Oxygen uptake was determined by the standard Warburg technique the animals being maintained in Salton Sea water with no addition of substrate: 10 mg/l chloromycetin was added to inhibit bacterial action. Readings were taken at hourly intervals.

Typical results are shown in the Figure. Since the mean oxygen uptake varies with temperature, the percentage deviations from the mean values over the whole period are given. The animals on which the results are quoted were large and of similar size; oxygen uptake is weight dependent, but no difference in relative behaviour was found with the different size groups. It is evident that while there is some variation in the oxygen uptake from hour to hour at 20° and 30°C, the changes are small compared with the hourly fluctuations when the animals are held at 10°C; these take the form of a damped oscillation. A large number of species of barnacles have been investigated under a wide variety of experimental conditions and marked oscillations of this type and magnitude have only been found in this species. (It must be pointed out that small oscillations particularly if they were of a short period would require a different technical approach.)

In intertidal species which are subjected to rapid changes of temperature in their natural habitat, the metabolic processes might be expected to be so coupled as to reduce the oscillatory behaviour, whether it is overshoot or response to shock. It is, however, not so evident why other sub-littoral species which live in a comparatively constant environment should not show a similar behaviour when subjected to equally large temperature changes. It is perhaps, therefore, of significance that *B. amphitrite* was the only warm water and tropical sublittoral species investigated. The fact that northern forms when subjected to a rise in temperature do not behave in an oscillatory manner comparable to that of this southern form when subjected to similar falls in temperature suggests that the coupling of the metabolic processes may be different in the two types of species; further work on this line would perhaps help to elucidate some of the factors concerned in determining their respective distribution.

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The Marine Station, Millport (Scotland), April 20, 1959.

Résumé

Chez *Balanus amphitrite*, espèce des eaux chaudes, l'utilisation de l'oxygène par des individus isolés (extraits de leur coquille), soumis à une température peu élevée est de forme oscillatoire, contrairement à ce que l'on observe chez des espèces des eaux froides. Il se peut que cette différence caractérise de manière générale deux types d'espèces.

Action of Insulin *in vitro* on the Glucose Uptake of the Spinal Cord of the Rat*

It is not yet clear whether insulin has any effect on the metabolism of glucose through the nervous system or not[1]. Recently Rafaelsen[2] published his results, according to which the spinal cord of the rat appears to be sensitive to this hormone. Since most authors[3], as also we ourselves[4], were unable to find any effect of insulin on the nervous system, we thought it interesting to repeat Rafaelsen's test in order to try to confirm his results.

Table I

Action of Insulin on the Glucose Uptake and Oxigen Consumption of the Spinal Cord. Krebs Buffer. Not anesthetised

	Mean	t	P	No. experiments
Glucose uptake mg × 100 mg of tissue				
Insulin 10^{-1} . .	0·236	0·000	> 0·9	8
Control	0·236			8
Oxygen consumption microlitres × 100 mg of tissue × h				
Insulin 10^{-1} . .	13·4	0·209	$0{\cdot}8 < P < 0{\cdot}9$	8
Control	13·5			8

Methods. We utilised rats with a body weight of 100 to 160 g, fasted for 24 h. The extraction of the spinal cord was made following exactly the same method as Rafaelsen[2], after a previous anesthetic by inhalation of a mixture of 50% CO_2/O_2. In some other groups of experiments, the rats were decapitated in order to elucidate the effect, if any, of the anesthetic.

The medullary portion was divided into two parts weighting 100–120 mg, their weight being determined through the weighting differences of the incubation flasks. These pieces were directly incubated in 2 cm³ of the Gey and Gey (Rafaelsen) or Krebs medium according to the cases. For this purpose, Warburg glasses with a total volume of 15 cm³ were employed and the incubation (Warburg S. L.) was made at a temperature of 37·5°C for 120 min, at 80–90 oscillations/min and 4 cm amplitude. The flasks were aerated with a mixture of 95% oxygen and 5% carbon dioxide, for 1 min. Glucose was determined by the glucose-oxidase method, with a maximum development of colour. The insulin employed was Lilly[5], free from glucagon, at a concentration of 10^{-1} I. U. per cm³.

The glucose uptake was calculated from the decrease of glucose level in the medium during the incubation and expressed as: mg glucose per 100 mg of tissue (spinal cord) per 60 min. The determination of the consumption of oxygen is expressed in microlitres consumed per 100 mg of tissue per 60 min. Student's 't' test was used for statistical analysis of the data.

Results. Table I contains the results obtained in tests made with rats decapitated utilising the Krebs buffer. There are no differences in the glucose consumption between the series treated with insulin and the control animals. There are also no differences in oxygen consumption. There is no sensitiveness to insulin.

Tests reproduced in Table II were made with rats decapitated utilising the same buffer as Rafaelsen (Gey and Gey). The difference is not significant, either in the consumption of glucose or in that of oxygen. There is no sensitiveness to insulin.

* Preliminary communication.

[1] H. E. Himwich, *Brain Metabolism and Cerebral Disorders* (The Williams & Wilkinks, 1951). – D. Richter, *Metabolism of the Nervous System* (Pergamon Press, 1957). – H. McIlwain, *Biochemistry and the Central Nervous System* (J. & A. Churchill, 1955). – A. E. Renold, J. Ashmore, and A. B. Hastings, Vitamins and Hormones *14*, 170 (1956).

[2] O. J. Rafaelsen, Lancet *1958*, 941.

[3] C. P. Park, L. H. Johnson, J. H. Wright, and H. Batsel, Amer. J. Phys. *191*, 13 (1957).

[4] D. Martin-Hernandez, R. R-Candela, and J. L. R-Candela, Rev. iber. Endocrin. *5*, 39 (1958).

[5] We are indebted to Dr. W. R. Kirtley for the generous supply of this hormone.

Table II

Action of Insulin on the Glucose Uptake and Oxigen Consumption of the Spinal Cord. Buffer Gey and Gey. Not anesthetised

	Mean	σ	t	P	No. experiments
Glucose uptake mg × 100 mg of tissue					
Insulin 10^{-1}	0·328	0·027	1·867	$0{\cdot}05 < P < 0{\cdot}1$	14
Control . .	0·304	0·033			14
Oxygen consumption: microlitres × 100 mg of tissue × h					
Insulin 10^{-1}	12·3	1·06	1·1211	$0{\cdot}2 < P < 0{\cdot}3$	14
Control . .	12·7	0·65			14

In Table III the results are obtained by exactly following RAFAELSEN's technique: that is to say, after previously anesthetising the rat with a mixture of 50% CO_2/O_2. In this kind of test, the spinal cord appears sensitive to the action of insulin because the difference in glucose consumption of the tissue with and without insulin is significant. There is, however, no significant difference in the oxygen consumption.

Table III

Action of Insulin on the Glucose Uptake and oxigen Consumption of the spinal Cord. Buffer Gey and Gey. Anesthetised with a CO_2/O_2 mixture at 50%

	Mean	σ	t	P	No. experiments
Glucose uptake: mg × 100 mg of tissue					
Insulin 10^{-1}	0·485	0·021	3·8693	$< 0{\cdot}01$	9
Control . .	0·290	0·029			9
Oxygen consumption: microlitres × 100 mg of tissue × h					
Insulin 10^{-1}	17·1	2·07	0·5263	$0{\cdot}6 < P < 0{\cdot}7$	8
Control . .	16·6	1·38			8

Discussion. We were able to confirm RAFAELSEN's results, although our own data give them a different interpretation, because we observed that the spinal cord is *only sensitive* to insulin when the rat has been previously anesthetised with CO_2. The basal glucose-uptake is the same in the experiments but in the test made without anesthesia, no effect is produced when adding this hormone. Similar results have obtained URELES and MURRAY[6], because they have found that r. c. b. uptake of I^{131} triiodothyronine increases either with marked CO_2 retention (patients) or when the blood has been bubbled with CO_2 into the flask *in vitro*.

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Instituto de Metabolismo y Nutrición, Madrid (Spain), June 9, 1959.

Résumé

La moelle épinière ne paraît pas sensible à l'insuline *in vitro* si elle provient d'un animal décapité alors qu'elle l'est de façon significative ($P < 0{,}01$) si l'animal était anesthésié avec un mélange de CO_2/O_2 à 50%.

[6] A. URELES and M. MURRAY, cited by M. W. HAMOLSKY *et al.*, J. clin. Endocrin. Met. *19*, 103 (1959).

Ion Adsorption and Excitation II

It has been shown in a previous communication[1] that the internal and external ion concentrations of nerve can be related to the net quantity of ions fluxing during the passage of a single impulse by the expression $f = A\,r\,(C_i - C_0)$. It was also deduced that the ions involved in these fluxes form a monolayer at the nerve surface and that they are not hydrated. This was taken as an indication that excitation may be accompanied by an adsorption-desorption process.

The quantity Ar has been defined as that volume of the nerve substance in which the ionic concentration corresponds to that of the nerve interior or to that of the bathing solution depending on the presence or absence of the flux quantity.

The advantage of this formulation is that by summating the flux values over all such unit volumes available, the gross ion concentration difference between the axoplasm and the bathing fluid can be expressed in terms of the flux quantity characteristic of a single discharge.

Since Ar can also be regarded as a sort of 'extended surface', being in fact only one half of an ion thick and situated presumably at the functional surface of the nerve, this surface may be considered as having the same ionic composition as the nerve interior. This suggests that the forces which operate in maintaining the ionic composition of the nerve interior are identical with those which maintain the ionic composition of the nerve surface. Conveniently these forces may be characterized as adsorption forces, opposite and equal in magnitude to the potentials as given for each ionic species by the relation $E = kT/e \ln (C_i/C_0)$.

It follows from such a view that since the surface monolayers of the major monovalent ions can be regarded as spatially superimposed, the resting potential of the cell may be assumed to correspond to the algebraic sum of the potentials due to each of the major ions represented at the surface. This leads to the expression

$$R.P. = kT/e \ln(K_i/K_0 \times Na_i/Na_0 \times Cl_0/Cl_i).$$

Taking the external concentrations of K and Na ions as $K_0 = 22$ mM/kg and $Na_0 = 440$ mM/kg[2] and the internal ones as $K_i = 345{\cdot}3$ mM/kg and $Na_i = 62{\cdot}5$ mM/kg[1], and using the value of 59 mV for the corrected resting potential of the squid giant axon[3], one obtains from the above formula the ratio $Cl_0/Cl_i = 4{\cdot}5$.

According to the data of BEAR and SCHMITT[4] this ratio is about 4, the agreement between this value and the one calculated above suggesting that the assumption involved in the formulation is essentially correct.

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Department of Pathology, New York University, Bellevue Medical Center, New York, May 25, 1959.

Résumé

Une façon simple de formuler le potentiel de repos de l'axone géant du Calmar est présentée. L'équation est

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[2] A. L. HODGKIN, Biol. Rev. Cambridge Phil. Soc. *26*, 339 (1951).
[3] A. L. HODGKIN and B. KATZ, J. Physiol. *108*, 37 (1949).
[4] R. S. BEAR and F. O. SCHMITT, J. cell. comp. Physiol. *14*, 205 (1939).