substrate liable to oxidation, such as succinate, glutamate, alanine, cytochrome c.

The manometric determination of O_2 consumption[16] showed a progressive increase of the $QO_2(N)$ from acid to alkaline pH.

We then considered the problem of the utilization of the energy freed from oxidative processes. The behaviour of oxidative phosphorylation and of ATPase was studied at various pH comprised between 6 and 8 according to the technique described in a previous paper[17]. It was observed that an increase in oxidation runs parallel to an increase in phosphorylation only at the pH between 6.6 and 7.4, so that at these pH the P/O ratio tends to be constantly coupled. At the pH from 7.4 to 8, the P/O ratio was more and more uncoupled. On the other hand, at the extreme pH of 6 and 8 the ATPase activity was greater.

It therefore appears that pH variations simultaneously control both the shape and enzymic activities of mitochondria.

Riassunto. È stato osservato che quando il pH intracellulare è più basso di quello plasmatico la forma dei mitocondri è prevalentemente a bastoncino, aumentando il pH (in condizioni sperimentali e patologiche) i mitocondri tendono a rigonfiare. Il fenomeno è anche dimostrabile spettrofotometricamente sui mitocondri isolati il cui rigonfiamento spontaneo regredisce abbassando il pH del mezzo. Il $QO_2(N)$ e l'attività ATPasica aumentano a pH alcalini parallelamente al rigonfiamento mitocondriale. Il rapporto P/O si mantiene accoppiato fra pH compresi fra 6.6 e 7.4.

È possibile che attraverso il pH si verifichi nella cellula un meccanismo di autoregolazione dell'attività funzionale dei mitocondri.

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[16] V. R. Potter in W. W. Umbreit, R. H. Burris, and J. F. Stauffer, *Manometric Techniques* (Burgess P., Minneapolis 1957).
[17] G. Nanni and A. Casu, Exper. *17*, 402 (1961).

Action Potentials of the Guinea Pig Heart in Sodium Deficient Solutions[1]

According to the 'sodium hypothesis' of electrical activity, the upstroke of the action potential depends on a substantial increase of the membrane permeability to sodium ions, allowing a positive charge to enter the cell[2]. Evidence in favour of the 'sodium hypothesis' is available for a variety of cardiac preparations: frog ventricle[3], sheep and calf ventricle[4], and mammalian Purkinje fibres[5]. The ventricle of the guinea pig, however, does not seem to follow this general rule. According to reports by two different groups[4,6], the amplitude of its action potential remains unchanged even if the sodium content of the bathing solution is reduced to zero. The present report is concerned with the effect of sodium-poor solutions on the *auricle* of the guinea pig.

Strips of the thin-walled right auricle, 8–10 mm in length and 2-4 mm in width, were immersed in a Tyrode bath and driven by a transistorized stimulator[7] at a rate of 50–100 per min. Ling–Gerard electrodes[8] were used to record from the inside of single fibres. Atrial fibres are relatively thin (diameter 8 μ[9]); therefore, it was difficult to change solutions and keep the tip within the same fibre until a new steady state was reached. Results were discarded if the action potential did not return to its original value upon return to normal Tyrode's solution.

Figure 1 illustrates the effects of reducing the sodium concentration from 100% to 27% (isosmotic saccharose was substituted for NaCl): the resting potential remained unchanged while the amplitude and duration of the action potential decreased. The upstroke velocity of the action potential, as seen on high speed records, decreased to about 30%. Reduction of the sodium concentration to zero resulted in a total loss of excitability.

Figure 2 is a plot of resting and action potential values as recorded with various extracellular sodium concentrations. The dashed line would be expected if the fibre membrane at the height of activity were exclusively

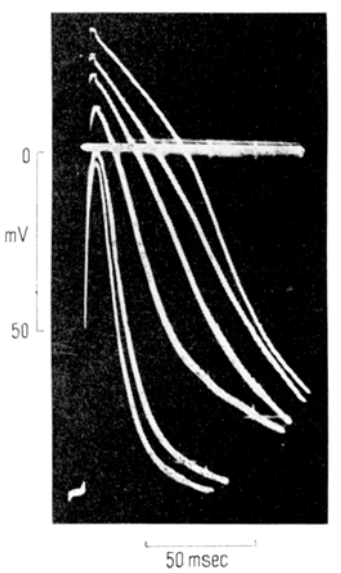

Fig. 1. Action potentials of the same auricular fibre, showing the effect of reducing the Na content of its Tyrode bath to 27%. Top trace: tissue in Tyrode's solution; lower traces: 4, 5, 10, 15 and 22 min after the change-over to Na poor solution. The rate of inflow was such that the bath was 50% exchanged at the end of 2 min.

[1] Aided by a grant from the Italian 'Consiglio Nazionale delle Ricerche'.
[2] A. L. Hodgkin, Proc. Roy. Soc. *B*, *148*, 1 (1957).
[3] A. J. Brady and S. W. Woodbury, J. Physiol. *154*, 385 (1960).
[4] J. Déléze, Circulation Res. *7*, 462 (1959).
[5] M. H. Draper and S. Weidmann, J. Physiol. *115*, 74 (1951).
[6] E. Corabœuf and M. Otsuka, C. R. Acad. Sci. Paris, *243*, 441 (1956).
[7] P. Colombino, A. Ferroni, and E. Meda, Arch. Physiol. *60*, 1 (1960).
[8] G. Ling and R. W. Gerard, J. cell. comp. Physiol. *34*, 383 (1949).
[9] E. Meda, Boll. Soc. Ital. Biol. sperim. *36*, 1167 (1960).

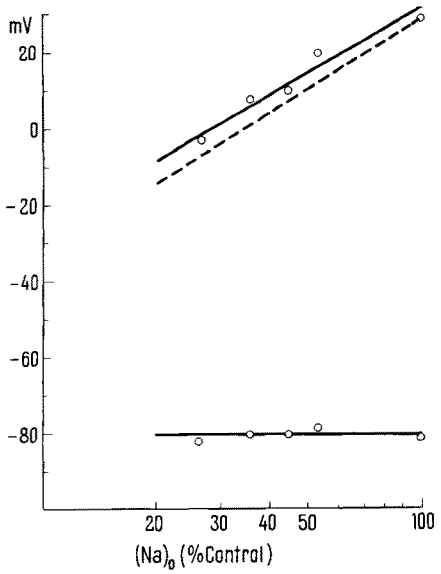

Fig. 2. Action potentials (top) and resting potentials (bottom) as a function of the extracellular sodium concentration. The dashed line is drawn through the mean overshoot of 29 mV with a slope equal to 61.5 mV/tenfold change of $[Na]_0$.

permeable to sodium ions. Clearly, the agreement between results and theory is as good as can be expected, thus justifying the conclusion that the atrium of the guinea pig heart, in contrast to the ventricle, follows the sodium hypothesis.

When the tissue was kept in a sodium-poor solution for more than 1 h, the resting potential dropped by 10 or 20 mV. This same effect has been reported for ventricular fibres of the guinea pig[4,6]; it may be due to a low intracellular sodium concentration, resulting in a decreased rate of sodium extrusion.

Zusammenfassung. Es werden Aktionspotentiale vom Meerschweinchenvorhof in Badelösungen verschiedener Na-Konzentrationen mittels intrazellulärer Elektroden registriert. Während das Aktionspotential des *Ventrikels* relativ unempfindlich gegen Veränderungen der Na-Konzentration bleibt[4,6], lässt sich die «Na-Hypothese» für den *Vorhof* anwenden.

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Heterophile Antigens in Ehrlich Ascites Tumor Cells

The study of the immunological reactions which can be obtained by injections of tumor cells has been, and remains, a matter of the utmost interest for several authors. As concerns the bibliography on this subject, we refer to the previous works made by one of us[1–4], while in the present research we wanted to investigate the possibility of seeking possible heterophile antigens of FORSSMAN[5,6] in common between normal and tumoral cells.

As we have not found in the ample bibliography of the Ehrlich Ascites Tumor[7–16] any reference to heterophile antigens with erythrocytes, either human or animal, we decided to investigate whether immune antisera for this tumor may contain antibodies capable of haemolyzing human and/or animal erythrocytes.

Materials and Methods. For our research we used ten normal rabbits of an average weight of about 2000 g each, divided into three groups. The first group, of four rabbits, was treated with eight intravenous injections of suspensions of living cells of Ehrlich Ascites Tumor, and further by three new call injections of cells, made about one month *after* the first treatment, for a total of eleven intravenous injections to each rabbit, corresponding to 30 millions of living cells. Suspensions of living cells were prepared according to the method described by ROSSI and DI VITA[17,18], while the living cells were tested by the method of NOVELLI[19]. The second group, of three rabbits, was treated by the same technique by injections of erythrocytes of normal albino mice of the same strain used for the transplantations of the Ehrlich Ascites Tumor, while the third group, of three rabbits, was treated by liver homogenate of albino mice. Activity of the immune antisera for Ehrlich Ascites Tumor cells was controlled *in vitro* by the method of LEE, RICHARDS, and KLAUSNER[20].

When immune antisera for Ehrlich Ascites Tumor cells were obtained, we investigated whether they could be haemolytic for human Blood Group 0 erythrocytes, as for mouse, rat, guinea-pig, sheep, ox and horse erythrocytes. 0.5 ml of decomplemented and diluted immune antisera were put with 0.5 ml of 10% suspension of erythrocytes and with 0.5 ml of fresh complement. Test tubes were put at + 37°C, and we read twice, after 30 and 60 min of incubation respectively; for each series of tests we always made a blank with normal rabbit sera. Table I

1 A. ZINNARI, Tumori *46*, 533 (1960).
2 A. ZINNARI, Tumori *47*, 76 (1961).
3 A. ZINNARI and F. PITTALUGA, Ital. J. Biochem. *4*, 221 (1961).
4 A. ZINNARI, Exper. *18*, 38 (1962).
5 J. FORSSMAN, Biochem. Z. *37*, 78 (1911).
6 U. DIANZANI, Exper. *12*, 462 (1956).
7 R. W. MCKEE, K. LONBERG-HOLM, and J. JEHL, Cancer Res. *13*, 537 (1953).
8 C. LANDSCHUTZ, Exper. *15*, 15 (1959).
9 S. TOLNAI, R. SCANTLAND, and J. F. MORGAN, Nature *193*, 591 (1962).
10 G. F. TOWNSED, J. F. MORGAN, and B. HAZLETT, Nature *183*, 1270 (1959).
11 G. F. TOWNSED, J. F. MORGAN, S. TOLNAI, B. HAZLETT, H. J. MORTON, and R. W. SHUEL, Cancer Res. *20*, 503 (1960).
12 J. F. MORGAN, S. TOLNAI and G. F. TOWNSED, Canad. J. Biochem. Phys. *38*, 597 (1960).
13 J. G. BENNETT, Nature *187*, 72 (1960).
14 J. B. NELSON and G. S. TARNOWSKI, Nature *188*, 866 (1960).
15 H. GOLDIE, M. WALKER, B. BISCOE, G. J. POWELL, and R. J. HOWSE, Proc. Soc. exp. Biol. Med. *107*, 838 (1961).
16 Symposium sponsored by the American Cancer Society, Cancer Res. *21*, 1165 (1961).
17 M. ROSSI and G. DI VITA, Boll. Soc. ital. Biol. sper. *36*, (1960).
18 G. DI VITA and M. ROSSI, Atti V° Congr. Nucl., Simp. Radiobiol. Med., Roma (1960).
19 A. NOVELLI, Exper. *18*, 295 (1962).
20 H. LEE, V. RICHARDS, and C. KLAUSNER, Cancer Res. *20*, 10, 1415 (1960).