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Method for Successive Absorptions with Intestinal Perfusion *in vivo*

F. Ponz, A. Ilundain and M. Lluch

Departamento de Investigaciones Fisiológicas, C.S.I.C.
Universidad de Navarra
Pamplona (España)

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A simple technique to study intestinal absorption *in vivo*, a modification of that of SOLS and PONZ (34), is described. It uses a perfusion pump, with or without recycling of the solution along the intestinal lumen, and allows the carrying out of a series of successive absorption periods with high comparative value on the same intestinal segment. The technique is applied to sugar absorption in rat and hamster, but it can be extended to other substrates and other animal species. For each concentration of sugar, the intestinal absorption rate of glucose remains constant along a number of 1 min successive periods.

There are numerous techniques to study intestinal absorption *in vivo* and *in vitro* (24, 33), each one suited for specific purposes.

Using *in vivo* techniques with anesthetized animals, SOLS and PONZ described in 1946 (34) a simple procedure by which an intestinal loop was isolated between two cannulae, in such a way that its lumen could be easily washed, then filled or perfused with substrate solution, and the amount of substrate absorbed in a given time exactly measured. It was further possible to practice on the same intestinal loop a certain number of successive periods of absorption under equal or different experimental conditions. Although the animal must be anesthetized and undergo surgery with this technique, the preparation preserves normal blood and lymph flows, avoids interference from gastric emptying, permits a correct measurement of the absorbed substance and in reference to intestinal length and weight, keeps almost the physiological conditions, and possesses a highly comparative value on using in the various successive periods the same absorbing surface.

This technique has undergone various modifications and adaptations since it was published, including different perfusion systems, with or without solution recycling (12, 14, 15, 23, 29, 30).

There follows a procedure incorporating a perfusion pump, based on the same principles as those of SOLS and PONZ, and equally simple, by which intestinal absorption can be studied in very short

successive periods as little as 1 minute. It offers high reproducibility, and is suitable for kinetic analysis.

Materials and Methods

Animals. Wistar rats *(Mus norvegicus albinus)* and hamsters *(Mesocricetus auratus)* of both sexes, weighing 150-230 g and 75-110 g respectively, were used after 24 h fasting.

Preparation. The various operations and steps of the experiment are carried out in a chamber at 28-30° C —a thermocontrolled laboratory glass case is adequate. The animal is anesthetized by injecting 1 ml of 12.5 percent urethane per 100 g of body weight. After 20-30 minutes it is fastened on a stretcher, fixed to a stainless steel frame, and a laparotomy is performed (3-4 cm) in order to locate the beginning of the jejunum and to select the desired intestinal segment. The inlet and outlet cannulae are inserted into the intestine at a convenient distance (between 12 and 25 cm) and then tied. The intestine immediately proximal to the inlet cannula and the distal to the outlet one are also tied to prevent the escape of intestinal content. The incisions for the cannulae must be done on the side opposite the mesentery, which is less vascular. The abdominal cavity, with the intestine inside, is now closed, and the protruding cannulae are connected to the perfusion system.

Perfusion system. Figure 1 outlines the perfusion system, made of the following parts: (a) reservoir for the substrate solution; (b) plastic tube (Potrex Portland, 2 mm diameter) which connects with the peristaltic absorption pump (Mod. 1201 Harvard Apparatus) (c); a thermostatic bath (d); and a temperature control (e) of liquid at the entrance of the intestine. Glass cannulae (f) are connected to the plastic tube by means of a thin tube of very flexible rubber (g). A stopcock (h) is located at the intestinal exit to direct the flow to reservoir (a) when recycling the solution or reservoir (i) to collect the effluent in perfusion without recycling. All parts are easily washed, assembled and replaced.

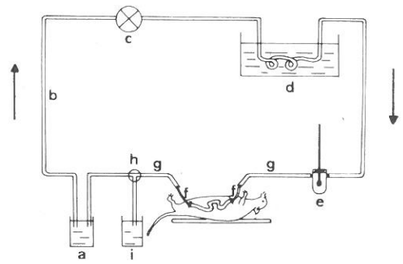

Fig. 1. *Scheme of perfusion system of an intestinal segment* in vivo.
(a) Solution reservoir; (b) plastic tube (Portex Portland, 2 mm diameter); (c) peristaltic absorption pump (Mod. 1201 Harvard Apparatus); (d) thermostatic bath; (e) temperature control; (f) glass cannulae; (g) thin flexible rubber tube; (h) key; (i) effluent reservoir.

The pump regulated the perfusion rate between 0.0025 and 13.6 ml/min. Minimum liquid volume in the circuit, including intestine, was 6 ml. Bath temperature had to be adjusted so that the solution entered the intestine at 38° C. Lateral pressure in the entrance cannula pulsated between 0 and 6-7 cm of water.

Carrying out the experiment. After connecting cannulae to perfusion system, the pump starts circulating saline solution (NaCl 0.9 %, about 40-50 ml) to wash thoroughly the intestinal lumen. Then, the pump blows air to eliminate any remaining solution. Next, reservoir (a) with the substrate solution is set in place, and the pump switched at the desired speed. Starting time for the absorption period is reckoned when the liquid front appears in the outlet cannula, if recycling; or at the end of the outlet tube (i), if not recycling.

In perfusion without recycling, once the time for the absorption periods is finished, the collection reservoir (i) is removed, and reservoir (a) is substituted for another with saline solution (20-25 ml) for washing the system. The remaining solution is blown with air and a new absorption period may begin under equal or different conditions. If we wish to know the absorption of a definite solution throughout a series of successive periods, it suffices to collect separately the fractions of effluent corresponding to each period.

In perfusion with recycling, after completing absorption time, the suction tube is removed from solution (a), and washing solution and air are let through to clear all remaining solution from the system, which is also collected in reservoir (a). A new period of absorption can be started right away under identical or different experimental conditions.

Bubbles that could lead to errors on reducing useful absorption surface, are not usually formed. Should they appear they could easily be detected through the translucent tubes, and eliminated.

If the operation is correctly performed, rectal temperature of the animal remains constant (± 0.2° C).

Perfused solution. As substrate solution, either Krebs-Henseleit-Bicarbonate or Krebs-Ringer-Phosphates at pH 7.4 (17) has been used indistinctively, but any other physiological saline solution even the simple isotonic solution of NaCl 0.9 %, is equally valid. No absorption differences were observed from the use of any one of these various absorption solutions. As there were no differences from gasifying or not the solution with O_2 or with O_2 and CO_2 (95 and 5 %), oxygenating is considered unnecessary.

The solutions to be used are kept in the bath at 38° C.

Expression of results. Since the quantity of substrate initially present in the volume of perfused solution is known, the amount absorbed is estimated by subtracting the remaining quantity at the end of the corresponding absorption period. In perfusion with recycling, the measure of initial and final volumes are estimated by weight with 1 mg approximation. When operating without recycling, only the effluent collected at the outlet, considered equal to that of the inlet, is estimated by weight.

Absorption rate is expressed in nmoles of substrate absorbed per minute and per centimeter of physiological length of intestine (or per gram of dry weight).

The accepted «physiological length» of the intestine was $l_{30\,g} \times 0.6$, were $l_{30\,g}$ represents the length of intestinal segment when separated from the body with one cannula secured to a stand and with a 30 g load hanging from the other one. The 0.6 factor corresponds to a somewhat arbitrary estimate of the relation between $l_{30\,g}$ and the length of the same segment under its physiological conditions during the experiment (37); its value has been corroborated by many years of experience, during which the approximate length of the segment was measured when the cannulae were inserted and related to the final one on being subjected to the 30 g load. Since segment length in *in situ* measurement shows great variability and errors on account of changes in muscular tone whereas the length measurement of the intestine outside the body and subjected to the 30 g load is much more constant, the latter system seems preferable, applying always the constant correction factor.

With animals of very similar age and working on homologous areas of the intestine, absorption per unit of physiological length or absorption per unit of dry

weight of intestine show a similar variation coefficient ($S_{\bar{x}}/\overline{X}$), clearly lower than the absorption expressed per unit of fresh weight of intestine. For reasons of convenience, relation to length is advisable. In a lot of 46 rats, the dry weight of jejunum was 24 ± 0.6 mg/cm; and the intestinal repletion capacity with the used perfusion pressures was 63 ± 2 μl/cm.

Determination of substrate concentration. This depends obviously on the substrate nature. With sugars, chemical (13, 22 ,27, 36), and enzymatic methods (16) have been used, as well as radioactivity countings for labelled substrates.

Results and Discussion

Minimum absorption time. Minimum absorption time is mainly conditioned by intrinsic errors in controlling effective absorption time and by the evaluation of differences between the initial and final substrate concentrations.

For sugar absorption in perfusion with recycling, periods may last from 2 min onward. A shorter duration, at least with certain perfusion rates, is inadequate for a proper recycling to take place. Furthermore, since the entire solution in the perfusion system must be collected, the exact ending of a period cannot be delimited, which would cause greater errors on shortening the reabsorption time.

In perfusion without recycling, periods may last as little as 1 min, provided perfusion rates are below 14-15 ml per min. With intestinal segments longer than 10 cm, the sugar absorbed in 1 min is usually more than 6-7 % of the initially present, an easily measurable difference in concentration.

Shorter periods, from 1 to 5 min, are doubtlessly preferable for studies of absorption kinetics *in vivo* over those of longer duration used until now (1-3, 6, 11, 19-21, 26), since with longer periods

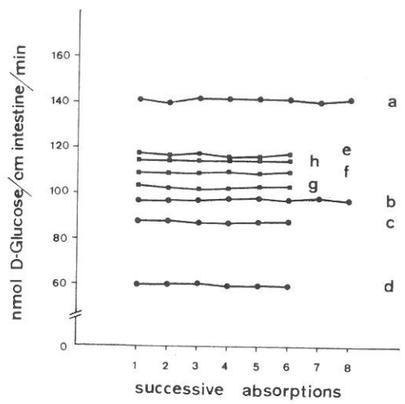

Fig. 2. *Steadiness of intestinal absorption of 2.77 mM D-Glucose in rat along various successive absorption periods.*
Perfusion (5.6 ml/min) with recycling (●) or without recycling (■). Periods of 1 (e), 2 (a, f), 5 (b, g), 10 (c, h) or 20 (d) minutes.

substrate concentration can decrease too much in the solution if working with recycling, or it can increase excessively in tissue or blood.

Steadiness of sugar absorption rate in successive periods. Absorption rate for the same sugar by the same intestinal segment under equal experimental conditions remains constant throughout a series of successive periods. This steadiness had already been established for long periods, 15-30 min, and constituted a solid base to study the effects of an experimental change among the various periods.

In rat, with perfusion recycling a solution containing 2.77 mM glucose, steadiness of absorption rate throughout the sucessive periods was practically complete. The same results were obtained with perfusion without recycling (fig. 2). Analogous steadiness was observed in hamster (fig. 3). It may be stated, as an index of that steadiness, that the variation coefficient (S_x/X) for the various periods in the same animal was practically 0 and reached at most, in some extreme cases, 3-4 %.

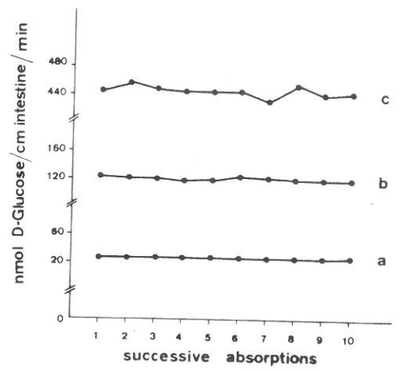

Fig. 3. *Steadiness of intestinal absorption of D-glucose in hamster with continuous perfusion without recycling.*
Perfusion rate, 5.6 ml/min. Sugar concentration, (a) 0.5, (b) 2.77 and (c) 10 mM.

In perfusion with recycling, mean absorption rate decreases on increasing the period duration, due to the decline of sugar concentration in the solution as the absorption progresses. Therefore, if experiments are to be compared, absorption rate must be measured in periods of identical duration.

In perfusion without recycling, instead, absorption rate is the same, independently of the time period employed for its measurement. It was interesting to know if the steady sugar absorption rate, observed during long time with a determined concentration of substrate, was reached from the first moment or if it was possible to measure an initial rate distinct from that of the steady state. Careful experiments were made in which the solution was continuosly perfused and successive fractions corresponding to periods of 1 minute were separated at different times. The results revealed an identical absorption rate of glucose or galactose along the whole experiment, from the 1st up to the 30th minute (fig. 4). This means that, *in vivo*, possible changes in the intracellular level of sugar upon starting absorption did not appreciably affect the net absorption rate, and that, if there was an initial rate distinct from that of the steady state, this one was reached almost instantly.

On the other hand, sugar concentration in blood increases during the experiment as further amounts of substrate are absorbed. When glucose is absorbed from 2.77 mM concentrations, the blood glucose level in rat, normally, about 5.5 mM, may reach up to 12-14 mM after a number of absorption periods. At any rate, this hyperglycemia does not affect the rate of glucose absorption.

Influence of perfusion rate. In perfusion with or without recycling, increase of the flow rate of the solution through the intestine between 2.8 and 13.5 ml/min, produced in rat and hamster a non-linear

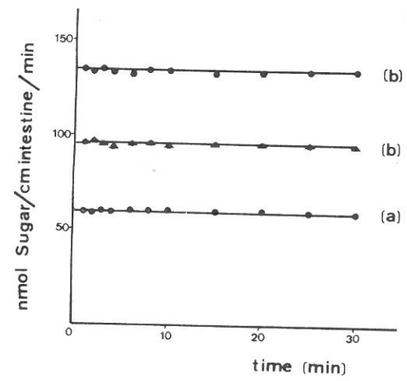

Fig. 4. *Intestinal absorption rate of D-glucose (▲) and D-galactose (●) in rat (a) and hamster (b) along the time.*
Sugar concentration 2.77 mM. Perfusion rate 2.79 ml/min.

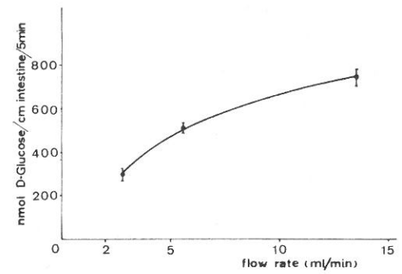

Fig. 5. *Effect of perfusion rate on intestinal absorption of 2.77 D-glucose in rat, with continuous perfusion with recycling, in successive periods of 5 min duration.*

increase in sugar absorption rate (fig. 5). This influence, previously noted by others (5, 8, 19, 25, 31), was not due to possible changes in hydrostatic pressure, which were negligible. Neither was it due to a lesser drop in substrate concentration along the intestine on increasing the speed of perfusion, since this factor was insignificant. Nor could it be explained by alterations in the epithelium or its permeability (19). It must be rather attributed to the effect of the greater speed of flow, which reduces the thickness of the unstirred layers close to the microvilli and which, in turn, facilitates absorption (4, 7, 9, 10, 25, 28, 32, 38-44).

Individual variability and statistical treatment of results. Contrary to the high steadiness of sugar absorption rate along successive periods practised on the same animal under identical experimental conditions, results obtained under the same conditions in different animals show an appreciable variability, even if their age and weight are similar, and the intestinal lengths approximately equal. The variation coefficient ($S_{\bar{x}}/\overline{X}$), that was less than 4 % among the different periods in the same animal, rose to 8-30 % when considering a set of absorption periods in a group of animals.

However, if the effects due to a change in the experimental conditions are expressed as relative deviations with respect to the control absorption in the same animal, the values for the various animals highly coincide. It is better, therefore, not to apply the statistical analysis, to the absolute values, but to the relative deviations observed in each of the animals of the group.

Applicability to different animal species. The afore described method has been applied to rat and hamster, but it can be adapted to any other species.

With hamster the dosage and course of anaesthesia must be carefully controlled to obtain a satisfactory preparation throughout the entire experiment.

Despite the rat's greater body weight, its glucose absorption rate *in vivo* per cm of intestine is usually inferior to that of hamster under equal experimental conditions.

Resumen

Se describe una técnica sencilla para estudios de absorción intestinal *in vivo*, modificación de la descrita por SOLS y PONZ (34), que utiliza una bomba de perfusión, con o sin reciclado de la solución por la luz del intestino y que permite realizar en un mismo segmento intestinal una serie de períodos sucesivos de absorción con alto valor comparativo. La técnica se aplica a la absorción de azúcares en rata y hamster, pero puede extenderse a otros sustratos y otras especies animales. La velocidad de absorción intestinal con glucosa para cada concentración se mantiene constante desde el primer período a lo largo de buen número de períodos sucesivos de 1 minuto y aumenta con la velocidad de perfusión.

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