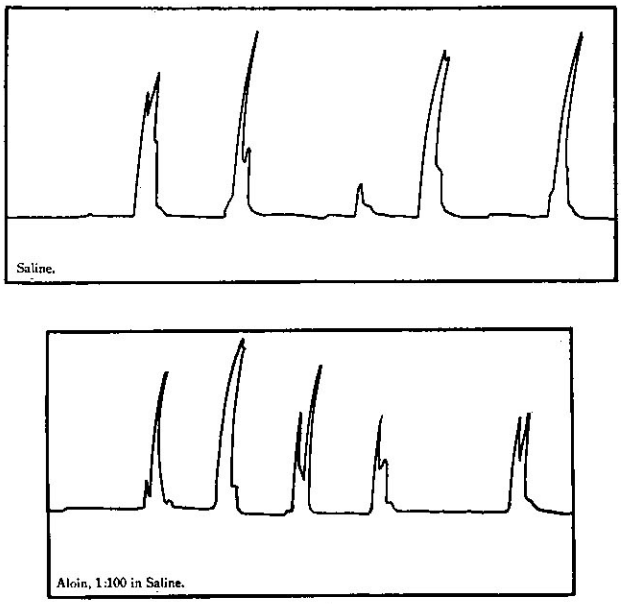


Fig. II. Colon.

These results strongly suggest that the reason why aloin fails to act on the small intestine under normal circumstances is because the drug as given is inert, and is only converted to the active form under the conditions prevailing in the large intestine. It thus becomes of interest to know what conditions are responsible for this "activation" of aloin.

In this regard, it is to be pointed out that one of the most striking characteristics of aloin, and of the anthraquionne derivatives generally, is their reaction to alkalies. The yellow or brown neutral solutions of the drugs on addition of a alkali become bright red in colour, the change taking place slowly (e. g. 10-20 minutes) if the alkalinity is very slight,

instantaneously in strongly alkaline solutions. The chemistry of the change is not understood, but probably involves an hydrolysis of the glucosides of which the drug is mainly composed, with liberation of free anthraquinone derivatives like emodin (trihydroxymethylanthraquinone) and chrysophanic acid (dihydroxymethylanthraquinone). These substances combine with the base to form bright red salts, there being probably some associated tautomeric change in the anthraquinone molecule. The reaction is reversible. It has been shown by MacClendon[15,16,17] that the small intestinal contents of man and animals are not alkaline in reaction, as has been generally supposed, but definitely acid (pH 5.6-6.6 in ileum of dogs). Unfortunately no direct data on the reaction of the large intestinal contents could be found in the literature. The faeces of man are normally slightly alkaline (pH 7.0-7.5)[18,19] London[20] found that the material escaping from a caecal fistula was strongly alkaline in reaction. It is easy to suppose that intestinal putrefaction could cause an alkaline reaction, if the easily diffusible acid products of protein deaminization are absorbed, while the slowly diffusible basic products of decarboxylation accumulate in the lumen of the colon. The possibility is therefore to be considered that the red alkaline form of aloin only is active, and this is formed in the large but not in the small intestine. In this connection it is interesting to remember that, as pointed out above, aloin acts on cats only when protein is fed.

The testing of this hypothesis was made difficult by the fact that the intestinal musculature is very sensitive to the reaction of the fluid which bathes it. Dilute acid relaxes and dilute alkali stimulates it. Excised intestinal muscle, for example, is thrown into a strong contraction if the pH of the solution in which it is suspended is changed from 7.0 to 8.0.

---

15 McClendon, *J. Am. Med. Assoc.* **75**, 1638 (1920).
16 McClendon, *J. Biol. Chem.* **38**, 535 (1919).
17 McClendon, *ibid.* **34**, 1 (1916).
18 Howe and Hawk, *J. Biol. Chem.*, **11**, 129 (1912).
19 Robinson, *J. Biol. Chem.*, **52**, 445 (1922).
20 London (quoted by Starling, *Human Physiology*, 5th ed. p. 613, Lea and Febiger, Philadelphia, **1930**).

Changes of hydrogen ion concentration on the mucosal side of the gut have much less effect, as is sufficiently evident from the case of the stomach. The introduction of slightly alkaline solutions into the lumen of the intestine does, however, considerably stimulate motility, either through diffusion of alkali into the muscle or reflexly through irritation of the mucosa. The problem thus is to compare the action of alkaline solutions of aloin with that of isotonic fluids of the same reactions.

Several sorts of alkaline solution were used for this purpose: (a) isotonic (1.3%) $NaHCO_3$, pH about 9.0; (b) sodium borate-boric acid solution of pH about 8.5; (c) in some experiments N/1,000 NaOH was added until the solution was just alkaline to phenolphthalein (pH about 8.5). Of these the most satisfactory was the borate buffer solution, and it was used in most of the experiments. As compared with 0.9% NaCl, all these solutions stimulated the intestine to some extent. Solutions more alkaline than pH 9.0 were strongly stimulant and damaged the mucosa considerably.

When aloin is added to such solutions it exerts a considerable buffering action, and the alkalinity is reduced. It was found, nevertheless, that the addition of aloin to such alkaline solutions greatly increased their activity on the intestinal musculature of both the large and the small intestine (Figs. III, IV, V). The stimulation was characterized by increased frequency and force of the peristaltic movements, inflammation and sloughing off of the mucosa, marked secretion of mucus (especially in the colon), and decreased absorption. In many cases, especially when the fluid contained 1 percent of aloin, there was transudation of fluid into the lumen, and the volume of the fluid withdrawn exceeded that introduced. There was tendency to colicky spasm of the gut, which sometimes contracted so forcibly that the vessels of the wall were squeezed empty of blood. Sometimes, especially in the colon periods of intense activity lasting two or three minutes alternated with periods during which the gut was inactive. There was usually a latent period of 20-30 minutes before the aloin action became marked. Shedding of the mucosa was a very

characteristic feature; it came off in large patches, dyed by the aloin solution. The stimulation persisted for hours after the purgative was thoroughly washed out of the loop, suggesting that reflex irritation caused by the damage to the mucosa was a factor in the stimulation.

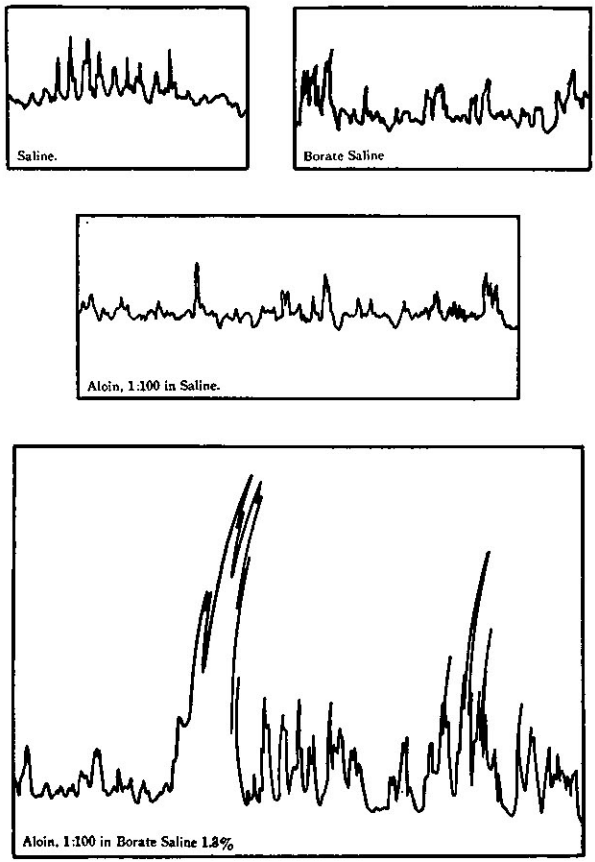
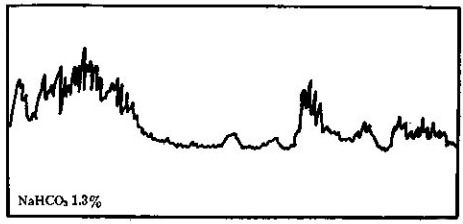


Fig. III. Ileum.



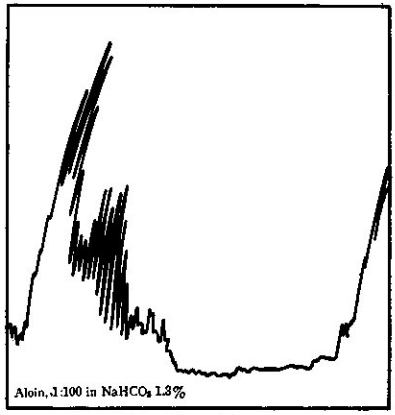
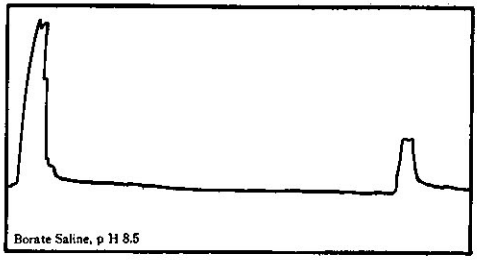


Fig. IV. Ileum.



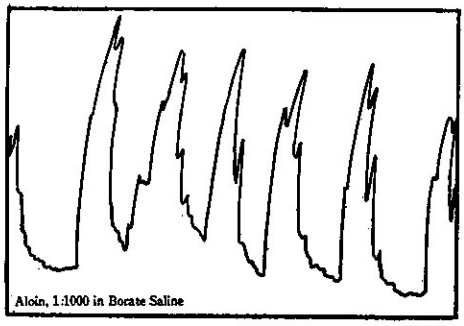


Fig. V. Colon.

In a few cases the increased motility did not take the form of peristalsis, as was expected, but of antiperistalsis, which was, however, far more powerful than the normal type seen in the proximal colon. It must be supposed that the direction of the movements was reversed by the obstruction of the lower end of the loop. In the majority of cases, however, the stimulation was featured by peristalsis, and frequently also by spasm of the circular coat. One or two typical experiments may be quoted to show the type of result obtained.

ILEUM.

Time	Fluid	Intr.	Withdr.	Movements.
11.44	Saline	20 cc.		Small movements, chiefly of longitudinal coat, with slow variations
11.14			18.0 cc.	of tone.
11.22	Borate saline,	20 cc.		Slight stimulation, rapidly falling off after 3-4 minutes.
11.52	pH 8.5		18.5 cc.	Remainder as above.
(Loop washed out)				
11.55	Aloin .1%	20 cc.		As in previous period.
12.25	in saline		18.6 cc.	
12.29	Aloin .1% in borate saline	20 cc.		No stimulation for 15 min., then sudden onset of vigorous peristalsis, with tendency to spasm, persisting to end of period
12.59			21.2 cc.	Much mucosa withdrawn.

ASCENDING COLON.

Time	Fluid	Intr.	Withdr.	Movements.
11.00	Saline	16 cc.		Occasional shallow antiperistalsis.
11.55			14.4 cc.	
12.00	Aloin 1%	16 cc.		2-3 larger movements during 1st
12.55	in saline		14.8 cc.	half hour, then quiet.
(Loop washed out)				
1.00	Borate saline	16 cc.		Gut quite idle throughout.
1.55	pH 8.5		14.9 cc.	
2.00	Aloin 1% in borate	16 cc.		No movements for 10 min., then gentle antiperistalsis changing to powerful peristalsis, lasting till end of period. Withdrew much
2.55			20.0 cc.	mucosa.
(Gut washed out)				
3.00	Saline	16 cc.		Persisting powerful peristalsis, fall-
3.55			18.0 cc.	ing off somewhat towards end.

ASCENDING COLON.

Time	Fluid	Intr.	Withdr.	Movements.
3.38	Saline	14 cc.		A few fairly large antiperistaltic
3.58	0.9%		10.6 cc.	movements.
4.00	.45% NaCl —.65%	14 cc.		Increase in frequency, slight increase in force. Antiperistalsis.
4.30	$NaHCO_3$		11.5 cc.	No mucosa withdrawn.
4.35	Aloin 1% in above	14 cc.		Strong peristalsis, with tendency to spasm, increasing during 1st
5.35			13.8 cc.	half hour, persisting to end.

One experiment may be described which would appear to show that the irritation of the mucosa is the factor chiefly responsible for the stimulation. A loop of large intestine which had been stimulated in the typical way by an aloin solution was washed out with saline, and the aloin replaced by a suspension of procaine, made by adding NaOH to a 1% solution of procaine hydrochloride until there was no further precipitation. The procaine suspension, probably on account of its alkalinity, strongly stimulated the gut. After 3 minutes it was withdrawn, and the loop was washed out and refilled with alkaline aloin solution. The loop thereupon became quite quiet and remained in that condition. It is of course possible that sufficient procaine might have penetrated to the muscular coat to paralyze Auerbach's plexus, though this is highly improbable because of the insolubility of the procaine base.

If aloin in neutral solution is permitted to stand exposed to the air, it becomes much darker in colour, but does not exhibit the typical deep red hue of alkaline solutions. A few experiments were performed to see if this ageing increased the activity of the aloin. The results obtained were somewhat contradictory. A stimulation was found in one out of three experiments on the ileum, and in one out of two experiments on the ascending colon, in which aloin solutions 1-2 weeks old were used. The stimulation was accompanied, as in the preceding experiments, by decreased absorption and damage to the mucosa. The darkening is presumably due to oxidation, but the chemistry of the change is unknown.

In view of the assertion made by Buchheim and others that the anthraquinone purgatives act only in the presence of

bile, one or two experiments were performed in which the aloin was dissolved in a neutral solution of 2% dried ox-bile. No stimulation was produced on either the large or the small intestine (Fig. VI). The theory of Buchheim was at any rate disproved by Stadelmann, who found that aloes, aloin, senna, and rhubarb caused normal purgation in a dog equipped with a complete biliary fistula. As the bile salts, which would presumably be the active factor, are absorbed almost completely in the small intestine, these results are not surprising. It is possible that bile may promote purgative action by increasing the alkaline reserve of the contents of the large intestine.

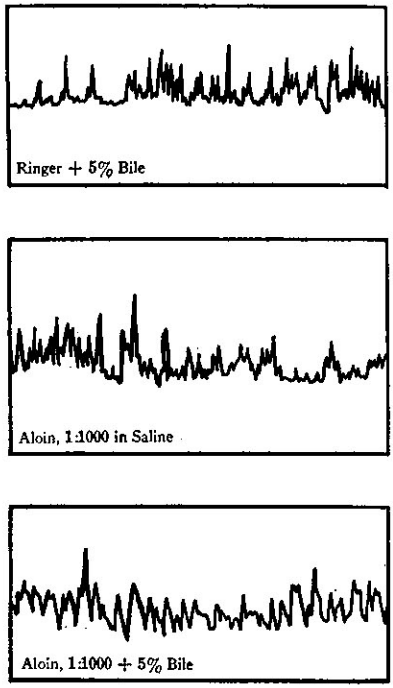


Fig. VI. Duodenum.

These experimental results may be briefly summarized and interpreted.

Fresh neutral solutions of aloin are without action on either the small or the large intestine. Alkaline solutions, however, act strongly on all parts of the intestinal tract, and the action is greater than can be accounted for by the alkalinity alone. There is evidence that the contents of the colon are more alkaline in reaction than the contents of the small intestine, and it seems probable that this is at least one reason for the fact that the anthraquinone purgatives normally act on the large intestine alone.

Ageing of aloin solutions also appears to increase their activity, and it is possible that various processes taking place in the large intestine may assist in the "activation" of the drug. In any case, the fact that the purgative action is normally confined to the colon is to be explained as due to a change undergone there by the drug itself, not to any peculiar sensitivity of the wall of the large intestine.

The purgative action appears to be due mainly to increased peristalsis, but secondarily to inhibition of the absorptive mechanism of the colon. The fluid character of the stool is thus due partly to this inhibition and partly to the decreased time given for absorption. The stimulation of motility is always accompanied by increased secretion of mucus and by damage to the mucosa. It is therefore likely that the augmented motor activity depends on a local reflex mechanism set in action by this irritation of the mucosa.

FURTHER OBSERVATIONS OF THE INFLUENCE OF ELECTROLYTES ON THE FORMATION AND DECOMPOSITION OF URATE GELS. E. Gordon Young and Harvey C. Graham, Dept. of Biochemistry, Dalhousie Univ. (Read March 14, 1932). The influence of electrolytes in promoting gelation of solutions of methyl amine urate has been extended and confirmed with solutions of lithium urate. The chlorides of potassium, ammonium, sodium and lithium have been used and their potency has been found to decrense in the order named. An optimal concentration exists for each electrolyte at which the gel is most stable and most quickly formed. At the upper limit of electrolyte at which gels will form it has been found that crystallization of the gel is accelerated.