elettronico in mitocondri incubati in soluzioni di Ca^{++} e da questi identificate come precipitazioni di calcio fosfato, ha dato valore di iniziali precipitazioni calcaree a funzione «nucleante». In conclusione, quindi, lo studio ultrastrutturale della calcificazione renale da sublimato ci ha consentito di precisarne la morfologia submicroscopia e l'assenza di relazione con le fibre collagene; restano confermati i dati topografici ricavati dallo studio al microscopio ottico (PALLADINI et al.[4]).

Summary. Carrying on the researches on the biological process of calcium deposition, the role of the substructures in the precipitation of calcium salts in the loci of distrophic calcification of kidney tubules following sublimate injections has been investigated. In agreement with the histochemical data previously found, the presence of collagen fibres in these sites is excluded; the morphological appearance of crystal deposits is described. The importance of mitochondria in the calcium ion deposition in kidney tubules is discussed.

G. PALLADINI e S. CARAVITA

Istituto di anatomia comparata G. B. Grassi dell'Università di Roma (Italia), 14 marzo 1966.

Failure of Iodoacetate to Protect the Antigenic Power of Insulin from Cystein Inactivation

The 'thiol' compounds (cystein, glutathione, dimercaptopropanol, etc.) promote[1], even non-enzymatically, the linked reaction of oxide reduction between the SS of the protein and the non-protein 'thiol' group.

$$\text{Protein}\begin{matrix}\text{S}\\\text{S}\end{matrix} + 2\,\text{RHS} \rightleftharpoons \text{protein}\begin{matrix}\text{SH}\\\text{SH}\end{matrix} + \text{R-S-S R}.$$

The reduction of the insulin by cystein, accompanied by a loss of its physiological activity, is also rapid.

YALOW and BERSON[2] state that plasmatic insulin disappears when the specimen is incubated at pH 8 in the presence of $0.02\,M$ cystein. The present paper reports observations obtained when studying the action of iodoacetate and cystein on the stability of bovine insulin's antigenic power under certain experimental conditions and using HALES and RANDLE's immunological method[3].

Method. Unlabelled insulin acts as a competitive inhibitor on the formation of the antigenic-antibody complex between labelled insulin and anti-insulin antiserum. Test samples of insulin are treated with iodoacetate and cystein, according to the conditions explained in the text and the previous paper[4].

Stability of the antigenic power of insulin in the presence of cystein: Concentrates of 250 and 500 μu/ml of bovine insulin are incubated for 2 h at 37 °C with $0.02\,M$ and $0.01\,M$ of cystein at pH 7, 7.5 and 8 respectively and subsequently dialysed for 3 h against a sodium phosphate buffer of $0.040\,M$ at pH 7.4.

Quantities of the same samples are treated likewise but without the cystein stage.

Results. Although the incubation and dialysis in themselves induce a significant loss of activity, Table I shows clearly that after addition of cystein, the insulin becomes quite inactive at pH 7.5 and 8. About 25% of the original activities, however, remains at pH 7.

Effect of iodoacetate and cystein on beef insulin stability at pH 7.5: In its role as a blocking agent of the -SH groups, the iodoacetate may react quickly with cystein[5], which suggests that an inhibitor of the insulin's biological action in vitro may be capable of protecting the hormone against the well-known inactivating effect of the amino acid. However, this does not occur, as can be seen in Table II, at least from the antigenicity viewpoint. In our experimental conditions with prior incuba-

Table I. Effect of cystein on beef insulin stability at different pH

pH	Control sample before incubation and dialysis	Control sample incubated and dialysed without cystein	Sample incubated with cystein and dialysed	
	μu/ml	μu/ml	μu/ml 0.02 M	0.01 M
7	500	400	48	100
7	250	180	10	25
7.5	500	390	0	20
7.5	250	195	0	0
8	500	420	6	0
8	250	210	0	0

Table II. Effect of iodoacetate and cystein on beef insulin stability at pH 7.5

Reagent present during incubation	Residual activity (μu/ml) time (min) 0	120
None (without incubation)	500	500
None	500	360
$1 \cdot 10^{-5}\,M$ iodoacetate + 0.02 M cystein	–	0
$1 \cdot 10^{-4}\,M$ iodoacetate + 0.02 M cystein	–	0
$1 \cdot 10^{-3}\,M$ iodoacetate + 0.02 M cystein	–	0
$1 \cdot 10^{-2}\,M$ iodoacetate + 0.02 M cystein	–	0
$1 \cdot 10^{-5}\,M$ iodoacetate + 0.01 M cystein	–	0
$1 \cdot 10^{-4}\,M$ iodoacetate + 0.01 M cystein	–	0
$1 \cdot 10^{-3}\,M$ iodoacetate + 0.01 M cystein	–	0
$1 \cdot 10^{-2}\,M$ iodoacetate + 0.01 M cystein	–	0

Incubation was carried out at 37 °C except where it is indicated. Tubes contained 500 μu/ml of crystalline insulin and the indicated additions in phosphate buffer $0.040\,M$, pH 7.5. After incubation the samples were dialysed out for 3 h and assayed for activity.

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tion of iodoacetate and cystein, the stability of the hormone is not maintained.

Discussion. The loss of the insulin's antigenic power (coinciding with the disappearance of its biological function) in the presence of cystein, implies the possibility that the disulphide groups of the molecule are also somehow involved in the control over its antigenic power. It is an established fact that the addition of OH ions to an isoelectric solution of cystein liberates protrons of the NH_3 group, with the consequent ionization of the 'thiol' group. In 1956 DE DEKEN et al.[7], studying the displacement of the UV-spectrum of absorption from the ionized cystein, suggested this mechanism. A consequence of this reaction are certain modifications directly connected with the structure of the hormone. At pH 7 these modifications are less profound but increase as the pH is increased.

The fact that iodoacetate is unable to protect the insulin from the inactivating effect of cystein, confirms the possibility that the amino acid action exceeds its reducing power to a much larger degree than the amount of reduced insulin present would seem to justify. It is necessary to account for the fact that reduction of only 10% of the total number of the disulphide bridges present in the molecule is capable of reducing the biological activity of the insulin by 87%[8]. On the other hand, iodoacetate behaves as an inhibitor of the antigenic power of the insulin[4]. The observations compiled in this work tend to confirm the reasonable supposition that the structural formation of insulin governs its antigenic potential.

Resumen. La capacitad de reacción de la insulina de bovino con un anticuerpo especifico desaparece completamente cuando, previamente, se incuba la muestra en presencia de Cisteina. Concentraciones de iodoacetato hasta $1 \cdot 10^{-2} M$ no son capaces de proteger a la hormona contra la acción inactivadora del aminoacido.

C. LOPEZ-QUIJADA

Instituto «G. Maranon», Velazquez 144, Madrid 6 (Spain), April 26, 1966.

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The Effect of a Sudden Rise in Temperature on Strobilae of *Aurelia aurita*

A certain low critical temperature has been listed as one of the criteria for strobilation to occur in scyphistoma larvae of various species from temperate regions[1,2]. During the winters of 1962/63 and 1963/64, scyphistoma larvae of *Aurelia aurita* which had been induced to strobilate at a temperature below 8 °C continued to do so even when the temperature rose as high as 12 °C. As this temperature is higher than that recorded when strobilation occurs naturally, a series of experiments was conducted to see how far strobilation was temperature sensitive and whether scyphistoma larvae could adapt themselves to a rise in temperature once the strobilation mechanism had been initiated.

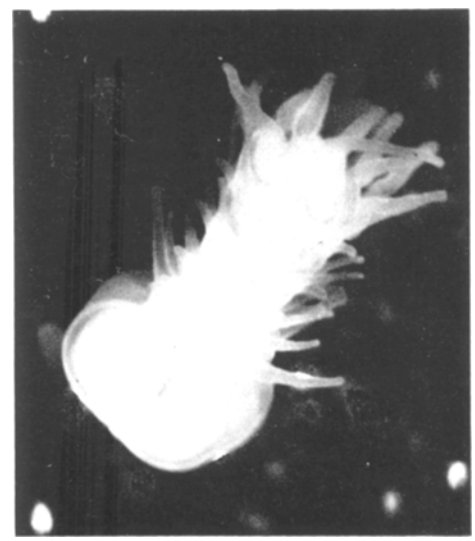
The collection and rearing of scyphistoma larvae has been described in an earlier report[3]. Strobilating individuals were taken either from the stock aquaria or from a low temperature experimental apparatus and were placed in darkened vessels in a water bath of the required temperature. In all other respects the conditions were identical to those the animals had previously been experiencing. It was found that while these animals were generally able to adapt themselves to a gradual rise in temperature up to 12–14 °C, a sudden rise produced the formation of tentacles characteristic of the scyphistoma in place of the ephyral lappets. Such a condition is shown in the Figure, where a 5 ringed strobila has produced 8 tentacles in place of the lappets in each ring.

The results of experiments where there is a sudden change in temperature are shown in the Table.

There are 3 main observations which can be made from these results: (1) Tentacles are formed most rapidly when the temperature is raised to 15 °C or above. If the temperature is not raised so high the effect is either absent or takes much longer to appear. (2) This effect is much more marked in individuals in which strobilation has not proceeded far, i.e. up to about 5 rings. In later stages the effect may take longer to appear, or may be completely absent. (3) There may also be a resorption of some of the proximal rings.

It is interesting to note that in all cases these changes tend to start at the proximal end of the strobilating portion of the scyphistoma and spread distally.

This apparent reversal of the strobilation mechanism has been observed to occur naturally by HAECKEL[4], who lists this tentaculate form as one of 8 variations of strobila. THIEL[5] in his observations on scyphistoma larvae in



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