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Fisiologia. — *Sugar reabsorption and its relation to sodium transport in isolated rabbit kidney perfused* in vitro (*). Nota di ELOISA MILLA e FRANCESCA ZANOLI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Su reni isolati di coniglio perfusi *in vitro* con sangue intero omologo a crescenti concentrazioni di glucosio (G_p) è stato determinato il massimo trasporto di glucosio per unità di ultrafiltrato $(TG/GFR)_m$ e si è descritta la relazione fra i flussi netti transtubulari di glucosio e Na^+ (TG e TNa^+).

La quantità di glucosio riassorbito per ml di ultrafiltrato, TG/GFR, cresce con G_p, secondo l'andamento tipico di un processo di trasporto a saturazione, che segue la cinetica di Michaelis-Menten, tendendo ad un valore massimo, $(TG/GFR)_m$ = circa 24 μ moli/ml. La concentrazione plasmatica di glucosio che corrisponde a 1/2 $(TG/GFR)_m$ è uguale a circa 11 μM/ml.

I valori di TG sono linearmente correlati coi rispettivi TNa^+ in ogni gruppo di perfusioni. Il valore del rapporto TG/TNa^+ aumenta con l'aumentare di G_p, tendendo ad un valore limite quando TG ha raggiunto il valore massimo (TmG).

The dependence of D-glucose and other sugar transport on Na^+ and the mechanisms of this relation have been investigated also for the reabsorbing proximal renal tubular epithelia by means of very widely varying techniques.

The presence of Na^+ is necessary for the uptake of D-glucose and other monosaccharides by slices of rabbit kidney [6, 7].

In later experiments it was shown that by increasing the Na^+ concentration in the incubation medium of tubular brush-border microvilli and of cellular baso-lateral membranes or in the perfusing luminal fluid of a single proximal tubule of rat [3, 5, 15] the values of the Km parameter in the Michaelis-Menten kinetics, both of the D-glucose uptake as well as of the local reabsorption of α-methyl-glucoside (which in the rat tubule is absorbed at the same rate as D-glucose), are lowered: this means that the affinity of the binding sites of the transporting system for these sugars is increased.

In the *in toto* kidney a linear relationship between the contemporaneous net Na^+ and D-glucose fluxes was seen in amphibian kidney [16] perfused by solutions at different Na^+ concentrations, varying between 10 and 75 mEq/l.

In the whole mammalian kidney the results are not univocal.

In isolated and perfused rat kidney [10], glucose reabsorption was decreased only when the Na^+ concentration in the perfusing fluid was lowered below 50 mM/l; the presence of ouabain, which inhibits the Na^+–K^+ activated ATPase pump, had the same result, but a strict relation between the decrease of Na^+ and glucose transport has not been found. In other experiments on

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(**) Nella seduta del 14 febbraio 1976.

isolated rat kidney [1] perfused with solutions at glucose concentration above the excretion threshold and at changing Na^+ concentrations (from 25 to 140 mEq/l), the two transtubular net fluxes appeared to be independent. On the other hand, in the *in vivo* kidneys of hyperglycemic dogs, the glucose reabsorption was increased or decreased, when Na^+ reabsorption was enhanced or impaired, respectively by clamping the inferior vena cava or by Ringer-lactate infusions [8]. These conditions are very far from the physiological ones.

No data are available, to our knowledge, on the relationship between the contemporaneous transtubular net flows of Na^+ (TNa^+) and glucose (TG) in the whole kidney, at progressively increasing TG up to the point of the maximal glucose reabsorption (TmG), but at constant Na^+ concentration of the perfusing fluid and at renal functional conditions which are homogeneous and as near as possible to the physiological performances.

This investigation forms the object of this paper [1].

For this purpose the values of glomerular filtration rate (GFR), of the filtered fraction (FF), and of the Na^+ fractional reabsorption (FNa_r^+) must be of the same magnitude, in spite of changes of the glycemic and the TG values; furthermore the total Na^+ reabsorption has to be always strictly related to GFR. For these purposes the technique of the isolated rabbit kidney perfused *in vitro* with whole blood, as described elsewhere by us [13] seems particularly suitable, since its functional performance is quite similar to that of kidneys perfused *in vivo* [12, 13]. Different filtered loads are easily obtained by changing plasma glucose levels. The TG can be measured in kidneys at a comparable level of functional performance, as indicated by FNa_r^+ and FF respectively not less than 0.7 and 0.14. In this way the TG should depend only on the glucose delivery and the specific reabsorption capacity of the tubules. Also the Na^+ reabsorption by different kidneys should be considered as homogeneous if the FNa_r^+ is constant and the total reabsorbed amount is strictly related to GFR. Therefore it seems correct to relate the single TG values to their corresponding TNa^+.

Methods

Groups of rabbit kidneys were perfused by whole homologous normo- hyper- or hypoglycemic blood: The latter was obtained by fasting and insulinating the animals with 1/3 I. U. pro Kg b.w.

(1) *List of symbols*: G_p: Plasma glucose concentration (μM/ml); GFR: Glomerular filtration rate (ml/g·h); TG and TmG: Glucose reabsorption and maximal glucose reabsorption rate (μmoles/g·h); TG/GFR and $(TG/GFR)_m$: Glucose reabsorption and maximal glucose reabsorption per ml of ultrafiltered fluid (μmoles/ml); RPF: renal plasma flow; FF: Filtration fraction (GFR/RPF); FG_r: Fractional glucose reabsorption (TG/ultrafiltered glucose); TNa^+ : Na^+ reabsorption rate (mEq/g·h); TNa^+/GFR : Na^+ reabsorption per ml of ultrafiltered fluid (mEq/ml). FNa_r^+: Fractional Na^+ reabsorption(TNa^+/ultrafiltered Na^+). $\dot{V}$: urine output (ml/g·h).

TABLE I.

Mean of the data ± s.e.m. *from 7 groups of isolated rabbit kidneys perfused with whole blood at increasing glucose concentration* (G_p).

Number of experimental periods for each group in parentheses.

G_p μM/ml	GFR ml/g·h	FF	TG μmoles/g·h	TG/GFR μmoles/ml	FG_r	TNa^+ mEq/g·h	TNa^+/GFR^+ mEq/ml	FNa_r^+	$\dot{V}$ ml/g·h
1,7±0,2 (6)	20,9±1,3	0,23±0,09	36±3	1,70	1,00	2,3±0,01	0,114±0,003	0,76±0,01	3,5±0,4
6,0±0,4 (18)	22,7±1,2	0,19±0,01	140±15	6,00±0,40	1,00	2,57±0,16	0,112±0,001	0,75±0,02	5,8±0,3
16,2±0,7 (20)	34,5±2,4	0,22±0,02	536±44	15,32±0,35	0,92±0,1	4,03±0,32	0,112±0,001	0,77±0,01	8,2±0,7
20,2±0,5 (10)	37,1±3,9	0,27±0,03	643±37	16,04±0,31	0,80±0,02	4,29±0,07	0,114±0,002	0,77±0,02	10,7±0,9
29,6±0,5 (20)	31,2±2,8	0,31±0,02	639±58	17,50±0,55	0,66±0,02	3,90±0,35	0,119±0,002	0,77±0,01	8,8±0,56
42,0±0,7 (25)	24,3±2,3	0,24±0,01	566±35	23,20±0,50 (*)	0,54±0,02	2,77±0,24	0,112±0,002	0,73±0,09	7,9±0,5
53,6±1,2 (10)	33,0±2,0	0,23±0,01	785±33	23,75±0,89 (*)	0,44±0,02	3,63±0,26	0,114±0,004	0,72±0,01	12,40±0,8

(*) Probability level for the significance of the difference, as from the Student's "*t*": $P > 0,1$.

In order to keep the hyperglycemic initial plasma glucose concentration (G_p) (up to 54 mM) unchanged, Krebs-Henseleit solution of proper glucose concentration was dropped throughout the whole experiment into the venous reservoir at the same rate as the urine flow. Krebs-Henseleit solution added with ^{14}C-inulin was also infused at a constant rate by a Harvard pump; a steady inulin concentration was reached after about 15′ of infusion. The perfusion pressure was carefully controlled and kept between 100 and 110 mmHg throughout the experiment.

At the beginning and at the end of each experimental period (from 2 to 5 in the different kidneys and each of 10′) arterial blood samples were taken for the analysis and the urine volume collected and measured at the end of of each period.

The analyses for glucose were immediately carried out by an enzymatic method [4]. ^{14}C inulin in deproteinized urine and plasma was detected by a liquid scintillation spectrometer (Tri-Carb, Packard Instr. Co. mod. 3315) and plasma and urine Na^+ concentration spectrophotometrically determined. From the GFR values, measured as inulin clearance, and the plasma and urine concentrations of glucose and Na^+, the corresponding TG and TNa^+ were calculated as the differences between the respective ultrafiltered and urinary excreted amounts.

Results

The mean values of the data obtained from clearance periods (in which FNa_r^+ and FF were not less than 0,7 and about 0, 17) for the most relevant functional parameters in the different groups of kidneys are given in Table I.

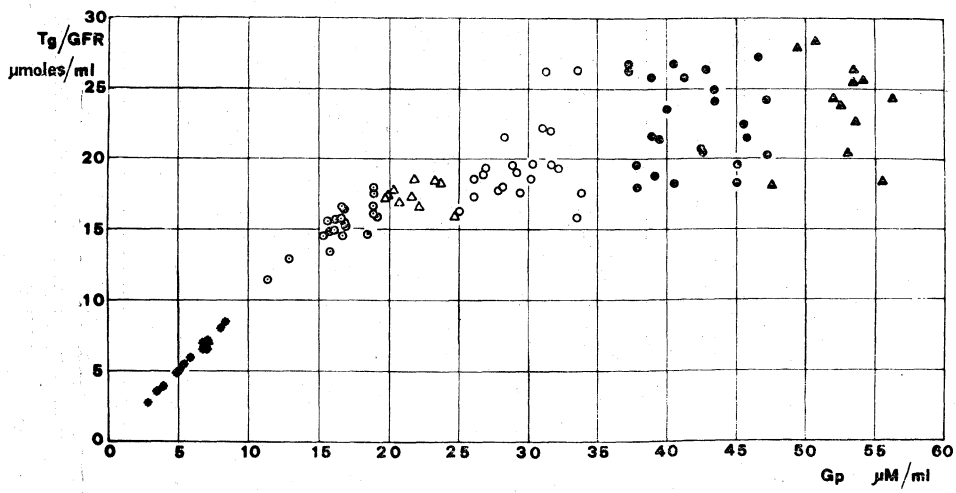

Fig. 1. – Glucose reabsorbed per ml of ultrafiltrate as function of plasma glucose concentration. TG/GFR (µmoles/ml) on the ordinate: G_p (µM/ml) on the abscissa. Symbols refer to the kidneys perfused at different mean G_p (µM/ml): $*$ 6 ± 0,4; ⊙ 16,2 ± 0,7; △ 20,2 ± 0,5; ○ 29,6 ± 0,5; ● 42 ± 0,7; ▲ 53,6 ± 1,2.

They show the functional homogeneity of the kidneys perfused at increasing G_p values. The mean GFR is of the same magnitude, though spontaneously changing within the physiological range [11], in all groups . FNA_r^+ and FF are also practically equal, as well as at normal or high G_p values to the mean total Na^+ reabsorbed amount, which is strictly related to GFR, thus meaning a well regulated glomerulotubular balance for Na^+ and fluid.

In relation to the glucose reabsorption, the data of Table I show that with increasing G_p the mean total TG values do not tend to a maximum, while this trend is evident for the mean TG/GFR ratios, not significantly different in the two last groups of perfusions at the highest G_p. This appears too from fig. 1, in which the values of TG/GFR ratio of all the experiments were plotted against the corresponding G_p. This behavior is typical of a satu-

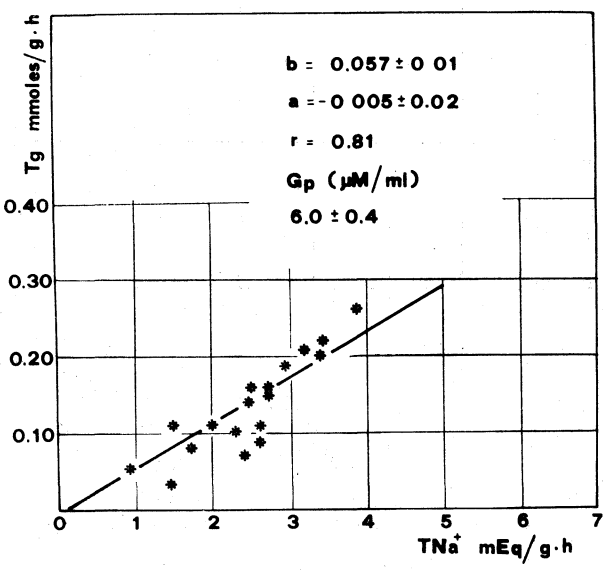

Fig. 2. – Relationship between total glucose reabsorption rate (TG mmoles/g·h, ordinate) and Na^+ reabsorption rate (TNa^+ mEq/g·h, abscissa). * $G_p = 6{,}0 \pm 0{,}4$ µM/ml.

ration transport process and suggests that the glucose transtubular transport in the perfused rabbit kidney obeys Michaelis-Menten kinetics. With reference to the rate of glucose reabsorption per ml of ultrafiltered fluid (TG/GFR), the maximal rate of reabsorption $(TG/GFR)_m$ is about 24 µ moles/ml and the G_p corresponding to 1/2 of $(TG/GFR)_m$ is about 11 µM/ml.

The figs. 2, 3, 4, 5, 6 show the relationship between all the TG of each group of perfusion at a given G_p and the corresponding TNa^+: the relationship is linear in each group, with a very high correlation coefficient and y intercepts indistinguishable from zero. This means that a constant ratio exists between TG and the corresponding TNa^+ and that, since TNa^+ linearly increases with GFR, also TG increases with GFR in proportion to the reabsorbed Na^+.

As shown by the curve of fig. 7, the ratios calculated as slopes of the lines of figs. 2 to 6, tends to a maximal value by increasing G_p. The TG/TNa^+ ratio approaches zero when G_p is very low, since TG becomes extremely small, while TNa^+ is in the normal range (Table I).

Discussion and conclusions

In our experiments a maximum value of TG/GFR ratio was found of about 24 μ moles/ml which represents the maximal amount of glucose reabsorbed per ml of ultrafiltrate; the plasma glucose concentration of about

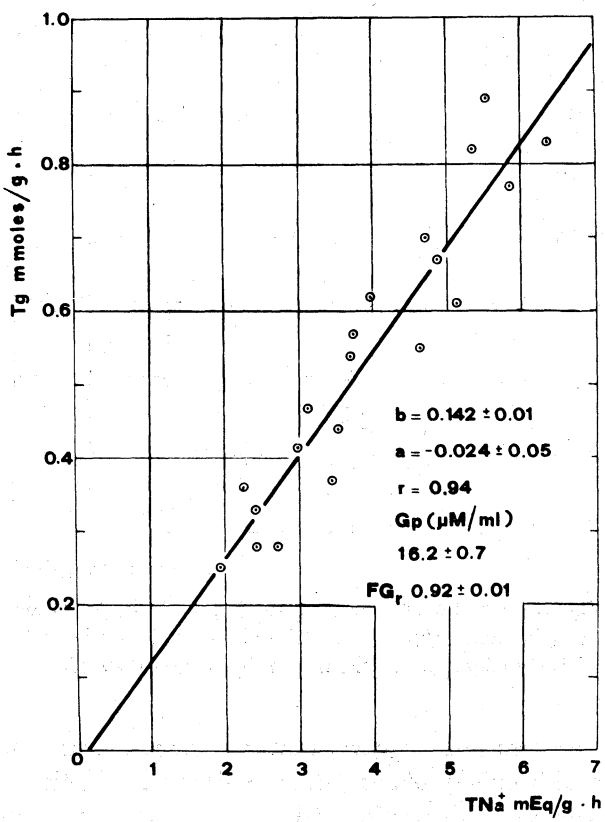

Fig. 3. - As in fig. 2. ⊙ $G_p = 16{,}2 + 0{,}7$ μM/ml.

11 μM/ml corresponds to 1/2 of this maximum. If the characteristics of the transtubular diffusion as determined in the single or isolated rat tubule [9] hold also in the whole rabbit kidney, in our experiments the diffusional component of the net transtubular glucose flow should be negligible, since the glucose concentration difference between the interstitial fluid or plasma and urine was never greater than 10 mM. Furthermore there are no reports of

any significant coupling to a solvent drag between the transport of glucose and water.

It has been clearly shown that the affinity of the sugar binding system in the sites of the microvilli membranes in the proximal renal tubule is related to the Na^+ presence and its concentration [3, 5, 15]. It seems obvious that also the performance of the Na^+ reabsorbing pump, the intracellular Na^+

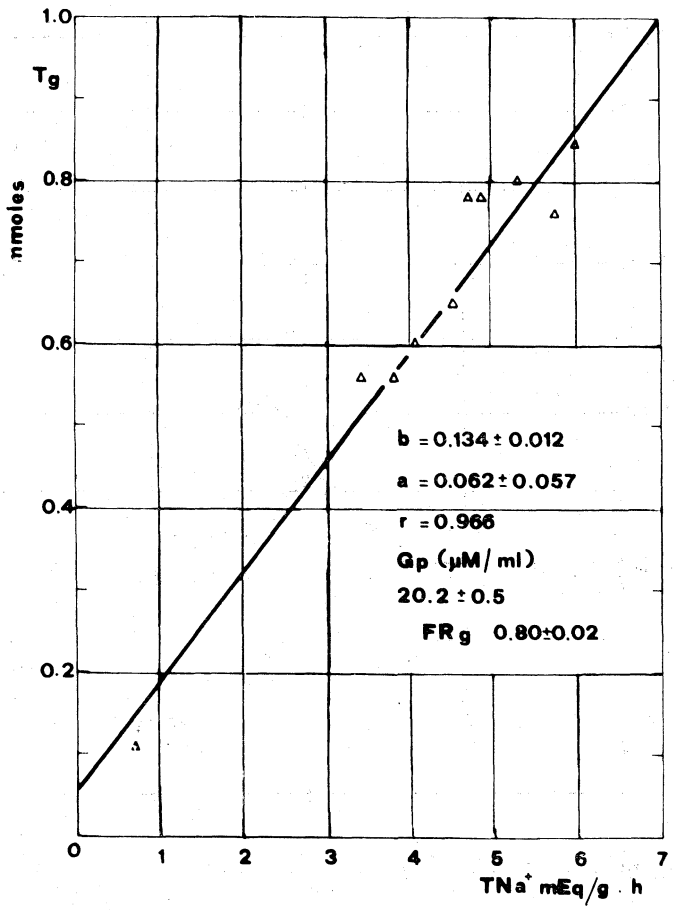

Fig. 4. – As in fig. 2 and 3. $\triangle G_p = 20{,}2 \pm 0{,}5$ µM/ml.

concentration, the maintenance of the Na^+ cellular gradient and finally a proper transluminal Na^+ flow must play a rôle in the transcellular movement of glucose and some references [10, 16] support this hypothesis.

Our data show that the contemporaneous transtubular net fluxes of glucose and Na^+ are linearly correlated in all groups of perfusions at increasing G_p (figs. 2, 3, 4, 5, 6) and that their ratio, calculated by the slopes of the lines, increases whith G_p, tending to a limiting value which can be geometrically obtained from the curve of fig. 7.

The linear relationship between the contemporaneous net fluxes and the trend to a maximum value of the TG/TNa^+ ratio suggests the hypothesis of a common carrier, which should be saturated with glucose, when the glucose transport is at its maximum. The maximal value of TG/TNa^+ ratio of about

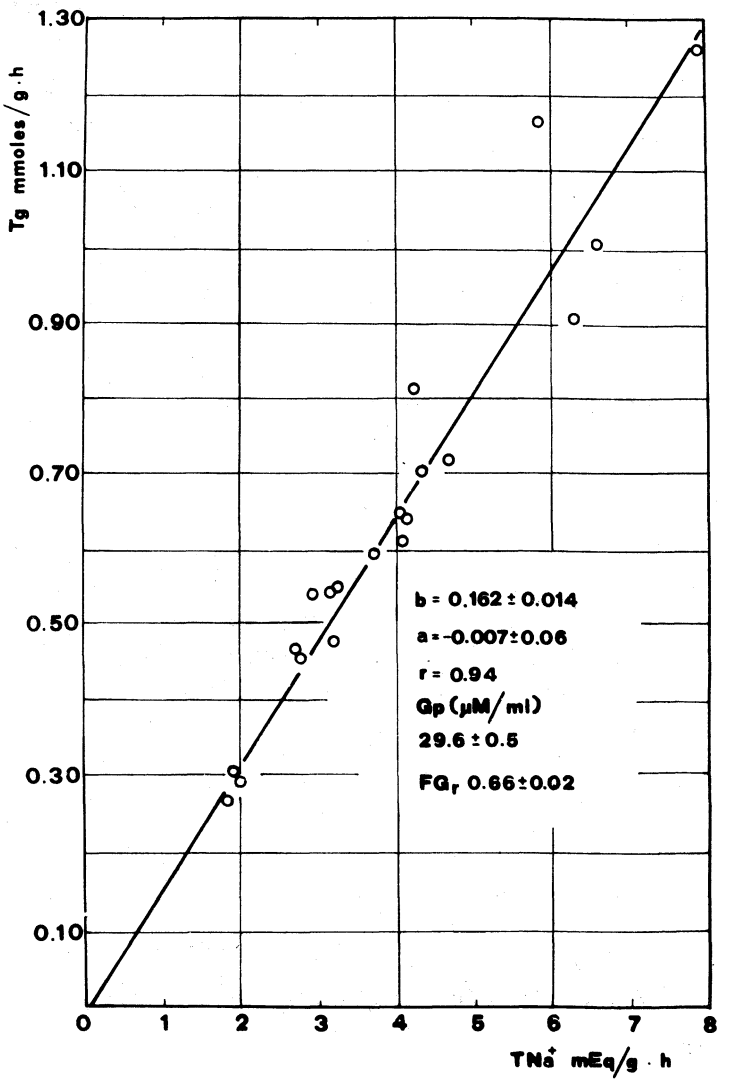

Fig. 5. – As in fig. 2–3–4. o $G_p = 29{,}6 \pm 0{,}5$ μM/ml.

0.2, as geometrically calculated from the curve of fig. 7, is not a real absolute value. It could be increased by about 2/3 or more since only the fraction of Na^+ actively transported by the proximal tubule (that is 1/3 of the total reabsorbed amount [14] should be taken into account.

In fact we can assume that, as FNa_r^+, FF, GFR are of the same magnitude and TNa^+ is always strictly related to GFR in spite of wide variations of G_p and TG, under our experimental conditions also the various mechanisms acting on the Na^+ reabsorption at the different levels of the

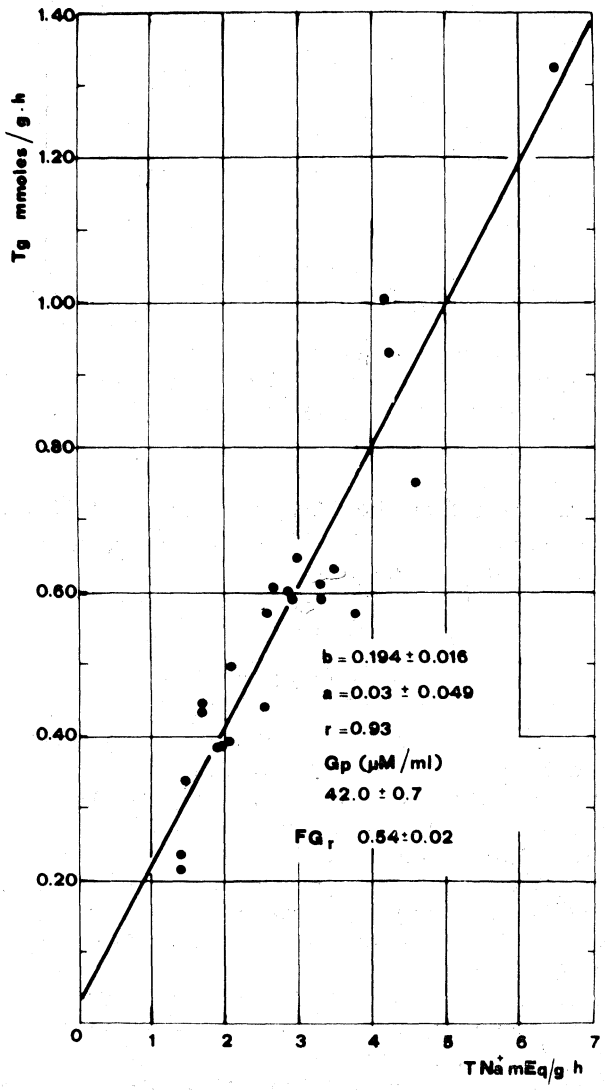

Fig. 6. – As in fig. 2–3–4–5. ● $G_p = 42{,}0 \pm 0{,}7$ µMml.

nephron should remain substantially unchanged and and in the same quantitative relationship to each other.

In these terms, the indication of the trend to a limiting value of the ratio between the contemporaneous net fluxes of glucose and Na^+ seems valid,

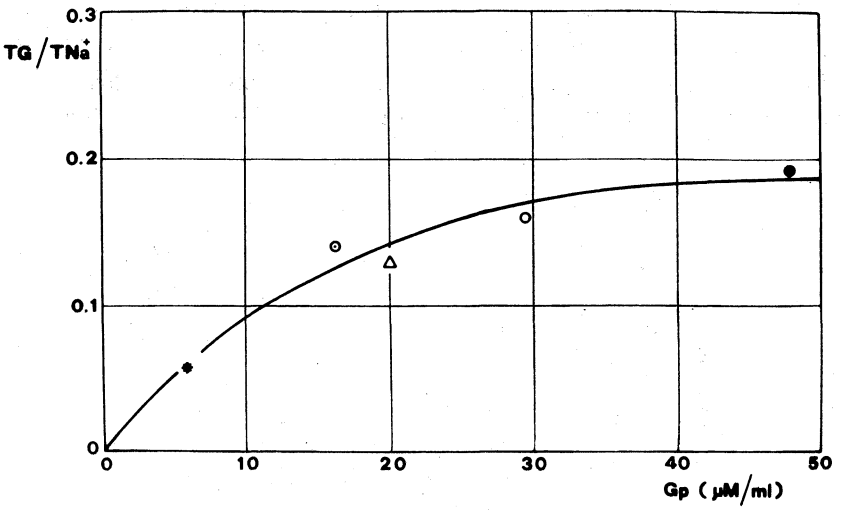

Fig. 7. – Slopes of the lines of figs. 2–3–4–5–6 on the ordinate; mean G_p (μM/ml) of the corresponding group of perfused kidneys on the abscissa (graphical interpolation).

notwithstanding the fact that the absolute value of this ratio cannot be found by experiments on the whole kidneys but only, hopefully, by investigation on isolated proximal tubules.

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