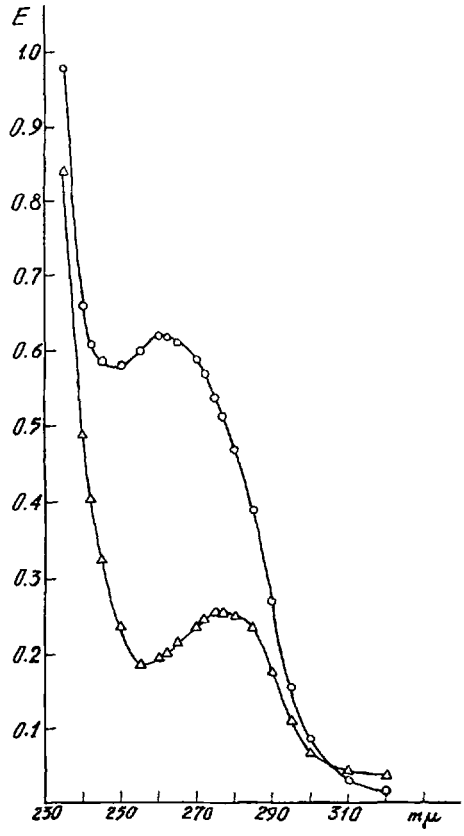
The extract includes carotenoids and phospholipids: indeed it contains 0·14% P. The delipidated material contains 10% N, 8·5% carbohydrates, and still 0·2% P. As no nucleic acid is present in such preparations, this residual P can be explained as being either strongly bound phospholipids or phosphoprotein-P. Paper chromatography has shown a complete assortment of amino acids.

The fact that no nucleic acid was present in our preparations seems to rule out the possibility of a contamination with mitochondria or microsomes.



Ultraviolet absorption spectrum of the chromoprotein of the pigment granules of unfertilized eggs (▵—▵) and of late blastulas (○—○) of *Paracentrotus lividus*.

The electrophoretic analysis of the granules in solution in phosphate buffer at pH 7·4 shows two main components and a third small one having mobilities of $u = -10$, $-4{\cdot}5$, and $-2{\cdot}9 \times 10^5/\mathrm{cm}^2/\mathrm{V/s}$. We cannot say at present whether the pigment is uniformly distributed in the three components, although there is some indication that the distribution may be unequal.

The fractional precipitation, using the salting out method of DERRIEN[1] (using $AmSO_4$ and estimating the amount of proteins in the supernatant from the absorption at 275 mμ), agrees with the results of the electrophoresis, showing the presence of three fractions with precipitation points at 60%, 70%, and 75% saturation of $AmSO_4$.

The same results were obtained with granules prepared from early stages of development and up to the early blastula stage. However, at about the time when migration of the primary mesenchyme takes place, the preparations appear to contain some nucleic acid. A comparison between the U.V. absorption spectrum of two preparations, one from unfertilized eggs and one from late blastula, shows this fact quite distinctly (Fig.). It is possible that the nucleic acid is a contamination, although the constancy of its quantity in different preparations (about 0·7% of nucleic acid P) may suggest that it is present as an integral part of the composition of the granules. The analysis shows that it is ribose nucleic acid, and that rules out a contamination with nuclear material. However, further analyses will clear up this important point.

Finally we might add that the chromoprotein in solution exhibits a type of reversible denaturation similar to that known for other proteins conjugated with carotenoids (Ovoverdin[1], Crustacyanin[2]): that is, the orange-red colour of our chromoprotein in solution in phosphate buffer at pH 7·4 turns to yellow when heated up to 85°C for 3–10 minutes and then, when the preparation is quickly cooled down, the colour returns to orange-red.

We wish to thank Dr. H. J. BIELIG for interesting discussions.

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Department of Physiology, Zoological Station, Naples, and Centre of Biology of the C.N.R., October 1, 1951.

Zusammenfassung

Die Pigmentkörnchen aus dem Ei von *Paracentrotus lividus* bestehen aus einem Chromoproteid. Mit Erreichen des Blastulastadiums ist in den Körnchen auch Nukleinsäure enthalten.

[1] K. G. STERN and K. SALOMON, J. Biol. Chem. *122*, 461 (1938).
[2] G. WALD *et al.*, Biol. Bull. *95*, 249 (1948).

Changes in Water Diuresis and Vasopressin Inactivation in Mice Fed on Protein Deficient Diets

It has been suggested by several authors (literature reviewed by EVERSOLE, BIRNIE, and GAUNT[1] and RALLI[2], that retention of water following liver damage is due, at least, in part to a decreased inactivation of the posterior pituitary antidiuretic hormone by damaged liver tissue. Pronounced depression of water diuresis has also been observed in animals kept on protein deficient diets (DICKER, HELLER, and HEWER[3], DICKER[4]) and in patients suffering from hunger oedema (GOPALAN[5]). There is ample evidence to show that the liver in such deficiency conditions may not be normal. It seemed of interest therefore to investigate whether the livers of protein deficient animals inactivate antidiuretic hormone at the normal rate, and secondly whether the fatty changes frequently found in the livers of such animals have a bearing on the impairment of water diuresis or on changes in the rate of inactivation of vasopressin.

[1] Y. DERRIEN, Svensk. Kem. Tid. *59*, 139 (1947).

[1] W. J. EVERSOLE, J. H. BIRNIE, and R. GAUNT, Endocrinology *45*, 378 (1949).
[2] E. P. RALLI, S. H. LESLIE, G. H. STUECK, and B. LAKEN, Amer. J. Med. *11*, 157 (1951).
[3] S. E. DICKER, H. HELLER, and T. F. HEWER, Brit. J. Exper. Path. *27*, 158 (1946).
[4] S. E. DICKER, Biochem. J. *46*, 53 (1950).
[5] C. GOPALAN, Lancet *258*, 304 (1950).

The experimental animals used were mice which were kept on various protein deficient diets. It could be shown that they developed signs of "protein deficiency" (loss of weight, anorexia, decrease of plasma proteins, fatty infiltration in the liver) much more rapidly than rats. In addition such animals excreted a standard water load at a lower rate than normals after only 6 to 7 days on the deficient diets: For example in a series of mice kept on a diet poor in protein and choline (= diet BB; see DICKER[1]) the mean volume of urine 90 min after water administration was only 17·5 per cent of that of the control series. When adequate amounts of choline were added to this diet (= diet BC) the mean urine volume rose to 47·4 per cent of that of the controls. A protein deficient vegetable diet (= TT) containing ample choline (DICKER, HELLER, and HEWER[3]), given to a third group of mice, lowered the mean urine volume excreted during 90 min to 33·2 per cent of the normal.

To study vasopressin inactivation, homogenates of mouse liver mixed with a standard dose of vasopressin and phosphate buffer of pH 6·5 were incubated at 37°C for 30 min. Rats to which water had been administered were injected intraperitonealy with the incubation mixtures and the antidiuretic effect compared with that of known doses of vasopressin. Expressed as percentage of the mean urine volume at 90 min of controls injected with an incubation mixture containing normal mouse liver, the results obtained with livers from animals on the protein deficient diets were as follows:

BB for 7 days = 73·7 per cent,
BB for 10 days = 61·5 per cent,
BC for 7 days = 81·2 per cent,
TT for 10 days = 60·6 per cent.

In other words, it would appear that more antidiuretic material remained active in the liver homogenates from protein deficient animals with the result that the inhibition of water diuresis in the test rats was more pronounced.

Fatty changes in livers of animals kept on the protein deficient diet were investigated by histological and by chemical methods. The histological results are summarized in the table. The chemical estimation of total liver lipids were in reasonable agreement with the histological findings. There would appear to be no parallelism between the results of the diuresis and inactivation experiments, and the intensity of fatty changes: Mice kept on diet TT for 10 days for instance showed a pronounced inhibition of water diuresis and their livers inactivated vasopressin less efficiently, even though no fatty changes could be found in the liver.

Assessment by histological methods of the intensity of fatty changes in the liver of mice on protein deficient diets. The degree of fatty vacuolation is recorded as 0, +, ++, +++ or ++++.

Days on Diet	Control Diet	Diet BB	Diet BC	Diet TT
1	0	+	0	0
2 to 3	0	+++	++	+
4 to 5	0	++++	+++	+
6 to 7	0	++++	no data	++
8	0	+++	+	+
10	0	++	+	0

[1] S. E. DICKER, Biochem. J. *46*, 53 (1950).

In view of the findings of LESLIE and RALLI[1] and of DICKER[2] that the urine of rats fed on a protein deficient diet contained amounts of antidiuretic activity which increased the longer the animals remained on the experimental diet, it would be tempting to associate the lack of the renal response to water in protein deficient mice with the decreased ability of their liver to inactivate vasopressin. However, such an association can at present, for several reasons, be regarded as no more than an inviting possibility.

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Department of Pharmacology, University of Bristol, October 22, 1951.

Zusammenfassung

Mäuse, die auf eiweißarme Kost gesetzt werden, zeigen wenige Tage später eine bedeutende Abnahme ihrer Fähigkeit zur Wasserdiurese. Die Leber solcher Tiere inaktiviert Vasopressin langsamer als Leberbrei von Kontrollen. Ein kausaler Zusammenhang zwischen diesen Befunden ist fraglich. Je nach der Zusammensetzung der eiweißarmen Kost (Cholingehalt, Kalorienwert) und der Versuchsdauer traten bei den Versuchstieren verschiedene Grade von Leberverfettung auf. Diuresehemmung und Verlangsamung der Vasopressininaktivierung konnten jedoch auch in Tieren nachgewiesen werden, deren Leber keinen erhöhten Fettgehalt aufwies.

[1] S. H. LESLIE and E. P. RALLI, Endocrinology *41*, 1 (1947).
[2] S. E. DICKER, Biochem. J. *46*, 53 (1950).

Antidiuretic Action of Small Doses of Enteramine Extracts in the Rat

Extracts of Posterior Salivary Glands of Octopus vulgaris

Alcohol and acetone extracts of posterior salivary glands of *Octopus vulgaris* and *Eledone moschata* inhibit diuresis in rats and in various other mammals, including humans. The antidiuretic action is due to enteramine.

Results obtained in dogs and rats with massive doses of salivary extract were reported in previous communications[1].

The purpose of present researches was to establish:

(*a*) the smallest dose of salivary extract of *Octopus vulgaris* still active on the diuresis of hydrated rats;

(*b*) the mechanism of action of small doses of salivary extract. In this connection it was important, above all, to confirm what had already been observed in dogs when using large doses of the same material.

The experiments were carried out on 560 adult albino rats of both sexes, weighing from 130 to 250 g. A first load of tepid tap water (2·5 cm³/100 g) was administered in the morning by stomach tube to fasting animals. After three hours a second water load (5 cm³/100 g) was given and, at the same time, the rats were injected subcutaneously with 1 cm³/100 g distilled water, in which, besides the salivary material (lacking, of course, in the controls), 50 mg sodium thiosulphate (500 mg/kg) and 5 mg *p*-aminohippuric acid (PAH, 50 mg/kg) were dissolved.

[1] V. ERSPAMER and A. OTTOLENGHI, Exper. *6*, 428 (1950), *7*, 191 (1951).