

Upon waking each morning, 15 menstrual women (ages 17–20 years) recorded their basal body temperature and placed a small amount of saliva (0.15 ml) at the top of a 45° inclined plane covered with wax paper. For 29 days each woman recorded the saliva flow in cm per min and oral temperature in °F. All women were asked by us to identify each day of menstruation and to indicate the days in which they felt abdominal pain. As far as we know, these women were healthy and had normal menstrual cycles.

The Figure shows a typical relationship between saliva flow and basal body temperature of 5 out of the 15 women which appears to reflect the time of ovulation. Ovulatory pain was also recorded as an aid in determining the ovulation time. The increase in saliva flow parallels basal body temperature from menstruation to the estimated time of ovulation and can be taken as the estrogenic phase whereas the subsequent saliva flow data seems to mimic the progestational phase. The estrogenic peaks of ovulation and corpus lutea are recorded in all 5 women so that saliva viscosity was altered in a characteristic manner by menstruation. In an attempt to correlate these data with previous work, two women (B. F. and L. C.) measured their daily saliva glucose by the Dextrostix blue color. A blue color was recorded each day between day 10 to 13 for

B. F. and between day 10 to day 15 for L. C. This agrees with our estimated time of ovulation obtained by saliva flow, basal body temperature and ovulation pain. Our previous work indicates that at this time the proportion of mesothelial cells drop to a minimum whereas polymorphonuclear leukocytes elevate to a maximum in peritoneal fluid². Urinary protein levels become elevated at the time of ovulation³. We feel that saliva viscosity can be used as an aid to distinguish the various phases of the menstrual cycle by women and to point out the time of fertility.

Résumé. Chez les femmes la viscosité de la salive mise en relation avec la température du corps révèle le temps de l'ovulation aussi bien que les phases variées du cycle menstruel.

R. H. DAVIS, D. KRAMER, J. SACKMAN
and H. BALIN

*Department of Obstetrics and Gynecology,
Department of Physiology and Biophysics,
Hahnemann Medical College and Hospital,
North Broad Street,
Philadelphia (Pennsylvania 19102, USA),
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The Effect of Intraluminal Hydrostatic Pressure on Intestinal Absorption in vivo

In view of the widespread use of perfusion techniques for the quantification of intestinal absorption in vivo, it is surprising that such little attention has been paid to the influence of intraluminal hydrostatic pressure on the parameters involved. Only an infinitesimal proportion of publications specify the pressure to which the intestine was subjected, and moreover in a recent review of perfusion techniques, experimental and clinical, the factor was not even mentioned¹. The present study indicates that the intraluminal pressure is a sensitive parameter which plays a significant role in the magnitude of the absorption obtained.

Methods. The experiments were performed on mongrel dogs fasted for 24 h and anaesthetized with pentobarbital. An ileal loop, 20–30 cm in length, was tied at both ends without interruption of any blood supply. Cannulae, of diameter 5 mm, were inserted into small incisions in the anti-mesenteric border at each end. The perfusion fluid

was passed through the intestinal lumen by means of a totally occlusive pump, and was returned to a reservoir for recirculation. The level of the aperture of the outflow tube with relation to the intestine was changed at will to produce the variations in hydrostatic pressure. The flow rate of the pump was always 3.3 ml/min. Each perfusion lasted for 30 min, after which the intestine was carefully drained of all liquid, the level of the outflow tube was changed, and the perfusion was re-started at another pressure. Generally, 4 different pressures were employed for each loop. After the final perfusion period, the experimental loop was excised and weighed. Samples of the tissue were desiccated overnight at 110°C so that the total dry weight of the experimental loop could be computed. The absorption was expressed per g of dry tissue and per h. In some experiments, the viability of the mucosa of the perfused loop was tested in vitro and compared with that of a contiguous control loop by determination of its capacity to absorb labelled phenylalanine or β -methyl-glucoside, according to previously published techniques².

The perfusion fluid consisted of Krebs bicarbonate buffer containing 0.2% glucose. Water absorption was assessed simply by noting the decrease in volume of the perfusate, whilst sodium, potassium, chloride and glucose concentrations were determined before and after the perfusion, using flame photometry, titration and glucose oxidase methods respectively. Absorption of the solutes was computed simply from the amounts perfused and the amounts recovered. Statistical evaluation was always performed by random-block analysis of variance, since the same loop was used for all determinations.

Results. The absorption of water by the perfused dog ratestate was doubled when the intraluminal pressure was inised from 0 to 10 cm of water, and a smaller but still

Table I. Influence of hydrostatic pressure on the absorption of water by the perfused dog intestine in vivo

Pressure (cm of water)	Absorption (ml/g dry wt./h)
0	3.82 \pm 0.53
10	6.06 \pm 0.68
20	6.96 \pm 0.89
30	6.80 \pm 0.70
^{0.05}	0.68
^{0.01}	0.90
^{0.001}	1.20

Results are the means of 11 experiments. Statistical analysis was performed by a random-block analysis of variance, each animal providing 1 block, since the same loop was used for all studies. The values of 'D', the least significant difference between means at a given significance level (as indicated by the subscript), were extracted from this analysis.

¹ R. MODIGLIANI, J. C. RAMBAUD and J. J. BERNIER, *Digestion* 9, 176 (1973).

² J. W. L. ROBINSON and V. MIRKOVITCH, *Gut* 13, 784 (1972).

very significant increase occurred when the pressure was increased to 20 cm (Table I). At this stage, a maximum appeared to be attained. Analogous results were obtained for the absorption of sodium, potassium, chloride and

Table II. Effect of hydrostatic pressure on the absorption of sodium, potassium, chloride and glucose from the perfused dog intestine in vivo

	Sodium	Potassium	Chloride	Glucose
Initial concentration (mM)	144.6	6.03	129.6	11.23
Effluent concentration (mM)				
Pressure 0 cm water	142.8	6.29	123.8	10.46
Pressure 10 cm water	142.8	6.36	124.6	10.60
Pressure 20 cm water	142.9	6.37	125.1	10.84
Pressure 30 cm water	143.2	6.36	125.2	10.68
Net absorption (μ moles/g dry wt./h)				
Pressure 0 cm water	0.574	0.0184	0.592	0.0550
Pressure 10 cm water	0.928	0.0294	0.890	0.0758
Pressure 20 cm water	1.008	0.0294	0.986	0.0838
Pressure 30 cm water	1.034	0.0296	1.016	0.0936
$p0.05$	0.130	0.0042	0.132	0.0130
$p0.01$	0.178	0.0058	0.186	0.0178
$p0.001$	0.242	0.0078	0.252	0.0242

Results are the means of 7 experiments. Statistical evaluation using a random-block analysis of variance provides the values of 'D' quoted. Concerning the effluent concentrations, an analysis of variance shows no differences, though each parameter differs significantly from its initial concentration. This implies equal absorption of water and solutes.

Table III. Effect of a pressure of 5 cm of water on absorption from dog ileum

Substance	Absorption (per g dry wt/h)
Water	4.12 \pm 0.69 ml
Sodium	0.654 \pm 0.110 μ moles
Potassium	0.0208 \pm 0.0027 μ moles
Chloride	0.604 \pm 0.099 μ moles
Glucose	0.0824 \pm 0.0032 μ moles

Perfusion performed for 30 min. Results are the means of 9 animals (\pm SEM).

Table IV. Control of the viability of the mucosa of perfused loops

Parameter	Control loop	Perfused loop
Tissue water (%)	79.47 \pm 0.62	79.88 \pm 0.88
Extracellular space (%)	16.3 \pm 0.86	15.7 \pm 0.74
Phenylalanine transport (distribution ratio)	9.85 \pm 0.91	9.41 \pm 1.29
β -Methyl-glucoside transport (distribution ratio)	8.24 \pm 1.16	7.77 \pm 1.95

Results are the means of 5 experiments (\pm SEM). After 4 perfusion sessions, the loop is excised, and compared with a contiguous control loop. Samples of mucosa are desiccated overnight for dry weight; transport is determined by incubating fragments of mucosa in 1 mM 14 C-labelled substrate for 1 h at 37°C; and extracellular space by incubating parallel fragments in inulin- 14 COOH.

glucose (Table II). There was no change in the effluent concentration of any solute when the pressure was varied, but the quantity absorbed increased in the same proportion as the water absorption. In a separate series of experiments, a pressure of 5 cm of water was used, since this probably represents a more physiological condition, and values intermediate between those of 0 and 10 cm were obtained (Table III). These results testify to the reproducibility of the experiments in different dogs, as well as to the importance of the smaller pressure increments.

At the end of the experiments, after a pressure of 30 cm of water for 30 min, the loop was examined histologically, and functional tests were performed in vitro to determine the viability of the mucosa. The histological investigations revealed no damage to the tissue and the results in Table IV indicate that such pressures do not harm the function of the mucosa.

Discussion. The results of this investigation demonstrate the importance of controlling the hydrostatic pressure both in animal experiments in vivo and in clinical investigations. Indeed this factor could account for the important differences reported in this field. An increase in pressure promotes a considerable rise in water and solute absorption; since the effluent concentration does not alter, this change must consist of increased absorption of isotonic fluid. It is therefore probable that the augmentation provoked by raising the pressure up to 20 cm of water is simply caused by opening up the folds of the intestine and widening the inter-villus spaces so that a greater absorptive surface is presented to the substrate.

In the literature of the last 25 years, we have only found 3 small studies that specifically investigated the effect of hydrostatic pressure on intestinal absorption in vivo³⁻⁵; in 2 cases, the results coincide closely with ours^{3,4}. In view of the widespread use of intestinal perfusion, both in animals and humans, we recommend that in the course of such studies the pressure should be controlled more systematically than appears to be the case.

Résumé. On a étudié l'influence de la pression hydrostatique intraluminal sur l'absorption d'eau, d'électrolytes et de glucose dans une anse perfusée d'iléon de chien in vivo. Une augmentation de la pression de 0 à 10 cm d'eau provoque une stimulation considérable de l'absorption isotonique. Un plateau est atteint à 20 cm d'eau, mais même après une perfusion à 30 cm d'eau, la muqueuse n'est pas lésée. Ces résultats suggèrent qu'il faut contrôler strictement les conditions de perfusion dans des expériences in vivo.

V. MIRKOVITCH, H. MENGE and
J.W.L. ROBINSON⁶

Département de Chirurgie Expérimentale,
Hôpital Cantonal Universitaire,
CH-1011 Lausanne (Switzerland), 7 May 1974.

³ D.D. BLICKENSTAFF, D.M. BACHMAN, M.E. STEINBERG and W.B. YOUNG, *Am. J. Physiol.* **168**, 303 (1951).

⁴ R. DENNHARDT and F.J. HABERICH, *J. Physiol.*, Paris **61**, 262 (1969).

⁵ M.G. COAN, F. COSTANTINI, J. FELLNER and H.K. WRIGHT, *Surg. Forum* **24**, 420 (1973).

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