has not been demonstrated for all the histones examined; but, as we have pointed out, such specificity is not excluded by our work.

A full account of this work is in course of preparation for publication elsewhere.

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Carbohydrate and Adenosinetriphosphate in Sea-Urchin Semen

SEA-URCHINS differ from mammals, in which fertilization is internal and the survival of spermatozoa largely depends on extraneous nutrients provided by accessory secretions, in that fertilization takes place after the eggs and sperm have been shed into sea water, which is a poor source of organic nutrients. Furthermore, unlike mammalian spermatozoa which in most species, including man, obtain their metabolic energy through anaerobic and aerobic glycolysis[1], sea-urchin spermatozoa depend on a non-glycolytic and strictly aerobic metabolism; sea-urchin semen is devoid of significant amounts of fructose or other fermentable sugars; and the spermatozoa are incapable of glycolysing sugars added *in vitro*[2].

In a search for glycogen or other intracellular reserve carbohydrate, seminal plasma and spermatozoa, obtained by centrifugation of semen (*Echinus esculentus*), were hydrolysed with potassium hydroxide; the ethanol-precipitable material obtained from the alkaline solution was analysed by several methods. Total carbohydrate was estimated by means of anthrone[3]; 'reducing sugar', 'yeast-fermentable sugar' and glucose were determined after acid hydrolysis of the ethanol-precipitable material; the amount of 'reducing sugar' was small, being 110 mgm./100 ml. spermatozoa (110 mgm. per cent), of which only 40 mgm. per cent was glucose, identified by the yeast-fermentation test and glucose oxidase[4]. The oxidation of this amount of glycogen or glycogen-like material would be inadequate to account for the high oxygen consumption of sea-urchin spermatozoa[5,6]. The seminal plasma of *Echinus esculentus* contains little free reducing sugar and no glycogen-like material, though about 200 mgm. per cent of some other anthrone-reactive carbohydrate is present.

The content of hexose-phosphate phosphorus in sea-urchin sperm was also determined and found to be only 2 mgm. per cent.

Studies on mammalian spermatozoa have established that both their glycolytic metabolism and motility are related directly to their content of adenosinetriphosphate; in normal ram semen the concentration of adenosinetriphosphate varies from 0·5 to 1·5 mgm. per cent of adenine amino-nitrogen[7]. So far, the presence of adenosinetriphosphate in sea-urchin semen has been deduced only from the occurrence of a 'barium insoluble, acid-labile ester'[8]. To obtain precise information about adenosinetriphosphate in sea-urchin semen, an attempt was made to isolate it, following the procedure of Parnas and Lutwak-Mann[9] for the quantitative estimation of adenosinetriphosphate in skeletal muscle. In a trichloroacetic acid extract from 20 ml. semen of *Echinus esculentus*, adenosinetriphosphate was separated as barium salt, and after removal of barium, the pentose, pyrophosphate-phosphorus and amino-nitrogen were estimated in the solution. There were 9·9 mgm. pentose, 3·8 mgm. pyrophosphate-phosphorus and 0·93 mgm. of adenine amino-nitrogen per 100 ml. semen. The molecular ratio of these three agreed satisfactorily with the theoretical ratio 1 : 2 : 1. Adenosinetriphosphate therefore occurs as a normal constituent of sea-urchin semen in a concentration similar to that found in ram semen.

Adenosinetriphosphate is usually associated with glycolytic metabolism. The existence of significant quantities of this substance in sea-urchin spermatozoa raises the question of the role of adenosinetriphosphate in these spermatozoa, the metabolism of which, being non-glycolytic, is very different from that of mammalian spermatozoa, muscle or yeast.

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Gluconokinase and the Oxidative Path for Glucose-6-Phosphate Utilization

STUDIES of the oxidation of glucose-6-phosphate and 6-phosphogluconate by extracts of many types of cells have indicated the existence of an oxidative pathway leading through pentose phosphate to triose phosphate, in addition to the anaerobic steps of the Embden–Meyerhof scheme. We have recently obtained an indication of the potential importance of the oxidative pathway in the economy of the cell from a study of gluconate utilization in *Escherichia coli*, strain B.

When a moist sediment of *E. coli* grown in glucose medium[1] was ground by hand for two minutes with a fine grade of alumina, essentially all of the organisms were disrupted and many enzyme systems could be extracted with 0·01 *M* phosphate buffer at *p*H 7·0. Among these systems have been demonstrated the oxidizing systems for glucose-6-phosphate and 6-phosphogluconate, hexokinase, the systems for anaerobic conversion of glucose-6-phosphate to triose phosphate, and a weak adenosine pyrophosphatase. These systems have been found to have essentially similar activity in the extract from comparable numbers of gluconate-adapted bacteria. In addition, this latter extract contained an enzyme, gluconokinase, catalysing the reaction:

gluconate + adenosinetriphosphate → 6-phosphogluconate + adenosinediphosphate

The acid liberated in this reaction has been studied in Warburg respirometers (see graph).

Aliquots of the products of anaerobic gluconokinase activity on gluconate and adenosinetriphosphate