In base ai fatti sopra riportati, quanto proposto da GONZALEZ-RAMIREZ per conciliare le diverse vedute sulle strutture del nucleolo, non è accettabile, e ciò specialmente tenuto conto della mutevolezza delle strutture e delle ultrastrutture, anche nell'ambito di uno stesso nucleolo in relazione ai vari momenti funzionali. In generale due principali funzionalità sono state riscontrate[7], una relativa alle granulazioni più grandi, una a quelle più piccole, funzionalità che, specialmente negli ovociti mononucleolati, hanno la loro esplicazione rispettivamente nella fase iniziale e nella fase finale di tutto lo sviluppo ovocitario. In una specie (*Patella coerulea*) con ovociti polinucleolati le due funzionalità si esplicano in due tipi distinti di nucleolo: il nucleolo primario con granulazioni più grandi (di 150 Å di diametro) e senza granuli nucleolonemali, e gli anfinucleoli con granulazioni più piccole (da 15 a 50 Å di diametro) e con granuli nucleolonemali[7,19]. Infine è bene far presente l'inopportunità di continuare a denominare nucleolonema il reticolo endonucleolare, che, come sopra detto, nulla ha a che vedere con la struttura che ESTABLE e SOTELO hanno erroneamente creduto di avere individuato.

Le osservazioni al microscopio elettronico, cui in parte si fa riferimento sopra, sono state compiute presso il Centro di Microscopia elettronica dell'Università di Padova.

A. BOLOGNARI

Istituto di Zoologia e di Anatomia comparata, Università di Messina (Italia), il 5 febbraio 1960.

Résumé

On donne certaines précisions en ce qui concerne le nucléolonema, qui en fait n'existe pas sous forme d'un peloton et qui ne correspond pas au réticule endonucléolaire décelé au microscope électronique. Dans le nucléole on peut reconnaître surtout deux formes d'éléments ultrastructuraux, c'est-à-dire les plus grandes granulations (environ 150 Å de diamètre), qui peuvent donner lieu au réticule, et celles plus petites, dont les accumulations correspondent aux granules nucléolonémales. A ces types de granulations il faut assigner deux rôles principaux.

Renal Tubular Transport of Sodium During Sodium Chloride and Sodium Bicarbonate Loadings in the Normal Dog

PITTS and LOTSPEICH[1] have demonstrated that bicarbonate reabsorption, expressed in *mM*/l of glomerular filtrate, remained constant when plasma bicarbonate concentration was increased above the threshold value of 26–28 *mM*/l by sodium bicarbonate infusion in the normal dog. No data have been published concerning the tubular reabsorption of sodium in experiments involving hypernatremia induced by $NaHCO_3$ loading. From current view on the $Na^+ \rightleftarrows H^+$ exchange mechanism, one would anticipate the tubular reabsorption of sodium to remain similarly constant if plasma sodium concentration was increased by $NaHCO_3$ infusion.

Data obtained in NaCl loading experiments concerning the influence of increases in sodium and chloride plasma concentrations on their tubular reabsorption are more conflicting. The studies of HARE and PHILIPS[2], and of BALDWIN, KAHANA, and CLARKE[3], seem to demonstrate that sodium and chloride reabsorption rates by the tubules rise linearly with their plasma concentrations. On the other hand, LEVY and ANKENEY[4], SELKURT and POST[5], GREEN and FARAH[6], and WESSON and ANSLOW[7] did not observe such a relationship.

These discrepancies led us to reinvestigate sodium tubular reabsorption during hypernatremia induced by NaCl and $NaHCO_3$ loadings in the normal dog.

Methods. The sodium salts were infused in isotonic or hypertonic solutions after the injection of suitable priming doses sufficient to induce frank sodium excretion (over 5.0 *mM* Na/l of glomerular filtrate). In 50 experiments plasma sodium concentration was maintained constant during 60–120 min by sustaining infusion of the sodium salt. In 11 experiments (5 with $NaHCO_3$ and 6 with NaCl) plasma sodium concentration was raised by 3 or 4 successive priming doses of sodium salt so that tubular reabsorption rate could be studied in the course of the same experiment at different plasma concentration levels.

1. *$NaHCO_3$ loading experiments.* 29 experiments were performed on 17 female dogs weighing between 12 and 28 kg. Sodium and bicarbonate reabsorption rates were calculated by the use of the exogenous creatinine clearance as a measure of the glomerular filtration rate (GFR). Hypernatremia was induced by priming doses of 150 to 250 *mM* $NaHCO_3$, and this state was further maintained by a sustaining infusion of isotonic $NaHCO_3$ (1.2–1.8 *mM* $NaHCO_3$/min). 134 anaerobic collection periods, ranging from 8–12 min, were secured during these experiments using the conventional clearance technique.

2. *NaCl loading experiments.* 32 experiments were performed on 10 dogs weighing 13–27 kg. Sodium and chloride reabsorption rates were estimated as in $NaHCO_3$ experiments. Hypernatremia was induced by priming doses of 100–250 *mM* NaCl followed by 1–2% NaCl infusion (1.5–3.0 *mM* NaCl/min). 161 urine collection periods were obtained during these experiments.

Results. It has been repeatedly demonstrated[7–10] that tubular reabsorption rates of sodium, chloride, and bicarbonate were markedly influenced by GFR changes. When GFR increases in the course of saline infusion, electrolyte tubular reabsorption rate rises proportionally so that transport rate values do not change when they are expressed in *mM*/l of glomerular filtrate. On the contrary, drops in GFR are not accompanied by changes in absolute tubular reabsorption rates. It follows that in such conditions tubular reabsorption rates increase when they are expressed in *mM*/l of glomerular filtrate.

The influence of GFR changes on the tubular reabsorption rates of the electrolytes made necessary the exclusion of some data from our results. We have deliberately omitted periods involving a drop of more than 5% in GFR in the course of a given experiment. The data to be presented are based on 295 urine collection periods.

[1] R. F. PITTS and W. D. LOTSPEICH, Amer. J. Physiol. *147*, 138 (1946).

[2] R. S. HARE and D. M. PHILIPS, Amer. J. Physiol. *140*, 334 (1943).

[3] D. BALDWIN, E. M. KAHANA, and R. C. CLARKE, Amer. J. Physiol. *162*, 655 (1950).

[4] M. N. LEVY and J. L. ANKENEY, Proc. Soc. exp. Biol. Med., N. Y. *79*, 491 (1952).

[5] E. E. SELKURT and R. S. POST, Amer. J. Physiol. *162*, 639 (1950).

[6] D. M. GREEN and A. FARAH, Amer. J. Physiol. *158*, 444 (1949).

[7] L. G. WESSON, JR. and W. P. ANSLOW, JR., Amer. J. Physiol. *180*, 237 (1955).

[8] W. D. LOTSPEICH, R. C. SWAN, and R. F. PITTS, Amer. J. Physiol. *148*, 445 (1947).

[9] D. D. THOMPSON and R. F. PITTS, Amer. J. Physiol. *168*, 490 (1952).

[10] D. D. THOMPSON and M. J. BARRETT, Amer. J. Physiol. *176*, 201 (1954).

1. *NaCl loading experiments.* Figure 1 shows tubular reabsorption rates for sodium (open circles), and for chloride (black circles), expressed in *mM*/l of glomerular filtrate, plotted with their respective plasma concentration values. It is readily apparent that the reabsorption rate is rising linearly with increasing plasma concentration for both electrolytes. The equations of the regression lines that can be calculated from all the NaCl data are respectively:

$$\text{NaR/l of GF} = 0.647\ P_{Na} + 32.7 \quad (1)$$

and

$$\text{ClR/l of GF} = 0.570\ P_{Cl} + 47.5 \quad (2)$$

where NaR/l of GF and ClR/l of GF represent reabsorption rates expressed in *mM*/l of glomerular filtrate, and P_{Na} and P_{Cl} plasma concentrations in *mM*/l.

The respective slope of both regression lines is significantly different from zero ($P < 0.001$). Moreover, application of the 't test' to the data used to construct both regression lines shows that their slopes do not differ significantly ($0.40 < P < 0.50$).

2. *$NaHCO_3$ loading experiments.* Figure 2 gives the data obtained during $NaHCO_3$ infusions, plotted as in Figure 1, the open circles representing sodium and the black circles bicarbonate transport rates.

The equations of the regression lines calculated from all the data are:

$$\text{NaR/l of GF} = 0.242\ P_{Na} + 89.2 \quad (3)$$

$$\text{HCO}_3\text{R/l of GF} = 0.154\ P_{HCO_3} + 18.7 \quad (4)$$

Variance analysis shows that the slope of the regression line differs significantly from zero in the case of bicarbonate ($P < 0.001$) but not in the case of sodium ($0.05 < P < 0.10$). On the other hand, 't test' application shows that the slopes do not differ significantly one from each other ($0.4 < P < 0.5$).

The range of sodium plasma concentrations explored during $NaHCO_3$ loading experiments is smaller than for NaCl because, due to severe alkalosis, it is impossible to raise natremia to such high levels with $NaHCO_3$. Nevertheless, comparison with sodium data obtained for a similar range of plasma sodium concentrations (147–169 *mM*/l) shows that the slope of the regression line is significantly different from zero in the case of NaCl ($0.01 < P < 0.02$), but not in the case of $NaHCO_3$ ($0.05 < P < 0.10$).

Discussion. The data obtained in NaCl loading experiments show that sodium and chloride reabsorption rates are intimately linked with the respective concentration of these ions in the glomerular filtrate for the plasma concentrations ranges explored (119–179 *mM*/l for chloride, and 158–232 *mM*/l for sodium).

Equation 1 would indicate that sodium tubular transport is the result of two different processes:

(1) 70–75% of the total transport are related to sodium concentration in the glomerular filtrate;

(2) 25–30% constitute a constant fraction dealing with some 40 *mM* of sodium/l of glomerular filtrate.

It is known that the reabsorption of the sodium fraction combined with bicarbonate is limited by a Tm process of some 26–28 *mM*/l of glomerular filtrate, independent of plasma sodium concentration. It follows that the reabsorption of sodium combined with bicarbonate might constitue the main part of the 25–30% fraction. On the other hand, the fraction of the tubular transport which is a function of natremia would essentially represent sodium combined with chloride in the glomerular filtrate.

Such a conception of the tubular transport of sodium would find support in a recent paper by MALVIN, WILDE, VANDER, and SULLIVAN[11]. Using their 'stop-flow' technique, these workers calculated that sodium concentration in the proximal reabsorbate was equal to sodium concentration in the glomerular filtrate for any amount of water reabsorbed in the proximal segment of the nephron. In other words, sodium reabsorption rate is a function of sodium plasma concentration. Our own data further show that a similar phenomenon is taking place for chloride.

Contrastingly, in $NaHCO_3$ loading experiments sodium reabsorption rate reaches a fixed Tm value of some 130 *mM*/l of glomerular filtrate (108–144 *mM*/l). This value could represent the sum of two different processes:

(1) 26–28 *mM* constituted by the $Na^+ \rightleftarrows H^+$ exchange mechanism responsible for bicarbonate reabsorption;

(2) most of the remaining 100 *mM* of the sodium transport would represent NaCl reabsorption as described above; this last fraction of the sodium reabsorption rate is relatively stable.

The slight increase of bicarbonate reabsorption rate observed at high plasma bicarbonate concentration levels is due to the simultaneous decrease of plasma chloride concentration which follows $NaHCO_3$ loading[12, 13]. The

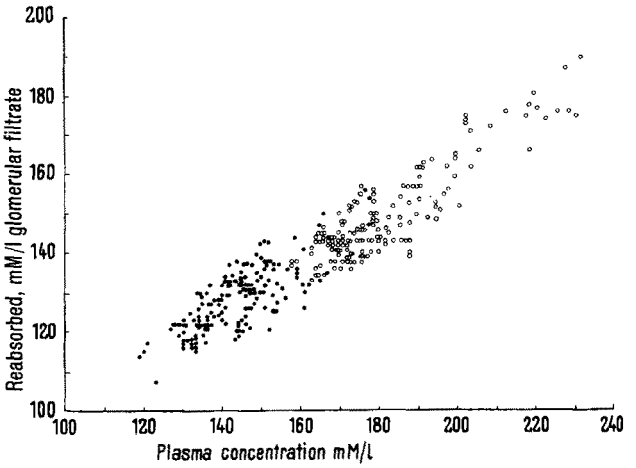

Fig. 1

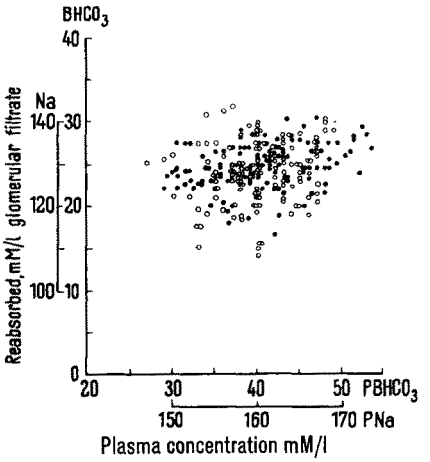

Fig. 2

[11] R. L. MALVIN, W. S. WILDE, A. J. VANDER, and L. P. SULLIVAN, Amer. J. Physiol. *195*, 549 (1958).

[12] CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN, Exper. *14*, 417 (1958).

[13] CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN, Exper. *15*, 434 (1959).

sligth but definite increments of pCO_2 observed in these experiments would also favour bicarbonate reabsorption.

CH. TOUSSAINT and P. VEREERSTRAETEN

Laboratoire de Médecine expérimentale, Université libre de Bruxelles et Fondation médicale Reine Elisabeth, Bruxelles (Belgium), March 3, 1960.

Résumé

La réabsorption tubulaire des ions sodium, chlore et bicarbonate a été étudiée au cours de perfusions de NaCl et de $NaHCO_3$ chez le chien normal. 61 expériences, comportant un ensemble de 295 récoltes urinaires, ont été pratiquées sur 21 animaux.

A filtration constante, la réabsorption du sodium exprimée en *mM*/l de filtrat glomérulaire demeure constante lorsque la natrémie est accrue par l'infusion de $NaHCO_3$. Elle est, au contraire, une fonction linéaire de la natrémie lorsque celle-ci est élevée par des perfusions de NaCl.

Dans le premier cas, la réabsorption du sodium s'effectue par un double mécanisme: l'un, lié à la réabsorption du bicarbonate, est fixé par un Tm constant, l'autre, lié à la réabsorption du chlore, dépend de la chlorémie. Lorsque la natrémie est accrue par l'infusion de NaCl, le second mécanisme l'emporte nettement sur le premier. Il apparaît alors une relation évidente entre la natrémie (ou la chlorémie) et la quantité de sodium (ou de chlore) réabsorbée par les tubes rénaux.

Physiology of Intracellular Symbiotes of *Stegobium paniceum* L. with Special Reference to Amino Acid Requirements of the Host

Stegobium paniceum L. is one of the few insects where intracellular symbiosis is somewhat better understood. The yeast-like symbiotes of this beetle supply their host with certain factors of vitamin B-complex and sterol[1,2]. The possible way by which the microbes may be supplying these growth factors has also been later suggested by GRÄBNER[3]. Recently it has been possible to extend such studies further with reference to amino acid requirements of larvae of this insect species.

Two batches of *Stegobium* larvae, one with and another without symbiotes, were grown on artificial diets containing various combinations of amino acids. The methods of making the basic artificial diet and eliminating the symbiotes by egg-surface sterilization have already been described earlier[2]. The diet consisted of an amino acid mixture 15 parts, starch 85 parts, salt mixture 1 part, cholesterol 2 parts, nucleic acid 0·1 part, and vitamin solution to give a concentration of 25 µg/g of thiamin, riboflavin, nicotinic acid, pyridoxin, pantothenic acid, and 500 µg/g choline, 0·01 µg/g biotin, 4 µg/g folic acid, and 250 µg/g inositol. 30 newly hatched larvae were put on diets deficient in one of the amino acids at a time and growth was observed. The tests were performed in small shell vials containing 2 g of diet.

The results are given in the Table, whence it can be seen that aposymbiotic condition led to an increased demand of the nutrient factors from external dietary source. For the sake of convenience, the various amino acids are so arranged in the Table that the first nine fall under the so-called 'non-essential' category while the remaining ten are under the 'essential'.

Growth of *Stegobium* larvae (with and without symbiotes) on diets lacking in one of the amino acids

Diet lacking in	With symbiotes		Without symbiotes	
	Number of adults out of 30 larvae	Developmental period in days	Number of adults out of 30 lavae	Developmental period in days
'non essential'				
DL-Alanine	29	31–36	10	72–118
DL-Aspartic acid	24	35–57	18	43–118
DL-Cystine	26	32–57	17	52–118
L-Glutamic acid	24	33–57	16	52–118
Glycine	14	64–94	0	–
L-Hydroxyproline	27	33–57	17	35–72
L-Proline	28	31–47	23	40–118
DL-Serine	28	31–50	23	43–108
L-Tyrosine	29	33–50	18	43–108
'essential'				
L-Arginine	2	51–78	0	–
L-Histidine	13	51–93	7	43–108
DL-Isoleucine	11	54–84	0	–
L-Leucine	9	54–88	0	–
L-Lysine	10	42–84	0	–
DL-Methionine	14	35–52	2	72–103
DL-Phenylalanine	18	35–86	0	–
DL-Threonine	7	57–78	0	–
L-Tryptophane	24	46–73	0	–
DL-Valine	13	42–75	0	–
No amino acids	26	38–57	–	–
Casein diet	27	27–37	29	28–40

The dietary deficiency in any one of the 'non-essential' amino acids did not adversely affect the growth of normal *Stegobium* larvae, with the single exception of glycine-deficiency which resulted in increased mortality and prolongation of larval period. Growth was, however, adversely affected when any one of the next 10 'essential' amino acids were omitted from the diet. Their deficiency resulted in marked disturbances in growth and a lower number of larvae completed metamorphosis. Without arginine only 2 larvae could become adults in 51 to 78 days and omission of threonine also proved highly detrimental. Except for tryptophane and probably phenylalanine, all the 'essential' amino acids proved comparatively more vital for larval growth than the first nine listed in the Table.

The reaction of aposymbiotic larvae was, however, entirely different. Individual amino acid deficiency became very much pronounced in the majority of cases, and was characterized by fewer larvae becoming adults and undue prolongation of larval period. In many cases, half-grown larvae survived even up to 118 days, when the experiment

[1] N. C. PANT and G. FRAENKEL, Science *112*, 498 (1950).
[2] N. C. PANT and G. FRAENKEL, Biol. Bull. *107*, 420 (1954).
[3] K. E. GRÄBNER, Z. Morph. Ökol. Tiere *41*, 471 (1954).
[4] H. J. MÜLLER, Z. Morph. Ökol. Tiere *44*, 459 (1956).
[5] R. GEIGY, L. A. HALFF, and V. KOCHER, Schweiz. med. Wschr. *83*, 928 (1953).
[6] V. B. WIGGLESWORTH, Tijdschr. Entomol. *95*, 63 (1952).
[7] N. C. PANT and G. FRAENKEL, J. zool. Soc. India *6*, 101 (1954).
[8] L. TOTH, Tijdschr. Entomol. *95*, 43 (1952); Arch. Mikrobiol. *18*, 242 (1953).