nearverstärker, Ratemeter, Impulszähler und Hochspannungsteil) betrieben, hierbei wird der Anschlußstecker (S) mit dem Vorverstärker des Zählgerätes verbunden.

Die Eingangsempfindlichkeit am Linearverstärker betrug 400 mV. Die Arbeitsspannung liegt bei 3,0 kV. Die Lage des Zählrohres (horizontal, vertikal) hat keinen Einfluss auf die Messeigenschaften.

Die Nachweisempfindlichkeit, bei stationärem Betrieb, beträgt 10 pCi bei einem Nulleffekt von 17 ipm. Die Messkammer wurde ohne Störung bei einer Durchflussmenge von 100 ml/min betrieben.

Unser Zählrohr kann nach Kontamination leicht zerlegt und gereinigt werden. Vorwiegend bei Gärversuchen zeigte sich nach Versuchsende ein Memory-Effekt (durch höher siedende markierte Gärprodukte verursacht), der sich durch Auswaschen der Messkammer mit Aceton und Alkohol beseitigen lässt. Zur Vermeidung von Kondensationen (Wasser, Aromastoffe) in der Messkammer kann der obere Zählrohrteil (Figur A und B, o) beheizt werden. Die Heizelemente (H) bestehen aus Thermocoax-Draht (Fa. Philips). Sie sind in eingefrästen Nuten eingelassen und werden über einen regelbaren Heiztrafo (Bereich 0–20 V) ans Netz angeschlossen.

Anwendungsbeispiele. 1. Respirationsmessung bei Rebläusen (*Dactylosphaera vitifolii* Shimer)[6]. 95 Rebläuse (Durchschnittsgewicht 220 µg pro Laus) nehmen in einem speziellen Fütterungsgefäss durch eine Parafilm-Membran[7] hindurch 14C-Saccharose auf. Die kontinuierliche Aufzeichnung der im Atmungs-CO_2 auftretenden Aktivität zeigt, dass, bei 23 °C Versuchstemperatur, bereits $^1/_2$ Stunde nach Beginn der Fütterung $^{14}CO_2$ von den Rebläusen ausgeatmet wird. Ab der 4. bis 5. Stunde wird von den Rebläusen in der Zeiteinheit stets die gleiche Menge $^{14}CO_2$ ausgeatmet und nach etwa 27 Stunden wird kein $^{14}CO_2$ mehr abgegeben.

2. Messung der CO_2-Fixierung von Weinbeeren (Dunkelfixierung). Der Verlauf der CO_2-Aufnahme durch Weinbeeren (etwa 10 g Beeren) ist anhand der Abnahme des im Kreislauf vorhandenen $^{14}CO_2$ zu verfolgen. Die Versuche zeigten, dass schon nach 3–4 Stunden die Hauptmenge des CO_2 von den Beeren aufgenommen wurde. Nach etwa 20 Stunden hat sich ein Gleichgewicht eingestellt. Wie dieser Versuch zeigt, kann mit dem Zählrohr auch die Photosynthese von geringen Mengen pflanzlichen Materials (z. B. Keimlinge, Triebe usw.) vefolgt werden.

In weiteren Versuchen wurde die Abbaugeschwindigkeit von ^{14}C-Äpfelsäure durch säureabbauende Hefen der Gattung *Schizosaccharomyces* und *Saccharomyces* verfolgt.

Summary. A fluid-measuring tube with separate cell measuring and counting chamber, is described with which metabolic transposition of small amounts of biological materials can be examined within an enclosed system. Tests were carried out on the course of the expiration of sucrose through phylloxers weighing 20 mg, and also on the fixation in the dark of CO_2 by 8 green grapes. Tests were also carried out on the expiration of sucrose and malic acid by yeast in fermentation. The course of metabolism can easily be seen by means of the radioactivity curves.

A. Rapp, H. Steffen, H. Ullemeyer und G. Rilling

Bundesforschungsanstalt für Rebenzüchtung, Geilweilerhof, D-6741 Siebeldingen über Landau/Pfalz (Deutschland), 7. Oktober 1971.

[6] G. Rilling und H. Steffan (unveröffentlicht).

[7] T. E. Mittler und R. H. Dadd, Nature, Lond. *195*, 404 (1962).

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An Appraisal of the Microinjection Technique Used in Investigating Sodium Efflux in Barnacle Muscle Fibers

Recent work by us on single barnacle muscle fibers loaded with radiosodium by microinjection has shown that the Na efflux always follows an exponential course, but that the rate constants plotted against time on a linear scale are seldom a constant. The question then asked was whether a declining rate constant for Na* loss was the result of gradual sequestration of a fraction of the microinjected ^{22}Na or the result of an extraleaky fiber membrane associated with damage to the T-system caused by the microinjector. The simplest way of testing the latter possibility was to reinsert the microinjector into the fiber shortly after equilibration of the injected radiosodium and to see whether this maneuver brought about any change in the kinetics of the Na efflux. Control experiments along these lines were done, and the results obtained show quite clearly that insertion of an inner capillary 110 µm in diameter, as well as ejection of a 1 cm column of distilled water were without any significant effect on the Na efflux. However, experiments designed to see if deeper penetration into the fiber with the microinjector modifies the Na efflux were not then done. This paper is hence concerned with the problem of whether greater damage to the T-system would result in a noticeable dilution of the specific activity of the internal Na*.

The experiments were carried out using single muscle fibers of the barnacle *Balanus nubilus* or *B. aquila*. These fibers measured approximately 3 cm in length and 1.5 mm in diameter. The method of loading with ^{22}Na by means of a modified Hodgkin and Keynes type of microinjector, and counting the activity of the washing-out specimens and the activity remaining in the fiber at the end of each experiment were essentially those already described[1,2]. In some of the experiments a microinjector having an inner capillary 130 µm in diameter was used in order to produce more damage to the fiber. The procedure consisted in axial insertion of the microinjector at least $^1/_2$ h following full equilibration of the injected ^{22}Na with the internal free Na, and in discharging a 1 cm column of distilled water. This was repeated in a few experiments by first ejecting a 1 cm column of distilled water, followed 30 min later by ejecting a further 1 cm column of distilled water.

The object of the first series of experiments was to check the finding that the Na efflux is unaffected by microinjecting 0.1 µl of distilled water, using an inner capillary 110 µm in diameter. Figure 1 shows that this is so (10 experiments). One sees that a steady state was achieved a few minutes after loading the fiber with ^{22}Na and that reinsertion of the microinjector, as well as the discharge of a 1 cm column of distilled water, failed to modify the behavior of the Na efflux. From this it may be deduced that damage of the T-system is not great enough to lead to a noticeable increase in influx of Na or that the damage done to the

[1] E. E. Bittar, J. Physiol., Lond. *187*, 81 (1966).

[2] E. E. Bittar and E. Tong, Life Sci. *10*, 43 (1971).

T-system leads to rapid plugging of the involved tubules and clefts.

In the next 3 experiments an inner capillary 130 μm in diameter was twice reinserted into the fiber. The depth of each insertion was about 1 cm. As illustrated in Figure 2 the slope of the efflux curve shows no step-down following completion of the procedure of ejecting 0.1 μl of distilled water. This again lends credence to the view that there may be plugging by debris of tubules and clefts lying in the pathway of the microinjector. Supporting evidence comes from unpublished electron microscopic studies which show accumulation of membranous elements and glycogen in tubules and clefts in the immediate vicinity of the track left by the microinjector. The question arises: Would still greater damage to the T-system lead to a discernible extraleaky fiber membrane? To try and answer this, another series of 3 experiments were done. The fibers were loaded by means of a microinjector having an inner capillary 110 μm in diameter. Next, an inner capillary 130 μm in diameter was twice reinserted, first by penetrating to a depth of 1 cm, and then 2 cm. The results obtained show that even this amount of extra damage failed to modify the course of the Na efflux. The last 5 experiments done involved the reinsertion of an inner capillary 110 μm in diameter to a depth of 2 cm instead of 1 cm, followed by the ejection of a 1 cm column of distilled water. As illustrated by Figure 3, the fresh damage caused by introducing the microinjector more deeply into the fiber brought about no change in the behavior of the Na efflux.

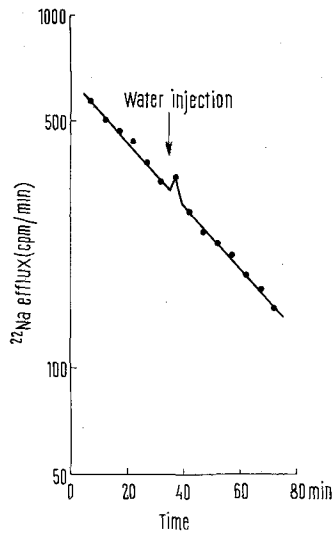

Fig. 1. Sodium efflux from a barnacle muscle fiber before and after the microinjection of 0.1 μl distilled water, plotted semilogarithmically. Inner capillary of microinjector measured ~110 μm in diameter. Temperature 22–23°C.

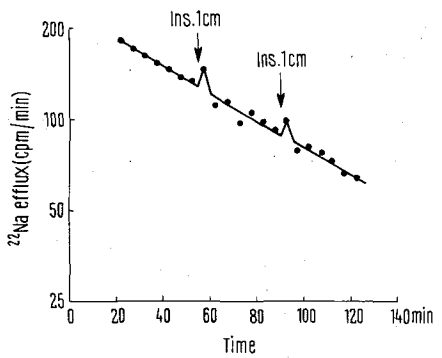

Fig. 2. Sodium efflux from a barnacle muscle fiber before and after a double injection with 0.1 μl of distilled water. Inner capillary of microinjecture measured ~ 110 μm in diameter. Temperature 22–23°C.

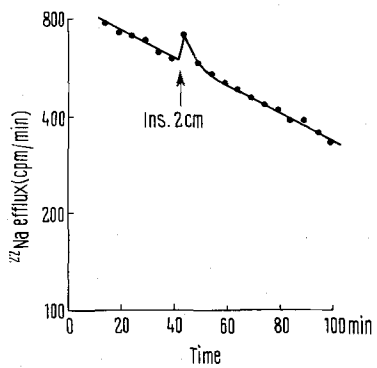

Fig. 3. Sodium efflux from a barnacle muscle fiber before and after penetration of the microsyringe to a 2 cm depth (and not 1 cm) and ejection of a 1 cm column of distilled water. The fiber used was 2.8 cm in length and 1.2 mm in diameter. Inner capillary of microinjector measured ~110 μm in diameter. Temperature 22–23°C.

The results described in this paper go a long way toward strengthening the view that the procedure of microinjection when properly used is in itself fairly benign. But they do not inform us why there is a slow decline in the rate coefficient for ^{22}Na loss in most of the barnacle fibers studied. One possible reason could be that the efficacy of the Na pump is gradually declining, so that the influx is greater than the efflux. That this is unlikely is suggested by the observation that acidification of the bathing medium causes a marked rise in the Na efflux, and by the observation that these fibers usually retain their marked sensitivity to K removal[3]. It is thus not easy to avoid the conclusion that the decline in rate coefficient is the result of the presence of a slowly exchanging Na fraction lying behind the inner membranes of these fibers. Attempts are now being made to mobilize this sequestered fraction of sodium[4].

Zusammenfassung. Die dargestellten Experimente zeigen, dass die Methode, welche durch Microinjection die Muskelfasern von Barnakel mit Na-22 belädt, keinen Schaden bewirkt, was die Efflux-Verhältnisse für Na anbelangt; zudem wird mit dieser Methode keine besonders durchlässige Muskelmembran erzeugt.

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11 October 1971.

[3] B. G. DANIELSON, E. E. BITTAR, S. CHEN and E. TONG, Life Sci. *10*, 437 (1971).

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