by the two experimental factors on the total water content of the cells. The results show that the deviation B due to the presence or absence of potassium is greater than the deviation A due to the presence or absence of the $0.2M$ saccharose. From the results obtained it may be concluded that the potassium uptake by cells of *Staphylococcus aureus* is accompanied by a decrease of intracellular water. The explanation of this is related to transport mechanism and consequent on the chemicophysical state of the potassium of the cells. The concept of an active carrier transport of the potassium[7,8] is necessarily linked to that of free potassium in the cells, and in concentrations greater than external ones[9]. If the intracellular potassium were in the free state, in the tests carried out there would have been an increase in water in conjunction with the stated increase in the intracellular potassium; on the other hand, there would not have been variations in water content if the potassium were exchanged with other intracellular substances. The average quantity of potassium in excess which enters into the cells in comparison with the controls is about 0.75 m-equiv./g of dry cells; this amount, considering a water content in the cells in a free state, corresponds to a solution of about $1M$, which in our experimental conditions would cause an osmotic pressure of about 25 atmospheres. The external osmotic pressure, however, in the presence of only $0.2M$ potassium citrate or added to $0.2M$ saccharose, is about 5 and 10 atmospheres respectively.

All this is without calculating the other intracellular osmotically active solutes or substances. Even admitting that only 30% of the potassium is in the free state[10], the external osmotic pressure does not exceed the internal one. It is therefore logical to conclude that the potassium is not in a free state but adsorbed by means of bonds of various kinds[11] on dissociated groups of the polypeptide chains of the protoplasmatic gel, the hydration of which is modified. Groups of the polypeptide chain with anionic dissociation, in fact, are hydrated groups which repulse each other. When potassium is adsorbed, which balances the negative charge, the repulsion ceases, the distance between the polypeptide chains is reduced, and the quantity of free water decreased. Such an explanation is in agreement with the modern theories which relate the phenomena of selectivity and permeability to all cell protoplasm, the polyelectrolytic behaviour which is subject to ever more investigations[10,12,13].

Riassunto. Cellule di Stafilococco aureo in fase di crescita logaritmica incorporano potassio eliminando acqua. Lo spostamento dell'acqua intracellulare dipende più dalla incorporazione del potassio che dalla pressione osmotica del liquido di sospensione.

F. GALDIERO[14]

Istituto di Microbiologia, Università di Napoli (Italy), November 24, 1964.

[7] P. MITCHELL, in *Membrane Transport and Metabolism* (Ed. A. KLEINZELLER and A. KOTYK, 1960), p. 22.
[8] E. J. CONWAY, *Regulation of the Inorganic Ion Content of Cells*, Ciba Foundation N.5 (1960), p. 2.
[9] A. KEPES and G. N. COHEN, in *Bacteria* (Ed. I. C. GUNSALUS and R. Y. STANIER, 1962), vol. 4, p. 179.
[10] A. KATCHALSKY, Biophys. J. *4*, 9 (1964).
[11] S. A. RICE and M. NAGASAWA, *Polyelectrolyte Solutions* (Academic Press, New York 1961), p. 427.
[12] A. S. TROSHIN, in *Membrane Transport and Metabolism* (Ed. A. KLEINZELLER and A. KOTYK, 1960), p. 45.
[13] G. A. KURELLA, in *Membrane Transport and Metabolism* (Ed. A. KLEINZELLER and A. KOTYK, 1960), p. 54.
[14] This work was carried out with the help of a Grant of the Italian Research Council (C.N.R.).

The Effect of Serotonine and 5-Hydroxytryptophan on the Erythrophores of Crustaceans

The colour pattern of the prawns *Palaemon serratus* (Pennant) and *Palaemon elegans* Rathke is composed of various types of chromatophores. Of these, two kinds of red pigmented cells, the large and small erythrophores, are the most numerous. Both species of prawns possess characteristic dark bands on the cephalothorax and abdomen. These consist purely of large erythrophores, while small erythrophores are scattered between these bands.

Eyestalk extirpation of the prawns results in pigment dispersion, while injection of extracts from the eyestalk or from the post-commissure organs (PCO) induces pigment concentration in both types of erythrophores of eyestalkless specimens of either species. In a study of the colour changes of *Palaemon adspersus* Rathke, ÖSTLUND and FÄNGE[1] noticed that 5-hydroxytryptamine (serotonine) induces pigment dispersion in the erythrophores of this prawn. AOTO[2] found that this substance causes pigment dispersion in the large erythrophores of the fresh water prawn *Palaemon paucidens* De Haan. To investigate whether or not serotonine also affects the colour change of other *Palaemon* species, the erythrophore-stimulating potency of serotonine was tested in *Palaemon serratus* and *Palaemon elegans*. Injection of serotonine in doses of 1 μg or higher into intact white-background-adapted *Palaemon* results in a distinct pigment dispersing reaction of the large erythrophores only. This selective action of serotonine is also visible following injection of a mixture of PCO extract and serotonine into eyestalkless prawns. If, for instance, a mixture containing 1/200 part of one pair of PCO and 1 μg serotonine is given to eyestalkless *Palaemon serratus* or *Palaemon elegans*, a strong pigment concentrating reaction of the small erythrophores sets in immediately following injection, while such changes do not occur in the large erythrophores of these two species.

The presented results are in agreement with those of ÖSTLUND and FÄNGE[1] and AOTO[2]. These data and the fact that the presence of serotonine in crustaceans has been demonstrated by WELSH and MOORHEAD[3] indicate that serotonine may play an important role in the colour change reactions of prawns.

[1] E. ÖSTLUND and R. FÄNGE, Ann. Sci. Nat. Zool. *11*, 325 (1956).
[2] T. AOTO, J. Fac. Sci. Hokkaido Univ. Zool. *15*, 177 (1963).
[3] J. H. WELSH and M. MOORHEAD, J. Neurochem. *6*, 146 (1960).

The results of many experiments have shown that in vertebrates 5-hydroxytryptophan is a precursor of 5-hydroxytryptamine (serotonine) (GADDUM and GIARMAN[4], UDENFRIEND et al.[5]). In view of the results obtained with serotonine in crustaceans it was considered worth-while to investigate whether the conversion of 5-hydroxytryptophan into 5-hydroxytryptamine takes place in these animals also.

As the number of specimens of *Palaemon elegans* available was rather limited and the mortality following eyestalk extirpation in this species much higher than in *Palaemon serratus*, it was decided to carry out the next experimental series with the latter species only. A number of eyestalkless *Palaemon serratus* were divided into three groups. The animals of the first group received 1/200 part of one pair of PCO only, those of the second a mixture of 1/200 part of one pair of PCO and 1 μg serotonine, and those of the third a mixture containing the same quantity of PCO and 10 μg 5-hydroxytryptophan. The small erythrophores of the animals of all groups reacted in a similar way, showing a strong pigment-concentrating reaction immediately following injection, which reaction reached its maximum after 15 min, whereupon the pigment dispersed again.

The pigment in the large erythrophores of the animals of the first group reacted in a similar way to that in the small ones. The degree of pigment dispersion in the large erythrophores of the animals of the second group did not change until about 15 min following the injection; at that time a weak pigment concentration was noticed. During the first 5 min following injection the pigment in the large erythrophores of the animals of the third group reacted in the same manner as those in the animals of the first group. Thereupon a strong pigment dispersion occurred. These results suggest that in the crustacean *Palaemon serratus*, like in amphibians and mammals, 5-hydroxytryptophan can be converted into serotonine, and moreover that the rate of this conversion is such that in about 5 min enough serotonine is formed to counteract the red pigment-concentrating activity of the PCO extract successfully[6].

Zusammenfassung. Es wird gezeigt, dass Serotonin (5-Hydroxytryptamin) beim Hervorrufen der Pigmentausbreitung in den grossen Garnelen-Erythrophoren eine wichtige Rolle spielt. Versuche zeigen, dass diese Crustaceen vielleicht 5-Hydroxytryptophan in Serotonin umwandeln können.

A. C. J. BURGERS

Zoological Laboratory, University of Utrecht (The Netherlands), January 25, 1965.

[4] J. H. GADDUM and N. J. GIARMAN, Brit. J. Pharmacol. *11*, 88 (1956).
[5] S. UDENFRIEND and H. WEISSBACH, J. biol. Chem. *224*, 803 (1957).
[6] The experiments were carried out in the Zoological Station at Napoli (Italy) during the summer.

Bestimmung der Zahl aktiver Zentren der Acetylcholinesterase in motorischen Endplatten

NACHMANSOHN[1] hat zuerst auf die hohe Konzentration Cholinesterase in den Endplatten hingewiesen und den enzymatischen Umsatz bestimmt. Es werden pro msec $1{,}6 \cdot 10^9$ Moleküle Acetylcholin hydrolisiert. Zur Hauptsache ist Acetylcholinesterase und nur wenig unspezifische Butyrylcholinesterase vorhanden[2]. Eine Möglichkeit zur Bestimmung der bis heute unbekannten Anzahl von aktiven Zentren der spezifischen Cholinesterase gibt die Bindung von radioaktiv markiertem Diisopropyl-Fluorophosphat (DFP) an den Rezeptoren des Fermentes.

Methode. Es wurden Zwerchfelle von Mäusen während 30 min in Badlösungen (Phosphatpuffer pH 7,4 0,015 m, NaCl 0,1 m, $MgCl_2$ 0,015 m) mit steigenden Konzentrationen ^{32}P-DFP (380 μC/mg) gebracht, dann zur Entfernung von nicht gebundenem DFP während 18 h mit Wasser gespült und zur Absorption schwacher β-Strahlen mit Aluminiumfolien (Dicke 40 μ) abgedeckt. Autoradiographien wurden durch Kontakt mit Ilford PM_2-Filmen erhalten. Mit ^{14}C-markiertem DFP (39 mC/mM) inkubierte Zwerchfelle ergaben Autoradiographien durch direkten Kontakt mit PM_2-Filmen ohne Aluminiumfolien. Mit einem Densitometer wurde die Schwärzung der Endplatten-Autoradiographien nach Abzug der Muskelaktivität ausgemessen und mit Eichpräparaten verglichen[3]. Daraus liess sich die Konzentration DFP in der Endplattenregion und die Anzahl Moleküle DFP pro Endplatte quantitativ berechnen.

Zum histochemischen Nachweis der Cholinesterase in den Endplatten wurde diese mit Pyridin-2-Aldoxim Methyljodid reaktiviert und dann nach KOELLE gefärbt. In einer zweiten Serie wurden die Zwerchfelle vor der Inkubierung mit DFP mit Mipafox ($2 \cdot 10^{-6}$ m Badkonzentration) zur Blockierung der unspezifischen Cholinesterase vorbehandelt.

Resultat. ^{32}P-DFP macht durch seine relativ energiereiche β-Strahlung eine diffuse Autoradiographie im Bereich des Endplattenbandes im Zwerchfell (Figur 1). Mit ^{14}C-DFP ist infolge der schwachen β-Strahlung die Auflösung viel besser und die Schwärzung auf einzelne Endplatten beschränkt. Bei steigenden Konzentrationen DFP in der Badflüssigkeit nehmen die Endplatten immer mehr DFP-Moleküle bis zu einem Sättigungswert von $2{,}4 \cdot 10^7$ auf. Vorbehandlung mit Mipafox verändert diesen Sättigungswert kaum (Figur 2). DFP scheint von der spezifischen Acetylcholinesterase sehr stark gebunden zu werden. Auch mit ^{14}C-markiertem DFP wurde eine ähnliche Sättigung erreicht ($1{,}2 \cdot 10^7$ Moleküle/Endplatte).

Diskussion. Der geringe Effekt des Mipafox zur Blokkierung der unspezifischen Cholinesterase lässt vermuten, dass diese in der Endplatte nur eine untergeordnete Rolle spielt[4]. Trotz der starken Bindung des DFP an beide Cholinesterasen kommt es wohl kaum zu einer Verdrängung des Mipafox von der unspezifischen Cholinesterase.

[1] D. NACHMANSOHN, *Chemical and Molecular Basis of Nerve Activity* (Academic Press, 1959).
[2] B. HOLMSTEDT, Acta physiol. scand. *40*, 331 (1957).
[3] P. G. WASER und U. LÜTHI, Arch. int. Pharmacodyn. *112*, 272 (1957); Helv. phys. pharmacol. Acta *20*, 237 (1962).
[4] G. B. KOELLE, Handb. exp. Pharmak. *15*, 187 (1963).