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Correlation of variations in intraluminal pressure and potential differences in the perfused colon

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SUMMARY To investigate the nature of variations in the large intestine potential differences, a continuous perfusion of isotonic saline was carried out in the colon of 14 rats. Intraluminal pressure and potential differences between the lumen and the peritoneal cavity were continuously and simultaneously recorded, while impedance of the system and respiration were also constantly monitored. To obtain a quantitative evaluation of the data, Fast Fourier Transform was performed on the signals and their derivatives which were auto- and cross-correlated. While there was no obvious relation between pressure and potential in the unperfused colon, there was clear visual qualitative evidence that, during steady state conditions of perfusion, an increase in intraluminal pressure was accompanied by a decrease in potential differences, while impedance of the recording system remained unchanged. Computer analysis disclosed four narrow ranges of stable frequencies for both pressure and potential. They were centred around 0·3, 1·75, 10·7, and 75 cycles per minute, the latter being synchronous with respiration. It is concluded that the variations of potential differences recorded during perfusion, a well-known phenomenon, are not electrical artefacts: the fast rhythm is probably induced by respiration, which increases intracolonic pressure and that, in turn, reduces the absolute value of potential differences, which remain negative mucosa *versus* serosa. The slower rhythms are synchronous for pressure and potential. Mechanisms reponsible for the decrease in potential related to the increase in pressure remain unknown.

Potential differences between mucosa and serosa of the intestine, described more than a century ago (Donné, 1834), oscillate with time (Cooperstein and Brockman, 1959; Geall *et al.*, 1969, 1970; Wingate *et al.*, 1974). During perfusion of the human (Chauve *et al.*, 1974) and rat (Postaire *et al.*, 1975) colon, potential differences were found to be not stable, but there was no change in impedance and thus poor electrical contact was no explanation for the variations. The nature of these variations has so far remained largely unexplored, and ignored by using planimetry (Geall *et al.*, 1969, 1970), scatter diagrams (Chauve *et al.*, 1974), or deleting experiments where the variations exceeded a preset range (Edmonds and Marriott, 1968). In only one study, fluctuations in potential differences were found to be qualitatively related to slow wave activity and not related to intraluminal pressure changes but this study was done in isolated vascular perfused preparations of canine stomach and duodenum (Wingate *et al.*, 1974).

Colonic perfusion has been shown to influence intraluminal pressure considerably (Chauve *et al.*, 1974, 1976). In this study, the simplicity of pressure recordings in the perfused colon has been used to show the existence of a very clear relation between changes in intraluminal pressure and apparent changes in potential differences.

Methods

Fourteen adult, male, Wistar rats, weighing 400 to 500 g, and maintained on Purina chow *ad libitum*, were anaesthetised with ethyl carbamate (1 g/kg) given intraperitoneally in a 20% solution. They were placed on a heated electrically isolated table, and maintained in isothermic conditions.

The colon was ligated 0·5 cm distally to the ileocaecal junction and a probe inserted 3 cm distally to

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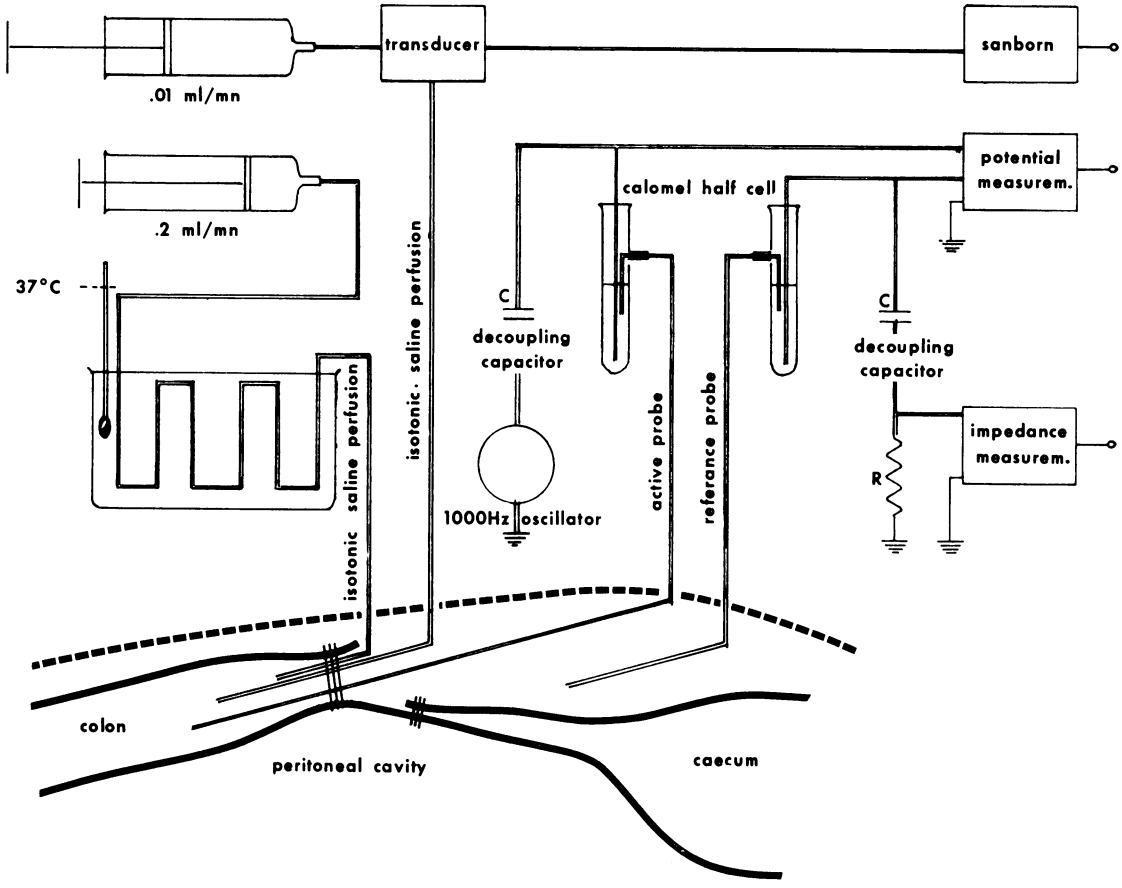

Fig. 1 *Experimental design to record simultaneously intraluminal pressure, potential differences, and impedance in the perfused rat colon.*

the ligature. It consisted in three polyvinyl tubing (OD:1 mm:ID:0·75 mm) cemented together with tetrahydrofuran, used respectively for colonic perfusion, intraluminal pressure recording, and potential differences and impedance measurements. The perfusion tube opened in the colonic lumen 1·5 cm proximally to the other tubes (Fig. 1).

Isotonic saline, maintained at 37°C, was perfused into the colon (infusion/withdrawal pump model 901—Harvard apparatus). After a colonic washing period of 10 minutes at 0·5 ml/min, 1 ml phenolsulphonphtalein (PSP) (1 g/l) was injected in the colon and immediately after followed by a perfusion at 0·2 ml/min. This rate was selected because it is 10% greater than the rate of rectal efflux, when the colon is full of fluid but not grossly dilated. Steady state conditions (Devroede and Phillips, 1969; Devroede and Soffié, 1973) were achieved when the bolus of PSP had passed completely through the colon. This was verified by the periodic addition of sodium hydroxyde 0·01 N to the rectal effluent collected *via* a tube inserted into the anus. Since the PSP total transit time was always less than 60 minutes, recordings were begun at that time for 50 minutes.

Potential differences and impedance were recorded through two electrodes. The electrode introduced in the colonic lumen and that introduced in the peritoneal cavity were both made of polyvinyl tubing filled with agar-agar 2%—saturated KCl, and connected to two calomel electrodes. To avoid mucosal damage by potassium chloride ions, a 10 cm saline bridge was interposed between the agar—KCl intraluminal electrode which terminated in a contact chamber (Chauve *et al.*, 1974) and the colonic lumen. The peritoneal electrode was placed in a small pool of isotonic saline in a fold of the peritoneal cavity.

The asymmetry induced by these bridges was negligible and without consequences on a potential recording because of the design of the equipment. Finally, the electrical zero was noted before and confirmed after each experiment, by placing both electrodes in a beaker containing isotonic saline. A detailed description of this instrumentation has been published (Postaire *et al.*, 1975). Briefly, the potential difference across the poles of the two probes was picked up by a high input impedance differential amplifier. Simultaneously, a sinusoidal signal of 1000 Hz frequency was sent through two decoupling condensors to the poles of the two probes. The variations in amplitude of this signal indicated the variations in impedance of the probes and the living material. With a system of filters, potential differences and impedance were recorded separately (Fig. 1). With this instrument, a variation in impedance of 100KΩ of the probes and living material induces a change in recorded potential of only $2 \cdot 10^{-2}$ mV (Postaire *et al.*, 1975).

The pressure recording tube opened in the colonic lumen at exactly the same level as that used for the recording of the electrical phenomena. Isotonic saline was perfused in the tube at a rate of 0·01 ml/min (infusion/withdrawal pump model 901 Harvard apparatus) through a pressure transducer (Hewlett-Packard 268 A Model). The electrical information was amplified (Sanborn 350-3000 C Carrier preamplifier). The position of the transducer relative to the colon was kept constant throughout each study. It was only approximately done, since only changes in pressure were to be analysed.

Respiration rhythm was recorded through a conventional pneumograph placed on the upper part of the abdomen and connected to a second pressure transducer.

The four electrical signals (pressure, potential, impedance, respiration) were recorded on a four-channel FM tape recorder (Sangamo/Tandberg TIR 100). During the experiments, they were also recorded on a four-channel strip-chart recorder (Brush 440) to maintain visual control of the experiments.

Automatic computer analysis performed on a PDP81 computer (Digital Equipment Corporation) was done in the following three steps: (1) selective data acquisition and sampling; (2) calculation of correlation functions; and (3) special analysis of the sampled signals and the correlation functions.

SELECTIVE DATA ACQUISITION AND SAMPLING

The recorded signals, taken two by two, were amplified and filtered according to the range of amplitude and frequencies to be studied. The tapes were played at the maximum speed of the recorder (19 cm/s) because the construction of filters is difficult for the low frequencies obtained in the present recording. This, in fact, multiplies all frequencies by four. To cover with good accuracy a large range of frequencies (Postaire, 1975), the tapes were sampled at three different rates, giving, for 1024 samplings, intervals of observation of 20 seconds, one minute, and 24 minutes.

The whole period of activity was covered. The signals were sent to a multichannel analogue-to-digital converter (Digital Equipment Corporation) and stored on magnetic tapes (Dectape) for further analysis.

CALCULATION OF CORRELATION FUNCTIONS

The autocorrelation function of the derivative of each signal was first calculated. This method allows the periodic events hidden by noise to be detected (Solodovnikov, 1964; Max, 1972). The programme was designed also to calculate cross-correlation between two simultaneous signals in order to investigate the possible correlation between two phenomena.

SPECTRAL ANALYSIS

Frequency—that is, characteristics of the wave form (and not the rate at which the wave form recurs)—was analysed. The Fast Fourier Transform (Cooley and Tukey, 1965; Rothman, 1968) made it possible to calculate the amplitude of the spectrum of the signals, their derivative, and the corresponding correlation functions. This spectral analysis was developed to determine the frequency content of the signals and quantify the results (Postaire, 1975).

In studying synchronism of appearance of waves in potential differences and pressure recordings, duration of the synchronism was calculated as the ratio of time during which waves of one of the four dominant frequencies (0·3, 1·75, 10·7, and 75 cpm) appeared in both types of recordings to the time during which they appeared in at least one of them. This was done with the aid of a computer. Listing of the programmes may be obtained upon request.

Results

Although in the unperfused colon, there was no obvious relation (Fig. 2) between potential differences and intraluminal pressure variations, after a 60-minute equilibration period during perfusion in 13 animals there was a clear visual, qualitative association between both types of recordings (Fig. 3): when intraluminal pressure increased, potential differences decreased, and *vice versa*, mucosa remaining always negative with regard to serosa, with a mean value ranging from -5 to -8 mV in the different animals. Calibration of the recorder was reversed for pressure and potential so that pheno-

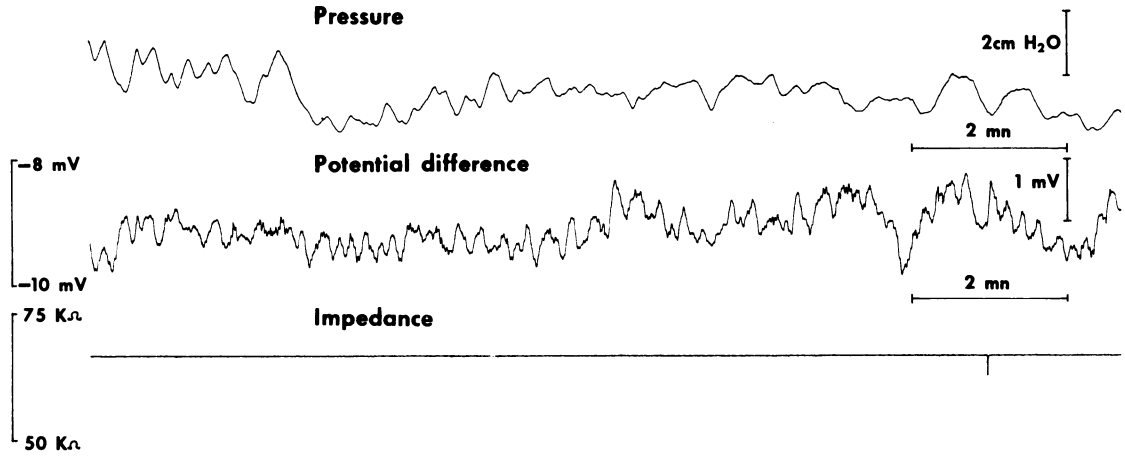

Fig. 2 *Potential differences, intraluminal pressure, and impedance recording in the unperfused rat colon.*

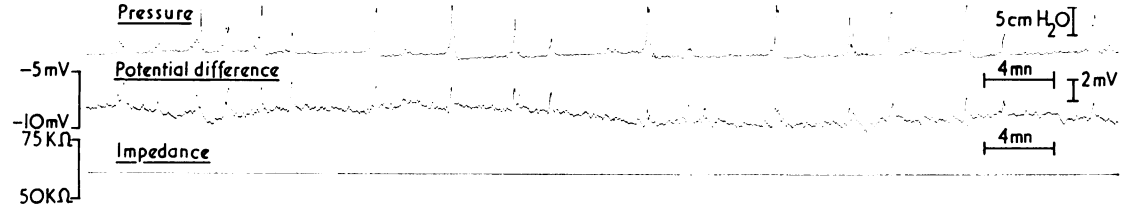

Fig. 3 *Prolonged recording of potential differences, intraluminal pressure and impedance recordings (about one hour).*

mena occurring in opposing directions were recorded in the same direction and a parallelism became obvious. In the fourteenth animal, potential differences remained at ±0·5 mV and were accompanied by a stable intraluminal pressure influenced only by respiratory artefacts. Results from this animal have been deleted.

All wave frequencies were normally distributed. They could be grouped with a 5% chance of error into four narrow ranges of stable frequencies both for pressure and potential differences centred around 0·3, 1·75, 10·7, and 75 cpm (Table). Respiration had a rhythm of 75 cpm (Fig. 4) which clearly influenced potential differences and intraluminal pressure. Mathematical analysis of this relation showed an excellent correlation between potential and pressure (Fig. 5). Less frequent waves occurred at about 11 cpm and a similar relation was shown between potential and pressure (Fig. 6 and 7). Finally, the same phenomenon was demonstrated for frequencies of 0·3 and 1·75 cpm. Of note, the slowest (0·3 cpm) rhythm was recorded in only 40% of the animals, in contrast with the three other rhythms which were present in all experiments.

The coefficient of variation of the wave frequencies was small (5%) for respiration frequency, but was, respectively, 26, 35, and 40% for the 10·7, 1·75, and 0·3 frequencies.

Impedance was relatively stable (Fig. 3) around a baseline ranging from 30 to 100 KΩ in different animals. There were, however, small cyclic variations correlated with respiration (Fig. 4). These never exceeded 2 KΩ.

Discussion

This study has clearly shown that variations in potential differences in the perfused rat colon are

Table *Numerical analysis of frequency and amplitude of pressure waves and potential variations occurring throughout experiments (X ± SD for all animals)*

Waves frequencies (cpm)	*Intracolonic pressure*		*Potential differences*		*Duration of synchronism (% time)*
	Duration (% time)	*Amplitude of variations (cm H_2O)*	*Duration (% time)*	*Amplitude of variations (mV)*	
75 ± 4	100 ± 0	0·21 ± 0·04	100 ± 0	0·21 ± 0·05	100 ± 0
10·7 ± 2·8	60 ± 30	0·46 ± 0·13	64 ± 30	0·34 ± 0·14	80 ± 12
1·75 ± 0·6	81 ± 24	2·71 ± 1·38	83 ± 21	1·16 ± 0·66	89 ± 10
0·30 ± 0·12	65 ± 16	7·10 ± 1·7	64 ± 13	2·31 ± 1·2	95 ± 1

$X \pm SD$ for all animals.

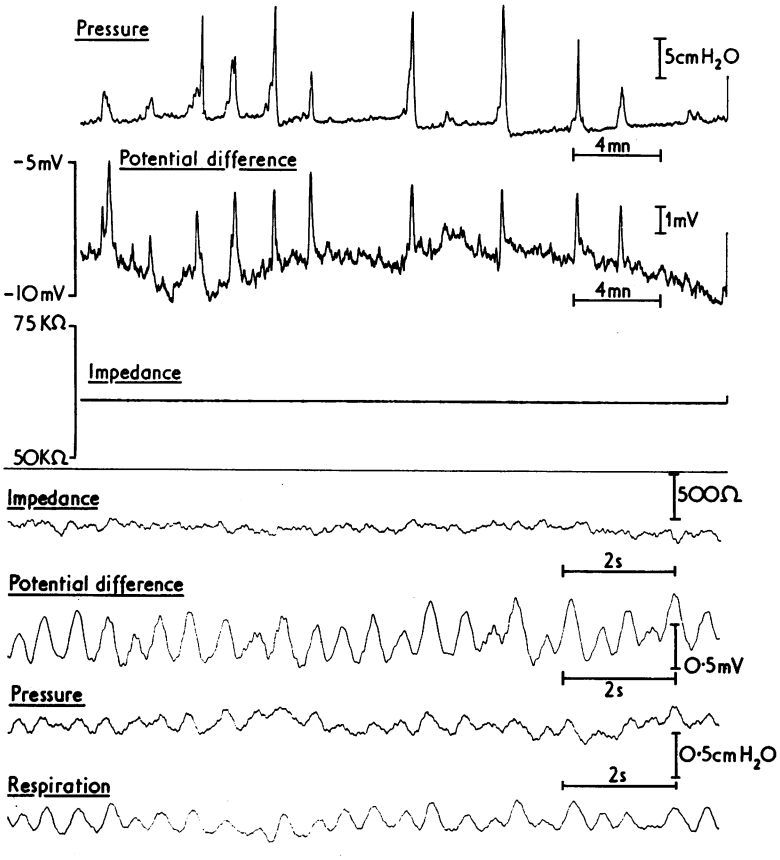

Fig. 4 *An example of potential differences, intraluminal pressure, and impedance recordings in the perfused rat colon (top). Below is a faster recording showing also respiration.*

concomitant with variations in intracolonic pressure. The relation is reversed: the greater the pressure, the lesser the potential. Underlying mechanisms of this relation remain to be investigated, particularly with regard to the electromyogram.

Impedance was very stable and variations never exceeded 2 KΩ. With the characteristics of the instrument used in this study (Postaire *et al.*, 1975), it can safely be said that changes in potential differences recording are definitely not artefacts induced by changes in impedance of the entire recording system. Other authors have also shown that fluctuations in potential differences are not random but related in time to the basal electric rhythm in the intestinal wall and they concluded from this that the variations were not a mere representation of the instability of the measuring circuit (Wingate *et al.*, 1974). On the other hand, tissue impedance is merely a small

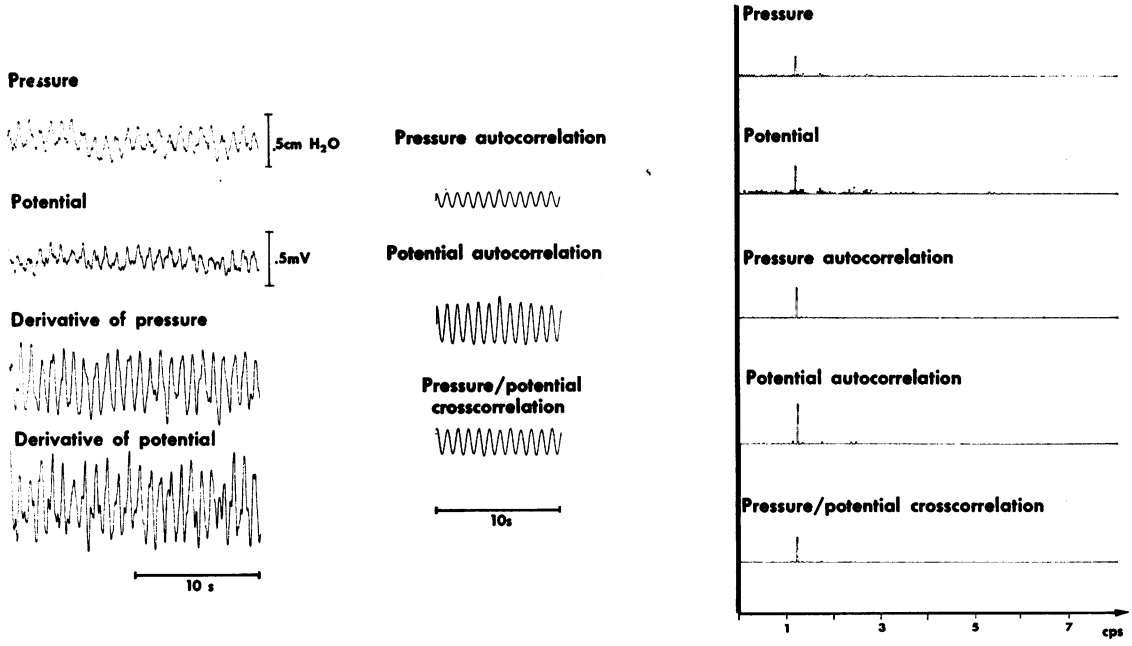

Fig. 5 *Correlation functions and power spectrum of recorded intraluminal pressure and potential differences in the perfused rat colon (20-second intervals of observation).*

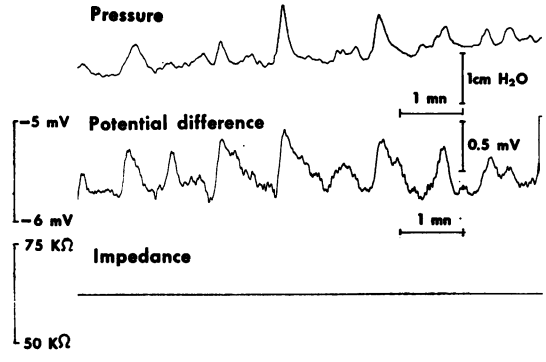

Fig. 6 *An example of potential differences, intraluminal pressure, and impedance recordings in the perfused rat colon (one-minute intervals of observation).*

fraction of the entire measured impedance, which also included the calomel half cells and the salt bridges. Thus, this study does not evaluate tissue impedance or its changes in a meaningful physiological fashion.

The relation between pressure and potential was best shown by their respective response to respiration. This could be expressed clearly graphically and the computer analysis indicated that there was practically no dispersion of waves outside of the respiration rhythm. Synchronism of the pressure and potential waves was perfect and the entire duration of recordings showed the existence of a respiratory influence on potential and pressure recordings. The image resembles closely that obtained in the oesophagus of man (Grantham *et al.*, 1970). For slower cycles of variations, it could not be shown whether changes in pressure induced changes in potential or *vice versa*, but only that they occurred simultaneously. Of course recordings of longer duration were more complex.

There is no theoretical objection which would preclude the recording of the colon electromyogram —that is, both action potentials and slow waves in addition to mucosal potential differences with the method used in this study. Bearing this in mind, it is interesting to note that cyclic changes in potential differences running at a frequency of 10·7 cps are very similar to electromyograms reported in the colon of other species (Couturier *et al.*, 1969) in shape, frequency, and amplitude. In another *in vitro* study (Wingate *et al.*, 1974), a rise in baseline pressure was accompanied by an increased potential difference. This phenomenon, however, was not consistently found. Moreover, in the same study, atropine abolished intraluminal pressure change but did

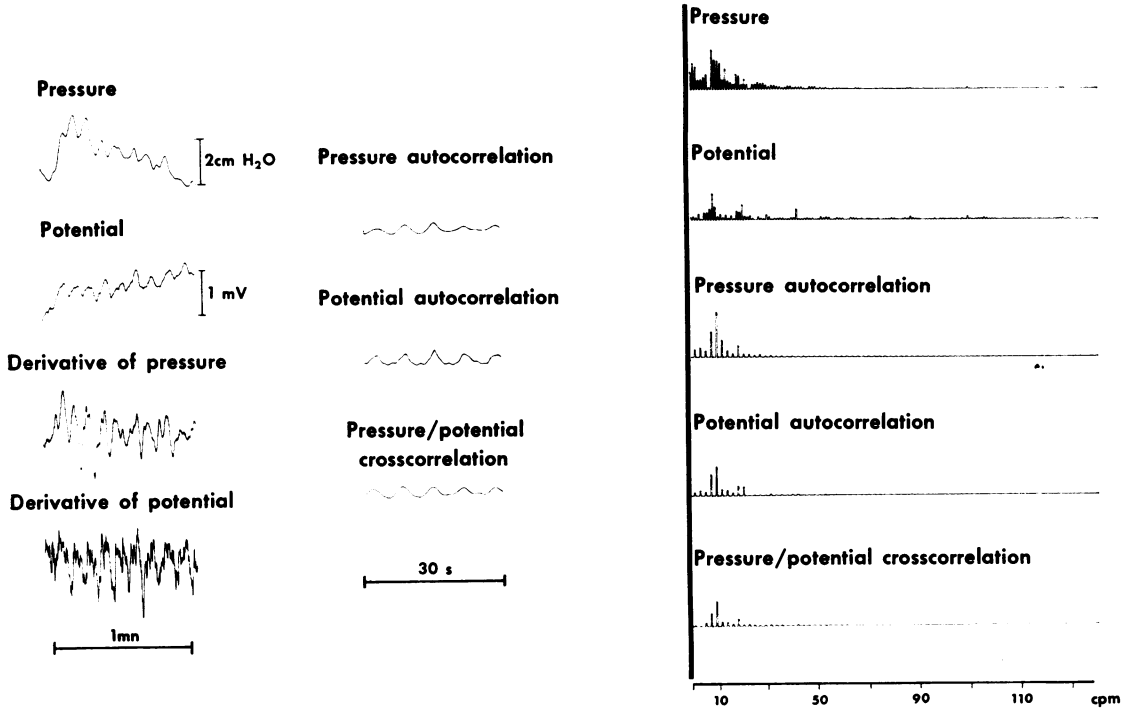

Fig. 7 *Correlation functions and power spectrum of recorded intraluminal pressure and potential differences in the perfused rat colon (one-minute intervals of observation).*

not affect potential differences variations and the electromyogram, while pentagastrin had a reverse effect. It was concluded that fluctuations of potential differences were related to duodenal slow wave activity. These authors did not find quantitative correlation between the magnitude of the intraluminal pressure change and the potential differences fluctuations, but the study was done on isolated vascular perfused preparations of canine stomach and duodenum and its results should be compared with those obtained *in vitro* from isolated mucosal membrane, where potential differences are very stable with time (White and Armstrong, 1971). They also explain why the variance of potential differences measurements obtained with perfusion methods are conspicuously greater than those obtained by direct contact of the unperfused mucosa (Rask-Madsen and Dalmark, 1973), as perfusions considerably influence intraluminal pressure (Chauve *et al.*, 1974, 1976).

The mechanism responsible for the correlation between pressure and potential waves cannot be derived from this study. The changes are a mirror image. It is thus interesting to speculate that changes in potential differences might be mediated *via* changes in colonic wall blood flow. Indeed, an increase in intraluminal pressure is accompanied by a corresponding decrease in arterial blood flow and a concomitant decrease in arteriovenous difference in oxygen (Hanson and Moore, 1969). Anoxia and low blood flow states on the other hand are known to decrease potential differences (Edmonds and Marriott, 1968).

This study clearly shows that mechanical events such as intraluminal pressure changes in the colon influence electrochemical events such as potential differences. Though it is logical to believe that motility influences absorption, relatively little work has been done to study this relation directly. Thus studies of this nature might prove of interest to develop an overall view of what goes on during absorption, taking all values into account.

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