lons of the exposed animals collected more dye on the average than did the colons of the controls. These differences of average values were significant at the 5% level of confidence under the null-hypothesis that no difference of averages would exist.

In sum, gastrointestinal transit data is in agreement with small intestinal motility patterns obtained from rats which were exposed to a high frequency electric field. The hypomotility and low responsiveness to acetylcholine which is manifested by isolated strips of large intestine from exposed animals is consistent with the observation that no animals exposed to these general radio-frequency parameters was ever observed to exhibit diarrhea. It is felt that these data definitely constitute the first demonstrable evidence warranting the inference of a possible relationship between exposure to a high-frequency electric field and gastrointestinal effects. No speculation upon the mechanistic aspect of this relationship is warranted at this time.

Zusammenfassung. Nachweis, dass die Exposition von männlichen Sprague-Dawley-Ratten im hochfrequenten elektrischen Feld zu Veränderungen der gastro-intestinalen Motilität führt.

M. F. TANSY, F. M. KENDALL, J. CHRYZANOWSKI, F. J. HOHENLEITNER and A. R. KALL[3]

Department of Physiology
Temple University Schools of Dentistry,
Pharmacy and Allied Health Professions,
Philadelphia (Pennsylvania 19140, USA),
22 March 1971.

[3] Present address: Ark Electronics, Willow Grove, Pennsylvania.

Sensitivity of Sodium Efflux in Barnacle Muscle Fibers to the Microinjection of Calcium Chloride

In the course of an enquiry into the behavior of Na efflux in barnacle muscle fibers bathed in artificial sea water it was found that the extrusion of sodium was greatly stimulated by an injection of $CaCl_2$. It was concluded from this that the Na efflux mechanism consists of a component which is Ca^{2+}-sensitive. However it was unclear whether injections of $CaCl_2$ would bring about a similar effect on Na efflux into K-free artificial sea water. In the present experiments, therefore, an attempt was made to uncouple the Na-K transport system of these fibers before microinjecting a solution of $CaCl_2$.

These experiments were done using single muscle fibers isolated by dissection from the depressor muscle bundles of the barnacle *Balanus nubilus* (or *B. aquila*). The fibers were cannulated in the same way as *Maia* fibers and then loaded with ^{22}Na by means of a HODGKIN and KEYNES[1] microinjector as modified by CALDWELL and WALSTER[2]. The injector discharged ca. 0.1 µl of test fluid/cm of micromanipulator. The experiments were done in artificial sea water as the bathing medium, the composition of which was: (mM) NaCl 465, KCl 10, $MgCl_2$ 10, $CaCl_2$ 10, $NaHCO_3$ 10, pH 7.8 and the temperature of this medium was between 22 and 23°C. K-free artificial sea water solutions were prepared by substituting NaCl in equimolar amount.

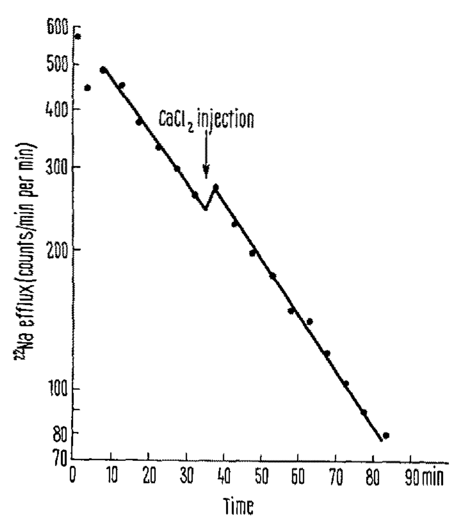

Fig. 1. The behavior of Na efflux from a barnacle fiber in artificial sea water before and after internal application of 1 M $CaCl_2$, plotted semilogarithmically.

The activity of ^{22}Na in the washings and the activity in the fiber remaining at the end of each experiment were measured using the procedures described by BITTAR[3] and BITTAR, CALDWELL and LOWE[4]. ^{22}Na (Code SKS. 1) was obtained from Amersham-Searle Corporation.

The effect on Na efflux of injecting a solution of 1 M $CaCl_2$ into barnacle fibers is illustrated by Figures 1 and 2. In the first Figure the Na efflux is shown to be relatively insensitive to a sudden rise in the sarcoplasmic free Ca^{2+} concentration. In the second figure however the Na efflux is shown to be greatly stimulated by a sudden rise in the internal free Ca^{2+} concentration. Also shown is that contraction (see legend) in itself leads to a slight increase in the Na efflux. This could be merely the result of the squeezing of some radiosodium out of the T-system.

The effect of K removal on the Na efflux and of injecting 1 M $CaCl_2$ on the residual efflux is illustrated in Figures 3 and 4. One can see that the removal of K ions from the bathing medium caused a large reduction in the loss of radiosodium. Furthermore, injection of 1 M $CaCl_2$ is shown to result in a rise in the Na efflux, the magnitude of the effect being quite great in Figure 4. This pattern of behavior parallels that of fibers injected with concentrations of $CaCl_2$ as low as 1 mM. The significance of this comparison lies in the fact that an initial

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[2] P. C. CALDWELL and G. E. WALSTER, J. Physiol., Lond. *169*, 353 (1963).
[3] E. E. BITTAR, J. Physiol., Lond. *187*, 81 (1966).
[4] E. E. BITTAR, P. C. CALDWELL and A. G. LOWE, J. mar. biol. Ass. U.K. *47*, 709 (1967).

sarcoplasmic Ca^{2+} concentration following injection of $4 \times 10^{-6} M$ (the dilution factor being 250-fold) is not very different from the threshold value for inducing contraction in barnacle fibers (see for example ASHLEY[5]).

The demonstration of a Ca^{2+}-sensitive component of the Na efflux and its activation by raising the internal Ca^{2+} concentration in the absence of external K ions is not altogether surprising in view of the observation of BITTAR and TONG[6] that lowering the external pH below 6.8 always stimulates the Na efflux into a K-containing or K-free medium. It thus seems not unreasonable to deduce that this part of the Na pump which is Ca^{2+}-sensitive on the inner side of the plasma membrane might be that which is sensitive to acidification of the bathing medium. That this is so is confirmed by more recent experiments in which the Na^+-K^+ ATPase system of barnacle fibers had been inactivated by applying ouabain. Both $CaCl_2$ injections and lowering the external pH were found to cause a large rise in the Na efflux (DANIELSON, BITTAR, TONG and CHEN, unpublished results). The significance of this ouabain-insensitive component and its newly discovered properties seems unmistakable. Whether this component is situated in the T-system and whether it is closely connected to the mechanism of excitation-contraction coupling are questions which await elucidation[7].

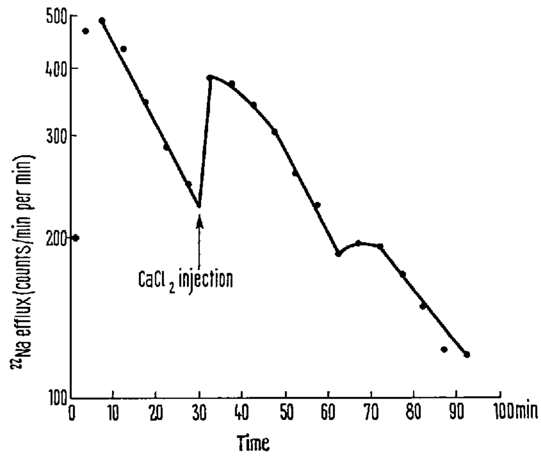

Fig. 2. To illustrate the marked stimulation by injected $1 M$ $CaCl_2$ of Na efflux from another barnacle fiber. The further rise in Na efflux occurring some 30 min affter injection was associated with more severe contraction. Thus, the increase in Na loss may have been due to the squeezing out of a fraction of the radiosodium present in the T-system.

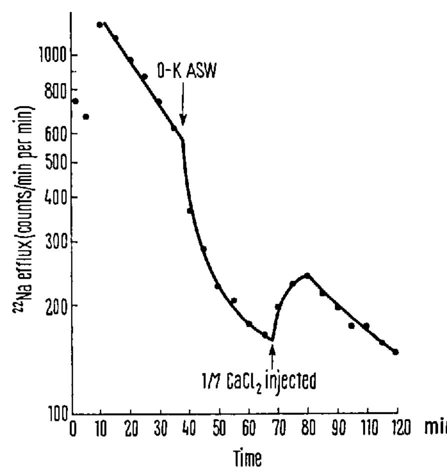

Fig. 4. The effect of injected $CaCl_2$ ($1 M$) on Na efflux from another barnacle fiber, following the removal of K ions from the bathing medium.

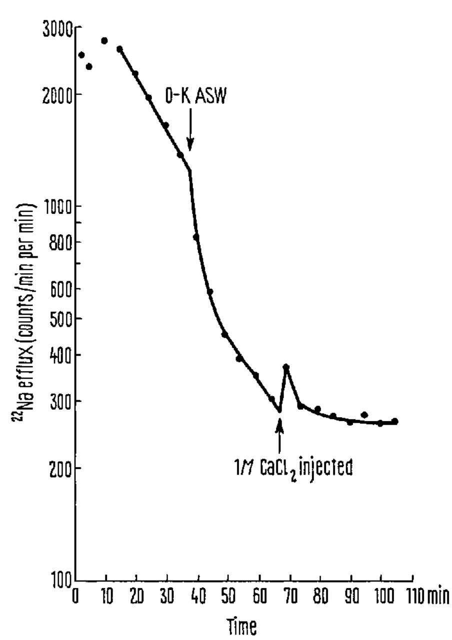

Fig. 3. The effect of injected $CaCl_2$ ($1 M$) on Na efflux following the removal of K ions from the bathing medium.

Zusammenfassung. Nachweis, dass der Auswärtsstrom von Natrium aus Muskelfasern der Arthropoden *Balanus nubilus* und *Balanus aquila* in künstlichem, kaliumfreiem Meerwasser durch Mikroinjektion von Kalziumchlorid aktiviert wird.

E. E. BITTAR, B. G. DANIELSON[8], E. TONG[9] and S. CHEN

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

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[8] Visiting Scientist of the American Heart Association. Permanent address: Institute of Physiology and Medical Biophysics, University of Uppsala, Uppsala (Sweden).

[9] Postdoctoral Fellow of the Wisconsin Heart Association.