worden war. Da 1 Tier keine, 4 Tiere eine geringgradige und nur 1 Tier eine etwas stärkere Fettaufnahme zeigten, nehmen wir an, dass die tagesrhythmischen Schwankungen nur zum Teil von der unterschiedlichen chemischen Zusammensetzung des Darmsaftes abhängen. In erster Linie dürften hierfür Leistungsunterschiede der Enterocyten selbst verantwortlich sein. Ungeklärt ist, ob die Schwankungen der Fettresorption auf Unterschiede in der Geschwindigkeit der Fettaufnahme oder der Resynthese der aufgenommenen Fette zurückzuführen sind[2].

Summary. The present light-microscopic study shows that, in the upper jejunum of the rat, fat absorption varies considerably in the 24 h period. The highest absorption was found at 02.00 h, the lowest at 14.00 h.

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3. Mai 1971.

[2] Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.

Tris as a Blocking Agent of the Proton-Sensitive Component of the Sodium Efflux in Barnacle Muscle Fibers

There is evidence that the mechanism of the Na efflux in barnacle muscle fiber consists of two components, one which is ouabain-sensitive and the other which is dilantin-sensitive[1]. In addition, there is evidence that the dilantin-sensitive component and the proton-sensitive component are the same[2]. However, the mechanism by which acidification of the external medium causes stimulation of the Na efflux has not yet been elucidated. One possibility considered by us is that the effect of protons on the Na efflux is dependent on the presence of bicarbonate in the bathing medium. This paper describing experiments which are based on the use of *Tris* as buffer brings forward evidence that *Tris* is an inhibitor of the proton-sensitive component of the Na efflux, and that in the absence of bicarbonate this component is not stimulated by acidification of the bathing medium.

Single muscle fibers measuring about 5 cm in length and 1.3 mm in width were isolated by dissection from specimens of *Balanus nubilus* or *aquila*. They were then cannulated and loaded with ^{22}Na by microinjection in the same way as *Maia* muscle fibers, using a modified Hodgkin and Keynes type of microsyringe (see Caldwell and Walster[3]). The procedure used for counting the activity of the wash-out specimens and the activity remaining in the fiber at the end of each experiment was basically that described by Bittar, Caldwell and Lowe[4].

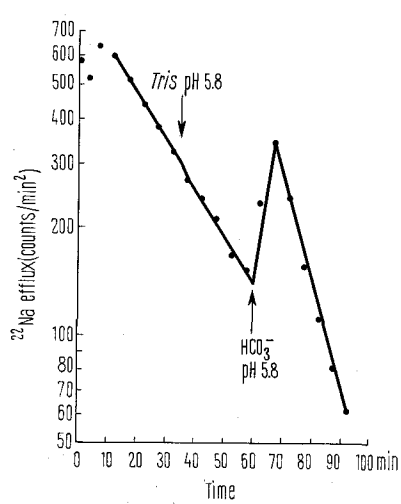

Fig. 1. Semilog plot showing the effect on Na efflux of external application of 3 m*M Tris* at pH 5.8, followed by replacing *Tris* with HCO_3^- as the buffer. Temperature 22–23 °C.

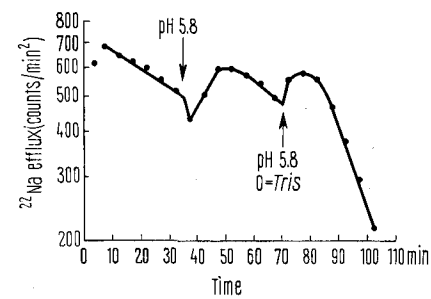

Fig. 2. The effect of reducing the external pH to 5.8 on Na efflux in artificial sea water containing at first 3 m*M Tris* (and HCO_3) and then, no *Tris*. Temperature 22–23 °C.

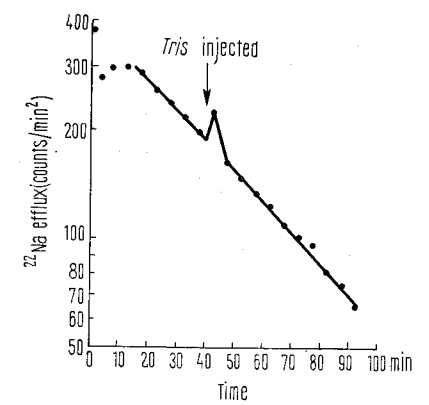

Fig. 3. Lack of effect on Na efflux of internal application of a 2.5 *M Tris* solution at pH 7.5. Temperature 22–23 °C.

[1] B. G. Danielson, E. E. Bittar, S. Chen and E. Y. Tong, Life Sci. *10*, 437 (1971).
[2] B. G. Danielson, E. E. Bittar, S. Chen and E. Y. Tong, Life Sci., in press (1971).
[3] P. C. Caldwell and G. E. Walster, J. Physiol., Lond. *169*, 353 (1963).
[4] E. E. Bittar, P. C. Caldwell and A. G. Lowe, J. mar. biol. Ass. U.K. *47*, 709 (1967).

^{22}Na in aqueous solution (SKS 1) was supplied by Amersham/Searle Corporation. This material was evaporated, and then made up in distilled water so that volumes of circa 0.1 µl gave about 50,000 cpm. The composition of the sea water was that given by BITTAR and TONG[5]. *Tris*-HCl was supplied by Sigma Chemical Company.

The experiments done by us fall into 2 groups. The first group of experiments was designed to see whether the response of the Na efflux to acidification of a bicarbonate-free medium, but using *Tris* as buffer, differs from that observed when using both *Tris* and bicarbonate. The second group of experiments was carried out by microinjecting *Tris* so as to substantiate the view that the main site of action of *Tris* following acidification of the bathing medium is the external surface of the fiber membrane.

Figure 1 illustrates the effect on Na efflux of adding protons to the bathing medium when only *Tris* is present (4 experiments). It can be seen that instead of there being stimulation there was a slight step-down in the rate of Na efflux. Furthermore, on replacing *Tris* with HCO_3- there was at once a marked rise in the loss of Na. The question then was whether the blocking action of *Tris* could be reversed by having HCO_3- in the bathing medium. Shown in Figure 2 is that acidification of the bathing medium in presence of 3 *mM Tris* and HCO_3- led to a sudden but the transitory fall in the Na efflux, followed by a rather substantial increase in the Na efflux. Also shown is that removal of *Tris* from the medium was found to bring about stimulation of the Na efflux (4 experiments). The fact that the response of the pump to *Tris* removal was prompt was taken as an indication that the point of action of the buffer was on the external surface of the fiber membrane. To verify this inference, a series of experiments were done involving the microinjection of *Tris*. In the first 3 experiments the external pH was not changed. As illustrated by Figure 3, the internal application of 2.5 *M Tris* solution at pH 7.5 failed to modify the Na efflux into artificial sea water at pH 7.8. However, this was not the case when the external pH was 5.8 (3 experiments). Such an experiment is recorded in Figure 4, where one can see that an injection of 2.5 *M Tris* at pH 7.5 caused a moderate fall in the Na efflux. A similar

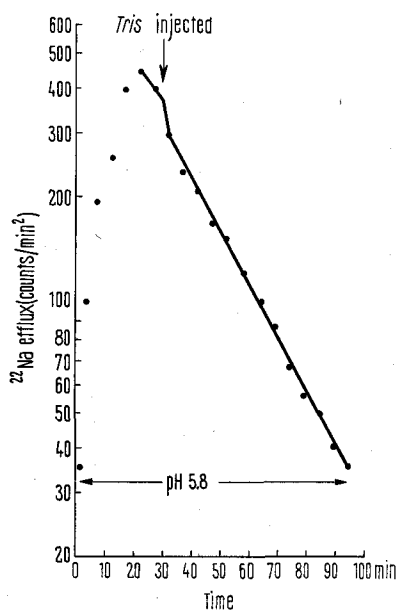

Fig. 4. The inhibiting effect of internal application of a 2.5 *M Tris* solution at pH 7.5. Temperature 22–23 °C.

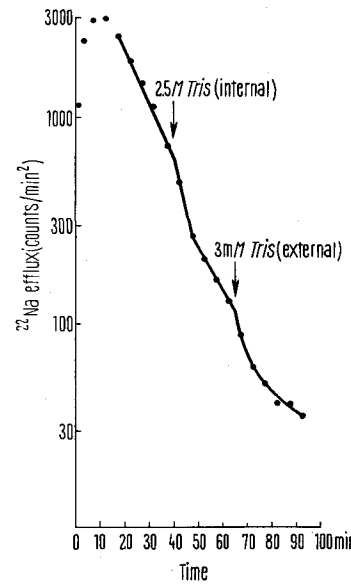

Fig. 5. The inhibiting effect of an internally applied 2.5 *M Tris* solution at pH 6.5, followed by externally applied 3 m*M Tris*. pH of artificial sea water throughout the experiment was 6.3. Temperature 22–23 °C.

reduction took place when the external pH was 6.3 (2 experiments). But as shown in Figure 5, there was a further and greater fall in the Na efflux when 3 *mM Tris* was added to the bathing medium. These results are interpreted as indicating that the principal site of action of *Tris* is the external side of the sarcolemma.

The present experiments have led to good evidence that the stimulating effect of acidification on the Na efflux in barnacle fibers is attributable to the H_2CO_3-HCO_3 system. Knowing from previous studies that changes in external HCO_3^- concentration do not modify the Na efflux, it would appear that CO_2 alone or pH and CO_2 are the cause of the large rise in Na efflux. The puzzling feature of these experiments is the inhibiting action of *Tris* on the pH-sensitive component of the Na efflux. Its interpretation is not straightforward. But what is tolerably clear is that studies involving the use of *Tris* rather than bicarbonate as buffer cannot give a true picture of membrane transport.

Zusammenfassung. Studien über den Natriumtransport in den Muskeln von *Balanus sp.* haben bewiesen, dass *Tris* den protonempfindlichen Anteil der Natrium-Pumpe verlangsamt und das *Tris* hauptsächlich an der Aussenseite des Sarkolemms aktiv ist.

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Department of Physiology, University of Wisconsin, Madison (Wisconsin 53706, USA), 15 June 1971.

[5] E. E. BITTAR and E. TONG, Life Sci. *10*, 43 (1971).

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