

generating animals is seen in Figure 2. Attempts to use media of lower concentration (e.g. 11179 ohm-cm) were prevented by drastically increased mortality. At no time were measurements made on groups with any evidence of histolysis.

It appears that the organism attains a state of equilibrium with the medium, the required time varying inversely with the concentration of the medium. Regression analysis, employing the *t*-test, indicates electrolyte loss and external concentration are independent variables at better than the 5% level of significance. The lack of a linear relation between electrolyte exchange and concentration of the medium implicates physiological mechanisms rather than passive ones, in accord with earlier findings<sup>4</sup>. On the basis of the direction and magnitude of the electrolyte exchange, *D. dorotocephala* indicates itself to be in physiological ionic equilibrium with the medium when the latter has a specific resistance of just slightly less than 988.02 ohm-cm.

The fact that regenerating animals attain a state of ionic equilibrium with the external environment implies that the internal resistance remains constant once this state has been reached. Also, it is implied that the in-

ternal resistance varies with the specific resistance of the medium, although to a lesser degree (compare with the conclusions of MARSH<sup>5</sup>).

**Zusammenfassung.** Der Elektrolytaustausch von vollständigen und regenerierenden *Dugesia dorotocephala* erweist sich als weitgehend unabhängig von der Konzentration im äusseren Medium. Die regenerierenden Tiere befinden sich in einem physiologischen, ionalen Gleichgewicht, wenn der spezifische Widerstand des Mediums etwas unterhalb 988,02 Ohm-cm liegt.

S. J. COWARD<sup>6</sup>

Department of Zoology, State University of Iowa, and  
Department of Zoology, University of California, Davis  
(U.S.A.), November 13, 1963.

<sup>4</sup> E. F. ADOLPH and P. E. ADOLPH, J. exp. Zool. 43, 105 (1925).

<sup>5</sup> Acknowledgment. The author is indebted to Prof. G. MARSH for his comments and discussions.

<sup>6</sup> Present address: University of California.

## Influence of the Transport of Amino Acids on Glucose and Sodium Transport Across the Small Intestine of the Albino Rat Incubated *in vitro*<sup>1</sup>

Previous investigations<sup>2-4</sup> have already shown that the absorption of glucose into the epithelial cells of the intestine does not seem to be correlated with the transport of sodium and that, on the contrary, the transport of glucose into the serosal side is closely linked with the transport of sodium.

As in a previous report<sup>4</sup> we shall call the material no longer recovered in the mucosal fluid 'absorbed substance'; the substance appearing in the serosal side will be called 'transferred' or 'transported substance'.

The present paper concerns the behaviour of the transport of the amino acids (L-alanine, L-valine, L-phenylalanine) with reference to the transport of glucose and to the transport of sodium.

Albino male rats (Wistar strain) weighing about 250 g were used. Under barbiturate anaesthesia the small intestine was removed and part of the jejunum about 20 cm

<sup>1</sup> This work has been supported by a research grant of the Consiglio Nazionale delle Ricerche, Roma.

<sup>2</sup> C. LIPPE, S. ROSSI, and V. CAPRARO, Boll. Soc. Ital. Biol. sper. 38, 956 (1962).

<sup>3</sup> S. ROSSI, C. LIPPE, and V. CAPRARO, Exper. 18, 325 (1962).

<sup>4</sup> V. CAPRARO, A. BIANCHI, and C. LIPPE, Exper. 19, 347 (1963).

Incubating mucosal fluid		Na net transfer $\mu\text{E g}^{-1} \text{ h}^{-1}$	Glucose absorbed $\mu\text{M g}^{-1} \text{ h}^{-1}$	Glucose transfer $\mu\text{M g}^{-1} \text{ h}^{-1}$	Amino acid absorbed $\mu\text{M g}^{-1} \text{ h}^{-1}$	Amino acid transfer $\mu\text{M g}^{-1} \text{ h}^{-1}$
Na conc.	143.5 mE/l	$182.0 \pm 15.5$	$145.7 \pm 8.4$	$37.4 \pm 3.4$		
+ glucose	13.9 mM/l					
n = 16						
Na conc.	143.5 mE/l	$242.7 \pm 23.9$	$104.6 \pm 10.6$	$29.9 \pm 4.6$	$112.8 \pm 11.3$	$56.1 \pm 7.9^a$ n = 12 $58.1 \pm 8.9^b$ n = 4
+ glucose	13.9 mM/l					
+ L-alanine	20 mM/l					
n = 12						
Na conc.	143.5 mE/l	$252.5 \pm 17.2$	$143.7 \pm 15.2$	$29.0 \pm 3.7$	$61.2 \pm 12.5$	$42.8 \pm 2.7^a$ $49.2 \pm 3.0^b$
+ glucose	13.9 mM/l					
+ L-valine	20 mM/l					
n = 12						
Na conc.	143.5 mE/l	$307.1 \pm 33.4$	$130.7 \pm 12.9$	$41.6 \pm 7.0$	$135.5 \pm 10.5$	$34.6 \pm 4.6^a$ $40.7 \pm 4.9^b$
+ glucose	13.9 mM/l					
+ L-phenylalanine	20 mM/l					
n = 12						

<sup>a</sup> = According to the method of MOORE and STEIN<sup>5</sup>. <sup>b</sup> = According to the chromatographic method<sup>6</sup>. The number of experiments (n) and the mean values  $\pm$  S.E., referred to 1 g fresh weight and 1 h, are reported.

from the pylorus was isolated. The isolated intestine was everted according to the technique described by WILSON and WISEMAN<sup>5</sup> and incubated *in vitro* for 1 h and 30 min at a temperature of 28°C in 50 ml of KREBS-HENSELEIT<sup>6</sup>

solution gassed with 5% CO<sub>2</sub> in 95% O<sub>2</sub>. To the mucosal incubation fluid, glucose at a concentration of 13.9 mM/l (control experiments) or, together with glucose, an amino acid (L-alanine, L-valine or L-phenylalanine) at a concentration of 20 mM/l were added. Before tying off the sac of the everted intestine, 0.3 ml of Krebs-Henseleit solution was introduced. To this solution the amino acid alone was added at the same concentration as in the mucosal perfusion fluid. The intestinal sac was weighed at the beginning and at the end of the experiment, as well as at the end of the experiment after its draining.

At the end of the experiment, the transport of sodium was determined in the serosal fluid by means of an 'Optica CF 4' flame spectrophotometer ( $\lambda = 589 \text{ m}\mu$ ).

The glucose disappeared from the mucosal fluid and the glucose appearing in the serosal fluid were determined according to the method of KING<sup>8</sup>.

The L-alanine, L-valine and L-phenylalanine disappeared from the mucosal fluid ('absorbed amino acids') were always determined according to the method of MOORE and STEIN<sup>9</sup>. The transferred L-alanine, L-valine and L-phenylalanine were determined by means of a column chromatography on ion exchange resins employing an automatic amino acid analyser of high precision<sup>10</sup>.

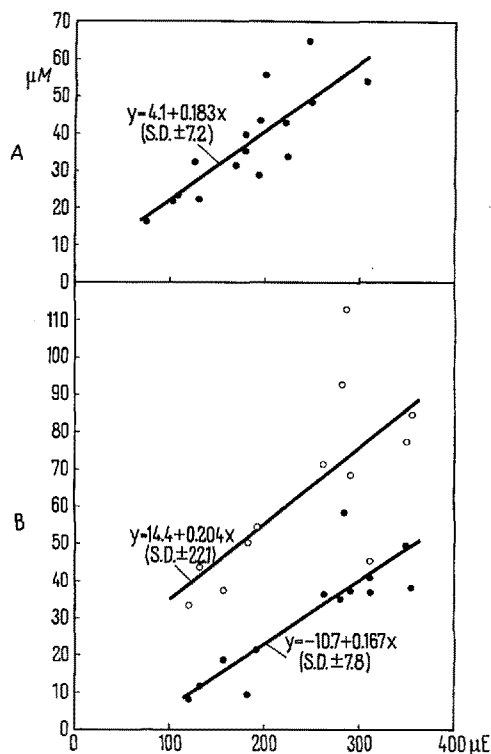


Fig. 1. Abscissa: Na net transport  $\mu\text{E g}^{-1} \text{ h}^{-1}$ . Ordinata: A - glucose transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  when glucose alone is present in the mucosal fluid. B - glucose transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  (●) and L-alanine transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  (○). Regression lines and standard deviation (according to CROXTON<sup>7</sup>) are reported.

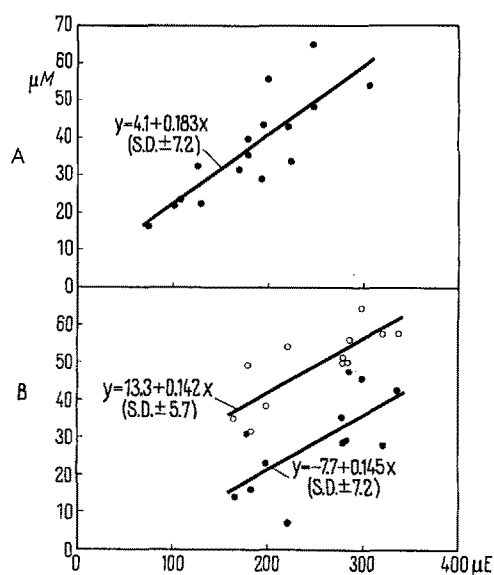


Fig. 2. Abscissa: Na net transport  $\mu\text{E g}^{-1} \text{ h}^{-1}$ . Ordinata: A - glucose transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  when glucose alone is present in the mucosal fluid. B - glucose transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  (●) and L-valine transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  (○). Regression lines and standard deviation (according to CROXTON<sup>12</sup>) are reported.

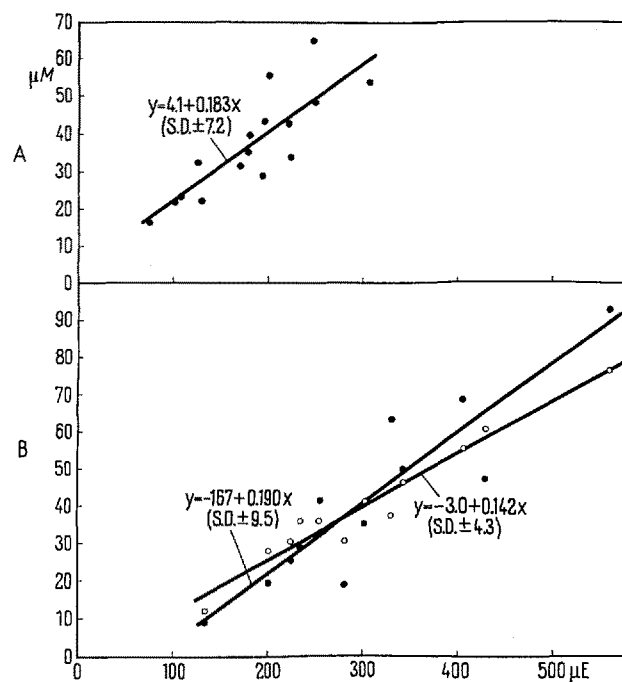


Fig. 3. Abscissa: Na net transport  $\mu\text{E g}^{-1} \text{ h}^{-1}$ . Ordinata: A - glucose transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  when glucose alone is present in the mucosal fluid. B - glucose transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  (●) and L-phenylalanine transport  $\mu\text{M g}^{-1} \text{ h}^{-1}$  (○). Regression lines and standard deviation (according to CROXTON<sup>7</sup>) are reported.

<sup>5</sup> T. H. WILSON and C. WISEMAN, J. Physiol. 123, 116 (1954).

<sup>6</sup> H. A. KREBS and K. HENSELEIT, Z. physiol. Chem. 210, 33 (1932).

<sup>7</sup> F. E. CROXTON, Elementary Statistics (Dover Publications Inc., New York 1959).

<sup>8</sup> E. Y. KING and I. D. P. WOOTTON, Microanalysis in Medical Biochemistry (Churchill, London 1956).

<sup>9</sup> S. MOORE and W. H. STEIN, J. biol. Chem. 211, 907 (1954).

<sup>10</sup> K. HANNIG, Clin. chim. Acta 4, 51 (1959).

The Table shows the results we obtained. It is interesting to observe that the transport of sodium is considerably enhanced by the addition of the amino acids to the glucose-Krebs solution. Among the tested amino acids, L-phenylalanine is presumably not metabolized by the intestinal tissue<sup>11</sup>.

Recently it has been reported that also sugars which are not metabolized but actively transported increase the active transport of sodium across the intestinal wall<sup>12</sup>. Perhaps any actively transported substance increases the transport of sodium. This is also supported by the observations that under the conditions we tested, there exists a linear correlation between sodium transport and glucose transport as well as amino acid transport (Figures 1–3).

However, with a given amount of Na transported, the amount of transferred glucose is less when an amino acid is transported at the same time than when glucose is the only substance transferred. Therefore, the correlation between sodium transport and glucose transport does not seem to be strictly chemical in nature. The existing correlation may simply be due to the fact that in all cases the transport of sodium is a linear function of the free energy at the disposal of the intestinal epithelium, but the coefficient of proportionality is different according to the number of substances that are available for transport. More precisely, the percentage of the total available energy employed for the transport of sodium seems to be smaller

the more the number of the transported substances increases.

The Table also shows that the absorption of glucose is smaller when L-alanine is present than when the amino acid is absent. Presumably L-alanine replaces in a more or less quantitative way the glucose as a supplier of the energetic requirements of the intestinal mucosa.

*Riassunto.* Proseguendo precedenti ricerche si mette in evidenza che esiste una correlazione anche tra trasporto di sodio e trasporto di vari amino acidi attraverso l'intestino tenue isolato di ratto albino. Se ne deduce che presumibilmente ogni sostanza capace di essere trasportata attivamente stimola il trasporto di sodio e che quest'ultima funzione diventa solo un indice dell'attività totale di trasporto dell'epitelio intestinale.

G. ESPOSITO, A. FAELLI, and V. CAPRARO

*Istituto di Fisiologia Generale dell'Università di Milano (Italy), October 10, 1963.*

<sup>11</sup> R. P. SPENCER and A. H. SAMIY, *Amer. J. Physiol.* **200**, 501 (1961).

<sup>12</sup> S. G. SCHULTZ and R. ZALUSKY, *Biochim. biophys. Acta* **71**, 503 (1963).

### Examinations of the Bactericidal Properties of the Serum Against Gram-positive Microbes in Patients Suffering from Leukaemia, Malignant Lymphoma, Myeloma and Myelofibrosis

Much less attention has been paid to the study of bactericidal properties of the serum against gram-positive microbes than to the study of bactericidal properties of the serum against gram-negative microbes (system of properdin-complement). A factor responsible for the bactericidal properties of the serum against gram-positive microbes has so far been studied to some extent<sup>1–6</sup>. There were various synonyms used for this factor, most frequently that of  $\beta$ -lysin. In accordance with STERZL's<sup>5</sup> view, we prefer to use the term bactericidin, which was coined previously by MACKIE and FINKELSTEIN<sup>2</sup>.

We examined the bactericidal capacity of the serum against gram-positive microbes in 137 patients suffering from haemoblastoses. Some of the patients were examined twice in an interval of 1 month. The group comprised 23 patients with acute leukaemia, 21 patients with chronic myeloid leukaemia, 23 with chronic lymphadenosis, 26 with Hodgkin's disease, 20 with reticulosarcoma along with lymphosarcoma, 10 with myeloma and 14 with myelofibrosis. The results were compared with a control group of 42 healthy subjects. *B. anthracoides* was used as testing microbe. A suspension of spores diluted in phosphate-saline containing a known number of spores (usually 50 000) was placed in 1 ml of saline and 1 ml of serum examined was added. This mixture was stirred and incubated at 37°C. At the end of 4, 6 and 24 h 0.1 ml of cultured mixture was pipetted to 4.9 ml of physiological saline and further diluted in geometrical series. From two adjoining test tubes containing dilutions presumed, by

experience, to contain countable numbers of cultured microbes, 0.5 ml were withdrawn and inoculated in 2 agar plates. The plates were dried and were incubated for 24 h at 37°C. Colony counts were then referred to the amount contained in 1 ml of serum. The mean value of both the results was taken. The bactericidin values were expressed by the index  $X_h/X_0$ , where  $X_h$  gave the number of microbes after incubation with serum at the end of the respective hour  $h$ , and  $X_0$  the original germ count entering the reaction. The more powerful the bactericidal effect of the serum, the more inhibited was the growth of the microbes, and the value of the index decreased. The highest bactericidal effect was observed in the first hours; after 24 h it was in most cases no longer notable.

*Results.* A statistically significant increase in the mean of bactericidin, i.e. lower values of bactericidal index, was found in almost all diagnostic groups when compared with a control group of healthy subjects. Only in chronic lymphatic leukaemia was the mean value of bactericidin not increased (Table I and II, Figure 1 and 2).

In most of the patients exhibiting increased bactericidin levels, no relation of the activity of the haemoblastic process to therapy, presence of an associated infectious process of bacterial origin, overall leukocyte counts or to abnormalities of differential leukocyte counts

<sup>1</sup> J. LIBÁNSKÝ and Z. JEŽKOVÁ, *Neoplasma*, in press.

<sup>2</sup> T. J. MACKIE and M. H. FINKELSTEIN, *J. Hyg.* **32**, 1 (1932).

<sup>3</sup> Q. N. MYRVIK, *Ann. N.Y. Acad. Sci.* **66**, 391 (1956).

<sup>4</sup> R. C. SKARNES and D. W. WATSON, *J. exp. Med.* **104**, 829 (1956).

<sup>5</sup> J. ŠTERZL, A. LANČ, and Z. TRNKA, *Čs. Mikrobiologie* **3**, 348 (1958).

<sup>6</sup> J. ŠTERZL, J. KOSTKA, and A. LANČ, *Folia mikrobiol.* **4**, 280 (1959).