Nettowassertransport mg/cm²-Stunde: + von aussen nach innen, — von innen nach aussen		Absolute Wasserdiffusion ml/cm²-Stunde von aussen nach innen	
Inneres Medium Ringerlösung Äusseres Medium NaCl 6,5°/oo	Inneres Medium Ringerlösung + Adrenalin HCl 10^{-5} Äusseres Medium NaCl 6,5°/oo	Inneres Medium Ringerlösung Äusseres Medium NaCl 6,5°/oo in H_2O + D_2O	Inneres Medium Ringerlösung + Adrenalin Cl 10^{-5} Äusseres Medium NaCl 6,5°/oo in H_2O + D_2O
$+3{,}48 \pm 1{,}12$ $m \pm 0{,}40$ $n = 8$	$-4{,}76 \pm 2{,}85$ $m \pm 1{,}16$ $n = 6$	$0{,}258 \pm 0{,}032$ $m \pm 0{,}019$ $n = 3$	$0{,}285 \pm 0{,}017$ $m \pm 0{,}008$ $n = 6$

$m \pm$ = Wahrscheinliche Abweichung des mittleren Wertes. $\pm$ = Wahrscheinliche Abweichung des einzelnen Wertes.
n = Zahl der Experimente.

wobei t_0 die Temperatur bezeichnet, bei welcher der Schwimmer im gewöhnlichen Wasser sich vom Boden löst; t ist die Temperatur, bei welcher der Schwimmer in der unbekannten D_2O-Lösung emporsteigt, d_0 ist die Densität des gewöhnlichen Wassers bei Temperatur t_0, d_1 die Densität des gewöhnlichen Wassers bei Temperatur t, d_2/d_1 ist das Verhältnis zwischen der Densität von D_2O und H_2O bei Temperatur t, M_2/M_1 ist das Verhältnis zwischen den 2 entsprechenden Molekulargewichten, 3β ist der volumetrische Dilatationskoeffizient des Pyrexglases ($= 10^{-5}$) und $c = 0{,}999984$.

Wenn wir als äusseres Medium eine D_2O-Lösung benutzen (3,5–5%) und mit C^0_2 und C_2 die D_2O-Konzentration des äusseren Mediums am Anfang des Experimentes und nach einer Stunde bezeichnen, kann man die absolute Wasserdiffusion von aussen nach innen in ml/Stunde (a_{21}) mit der folgenden Formel berechnen:

$$C_2 = \frac{C^0_2}{1 + \frac{V^0_1}{V^0_2}} + \left(C^0_2 - \frac{C^0_2}{1 + \frac{V^0_1}{V^0_2}} \right) e^{-\frac{V^0_1 + V^0_2}{V^0_1 V^0_2} a_{21}},$$

wobei V^0_1 und V^0_2 die Volumina des inneren bzw. des äusseren Mediums sind.

Um die obige Gleichung abzuleiten, setzten wir voraus:

a) dass die Wasserdiffusion während des Experimentes konstant sei;

b) dass die Wasserdiffusion in beiden Richtungen als gleich betrachtet werden kann.

c) dass man ohne grossen Fehler die Tatsache unbeachtet lassen kann, dass immer ein Gleichgewicht zwischen D_2O und H_2O besteht und dass die prozentige molare D_2O-Konzentration nicht ganz identisch ist mit der prozentigen volumetrischen D_2O-Konzentration.

Es wurden auf diese Weise die Werte der absoluten Wasserdiffusion von aussen nach innen, unter dem Einfluss des Adrenalins im inneren Medium, gesammelt (Tabelle).

Neben der Bestimmung der absoluten Wasserdiffusion von aussen nach innen haben wir auch den Nettowassertransport in derselben Richtung, unter dem Einfluss des Adrenalins im inneren Medium, bestimmt.

Das Verfahren, welches wir zur letzteren Bestimmung befolgten, ist schon veröffentlicht worden[1].

Alle Experimente wurden an Fröschen durchgeführt, die im selben Monat gefangen worden waren.

[1] V. Capraro und M. Tiengo, l. c.

Die restlichen hier wiedergegebenen Werte sind schon veröffentlichten Arbeiten entnommen[1].

Aus der Tabelle kann man ableiten, dass das Adrenalin die absolute Wasserdiffusion durch die Froschhaut nicht ändert und dass somit die Wirkung des Adrenalins auf den Nettowassertransport nicht einer Änderung der Wasserpermeabilität bei einem Diffusionsprozess zuzuschreiben ist, sondern einer Änderung des aktiven Wassertransportes[1].

Wir danken den Herren Prof. G. Bolla und Ing. M. Silvestri vom CISE in Mailand, die uns das D_2O zur Verfügung stellten und uns für die Technik mit ihrem Rate beistanden.

V. Capraro und J. Franceschini[2]

Biologisches Laboratorium des Institutes «C. Erba» für therapeutische Forschungen, Mailand, und Institut für allgemeine Physiologie, Urbino, den 16. Januar 1952.

Riassunto

Vengono riportati dei dati sul passaggio assoluto di acqua attraverso la cute di *Rana esculenta* sotto l'influenza dell'Adrenalina; questi dati sono stati ottenuti con l'aiuto del Deuterio come indicatore.

Da essi viene confermata l'ipotesi che l'Adrenalina non modifica la permeabilità passiva all'acqua, ma solo il trasporto attivo.

[1] V. Capraro und M. Tiengo, l. c.
[2] Mit der Hilfe von F. Poy, Chemotechniker.

On the Desoxyribonucleic Acid Content of Sea Urchin Gametes[1]

The far-reaching biological effects of the union between a sperm cell and an egg cell have prompted many investigations of the quantitative distribution of various components of these cells and especially of their nuclei. In view of the remarkable proportionalities, first suggested by the work of Boivin *et al.*[2] as characteristic of the desoxypentose nucleic acid (D.N.A.) contents of haploid and diploid nuclei of a species, it is not surprising

[1] This report is from a dissertation to be submitted by David Elson in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.
[2] A. Boivin, R. Vendrely, and C. Vendrely, C. r. Acad. Sci. Paris *226*, 1061 (1948).

that much interest has centered on this nuclear constituent.

Recent reports have indicated that the D.N.A. content of the unfertilized sea urchin egg exceeds that of the sperm cell of the same species by more than a hundredfold. The following values may be quoted from the literature (in 10^{-6} micrograms per single cell). (1) On the basis of phosphorus distribution[1]: *Arbacia*[2] sperm, about 21 to 30; egg, 700 to 1000. *Arbacia punctulata* egg[3], 90. (2) On the basis of the reaction of desoxy sugar with diphenylamine[4]: *Arbacia aequituberculata*[5] sperm, 0·7; egg, 220. *Paracentrotus lividus* sperm[5], 0·67. It is probable that methods of this type, when applied to biological material of such low D.N.A. content as the egg, yield extremely questionable results, mainly owing to their lack of specificity and to the interference by substances other than those assayed.

In the present study, an attempt was made to investigate the problem by a different and more specific method, namely, a microbiological assay of thymine. An *E. coli* mutant (American Type Culture Collection No. 11117[6]), kindly supplied by Dr. R. R. ROEPKE of the American Cyanamid Company, Stamford, Connecticut, that required thymine for growth, formed the basis of the assay. The organism used here was a variant isolated by Dr. S. ZAMENHOF from cultures of this mutant. When cultivated on the synthetic medium specified by ROEPKE[7], to which DIFCO Proteose Peptone No. 3 had been added in a 0·02% concentration, the growth response of this organism, greater than that of the parent mutant, was proportional to the concentration of the thymine supplement over a limited range (0·04 to 0·25 μg of thymine per cubic centimetre of medium). Unpublished work by P. L. FITZGERALD and S. ZAMENHOF in this laboratory has disclosed no other growth-promoting substances; but certain purine and pyrimidine compounds, at relatively large concentrations, were found to inhibit multiplication.

The biological materials studied were sperm cells and unfertilized eggs of the sea urchin *Paracentrotus lividus*[8]. After preliminary separation of the acid-soluble, lipid, pentose nucleic acid and D.N.A. fractions[9], the D.N.A. portion was further purified by three extractions at 90° with 7% trichloroacetic acid[10]. After the hydrolysis of the lyophilized extract with conc. formic acid (98-100%)[11], the mixture was evaporated and the residue washed with ether and then extracted four times with water. The combined aqueous extracts are designated as hydrolysate in the following discussion. When a pure D.N.A. preparation or a fraction rich in D.N.A., whose thymine content had been determined accurately by paper chromatography and spectrophotometry[1], was subjected to the same fractionation procedure, similarly prepared hydrolysates were found to contain about 80% of the thymine present in the starting material.

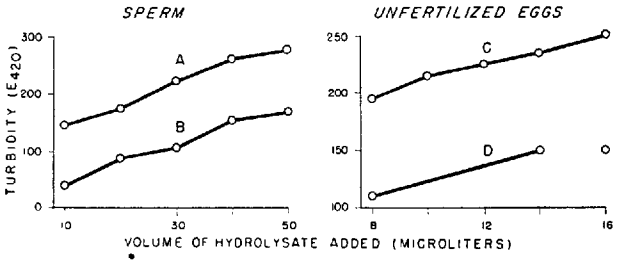

Fig. 1.–Each point represents optical density (at 420 mμ) of the bacterial culture in one tube containing (1) 0·4 cm^3 basal medium; (2) an aliquot of the hydrolysate assayed for thymine, the volumes being plotted as the abscissa; (3) an aliquot of an aqueous solution containing pure thymine in the following amounts per tube: *A*, 0·0430 γ; *B*, 0·0215 γ; *C*, 0·0784 γ; *D*, 0·0588 γ; (4) dist. water to final volume of 0·50 cm^3.

Each tube was inoculated with one drop of a 24 h nutrient broth culture of the *E. coli* mutant, diluted 10,000 times with physiological saline. In each assay, a third set of tubes from which the inoculum was omitted served as optical blanks for the turbidity measurements in the microcell attachment of the Beckman spectrophotometer. The cultures were incubated at 37°: *A* and *B* for 24 h, *C* and *D* for 15 h. Two intermediate points of curve *D* were lost by accident and a third (16 μl) was ignored in computing the results. One cm^3 of the hydrolysate represented 0·0439 mg of dry sperm (containing a total of 6·85 γ D.N.A.) and 150·4 mg of dry eggs (containing a total of 16·4 γ D.N.A.).

Since the hydrolysates prepared from the eggs contained unidentified substances causing partial inhibition of growth, the assays were conducted in a manner designed to diminish errors due to inhibitory or synergistic effects of substances other than tymine. The arrangement is exemplified in Figure 1 which shows both a thymine determination in sperm and the best assay of an egg hydrolysate. As can be seen, different aliquots of the hydrolysates were assayed, after the addition of known amounts of thymine, in two series, each at a different level of thymine concentration. When the turbidity of the bacterial culture (expressed as E_{420}) is plotted against the volume of the hydrolysate added, two parallel straight lines of positive slope should be obtained, affording a criterion of the validity of the assay. The differences in readings for adjacent points on the same curve are averaged to give the turbidity due to a known volume of hydrolysate. The differences between corresponding points on the two curves give the turbidity due to a known amount of thymine acting in the presence of whatever extraneous substances were introduced with the hydrolysate. Owing to the sensitivity of the method, a complete assay can be carried out with a hydrolysate containing a total of about 0·3 μg of thymine.

The thymine values so obtained were multiplied by 1·25 to correct for losses during the preparation and converted to values for total D.N.A. by multiplication with a factor of 8·2, derived from the detailed analysis of pure D.N.A. isolated from *Paracentrotus lividus* sperm in this laboratory[2]. Direct cell counts of sperm were made with an improved Neubauer hemocytometer. The lyophilized egg preparations could not be subjected to counting.

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A figure of 4500 eggs per milligram of dried *Paracentrotus* eggs was derived from data provided by ÖHMAN[1] and GUSTAFSON (private communication).

Paracentrotus sperm was analyzed by three independent methods, namely, by means of the microbiological assay described here, by the colorimetry of the reaction product with diphenylamine, and, most accurately, by the quantitative determination of the individual purines and pyrimidines[2] after the hydrolysis of the D.N.A. fraction with concentrated formic acid. The values found, as 10^{-6} µg D.N.A. per sperm cell, were, respectively, 1·0, 1·1, 1·0. This corresponds to a D.N.A. content of 15% of the dry weight.

The thymine content of *Paracentrotus* eggs was so low (corresponding to a D.N.A. content of about 0·01% of the dry weight) that only the microbiological assay could be employed; and even with this method the results are subject to some uncertainty. Five assays of two batches of unfertilized eggs, collected at different times, gave an average value of 28×10^{-6} µg of D.N.A. per egg. The most reliable individual determination corresponded to a D.N.A. content of 24×10^{-6} µg per egg. No significant difference between the two batches was observed.

The D.N.A. contents of the nuclei of sea urchin gametes are unknown, nor can the contribution by the polar bodies be estimated. When the sperm and egg analyses reported here are compared, it appears not unlikely that in sea urchin eggs D.N.A. is not limited to the nucleus. Indications of the existence of extranuclear D.N.A. in eggs have been reported recently[3]. What should, however, be stressed is that the differences in the D.N.A. contents of whole egg and of sperm recorded in the present study are of a less dramatic order of magnitude than those reported for sea urchins by other workers. It is obvious that the problem of the D.N.A. analysis of biological materials low in this constituent can hardly be solved in a general manner, before better methods become available[4].

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Résumé

Nous avons déterminé les quantités d'acide désoxyribonucléique contenues dans les spermatozoïdes et les œufs vierges de l'oursin *Paracentrotus lividus*. Ces déterminations, faites au moyen d'une méthode microbiologique pour le dosage de la thymine, ont donné les résultats suivants: sperme $1{,}0 \times 10^{-6}$, œuf environ 25×10^{-6} µg d'acide désoxyribonucléique par cellule.

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Zur Frage der Begleitstoffe in gonadotropen Hormonpräparaten

Es gibt eine Gruppe von Substanzen vom Charakter der Mucopolysaccharide, die von gewissen bakteriellen Enzymen und der «enzymatischen Komponente» der Viren der Influenzagruppe gespalten werden. Solche Stoffe können als Inhibitoren der Virus-Hämagglutination (HIRST) funktionieren und durch Einwirkung von «receptor destroying enzyme» (RDE.), einem Produkt von *Vibrio cholerae*, oder beim Kontakt mit Influenzavirus diese inhibitorische Eigenschaft einbüssen. Stoffe, wie Ovomucin[1], Francis Inhibitor (= unspezifischer Seruminhibitor der Influenzavirus-Hämagglutination[2]), Blutgruppensubstanzen, Ovarialzystenschleim[3], Serumgonadotrophin[4], diverse Urinextrakte[5], gelten deshalb als dem enzymatischen Abbau zugänglich. Im Falle des Ovomucins sind auch Abbauprodukte schon beschrieben worden[6].

Tabelle I

Inhibitorische Wirksamkeit verschiedener Harnextraktstoffe und gonadotroper Hormonpräparate gegen die Virus-Hämagglutination

Inhibitor	Inhibitionstiter, reziproke Verdünnungswerte
Extrakt aus Schwangerenharn: 400 Hormon-E/mg	64000
Extrakt aus Schwangerenharn: 750 E/mg	512000
Extrakt aus Harn klimakterischer Frauen, 200 E/mg	128000
Extrakt aus Männerharn	128000
Präparat aus Stutenserum: 200 E/mg	<1000
Präparat aus Schafshypophysen	<1000

Hemmung von 4 Hämagglutinationsdosen von erhitzter (30 min auf 56°C) PR8-Influenzavirus-Allantoisflüssigkeit gegen 0,8% Hühnererythrozyten.

In unseren chemischen Laboratorien von BENZ nach dem gleichen Verfahren gewonnene Harnfraktionen wurden auf ihre virus-hämagglutinationshemmende Wirkung untersucht, da nach dem auch für die Gewinnung gonadotropen Hormons geeigneten Verfahren vermutet werden konnte, dass sie mucopolysaccharidartige Stoffe enthalten könnten. Tabelle I zeigt, dass eine Reihe dieser Fraktionen stark hemmend auf die Influenzavirus-Hämagglutination wirken. Gonadotrop hormonal wirksame Harnpräparate unterscheiden sich hierin nicht von

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