areas the possibility of an artifact can be excluded. In addition, certain, probably malignant cells grown in tissue cultures also show very low enzymic activity:

(a) unclassified lymph node cells [10] S. A. = 0·33,
(b) cell culture derived from adenocarcinoma of human lung [11] S. A. = 0·53,
(c) Hela cells S. A. = 0·83,
(d) Ehrlich ascites cells S. A. = 0·59,
(e) Rat embryo fibroblasts S. A. = 4·9.

These preliminary observations suggest that the rate of anaerobic metabolism of cysteine is different in normal and in neoplastic cells. The possible biochemical and physiological significance of these observations is being studied.

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Zusammenfassung

Quantitative Analysen des β-mercaptobrenztraubensäure-spaltenden Enzyms zeigen, dass Zellen, die sich in aktivem mitotischem Prozess befinden (bzw. Krebszellen und Gewebekulturen), dieses Enzym in viel geringerem Masse enthalten als «normale» Zellen. Da dieses Enzym in dem anaeroben Stoffwechsel von Zystein eine wichtige Rolle spielt, lässt sich vermuten, dass sich der Zysteinstoffwechsel der Tumorzelle quantitativ von dem der Normalzelle unterscheidet.

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On a Glycoprotein of the Sea Urchin Eggs and its Changes Following Fertilization

Shaking with ether the trichloroacetic acid (TCA) soluble fraction of the unfertilized egg of *Paracentrotus lividus* results in the formation of a fluffy precipitate at the interface between the water and the ether phases. When the same procedure is applied to the TCA-soluble fraction of newly (5 min) fertilized eggs or developmental stages, no such precipitate appears. The analysis of the fluffy precipitate has given the following results (as % of dry weight):

Nitrogen:	5·6–7·4
Carbohydrates:	43·0–52·7
Glucosamine:	1·6

No phosphorus has been found and 2 h extraction with boiling alcohol-ether (3:1) resulted in no change in dry weight.

Only one carbohydrate, glucose, was identified by paper chromatography and this is considered as a good evidence that the fluff is not a contamination from material of the jelly-coat incompletely removed. In fact, the main carbohydrate component of the jelly-coat of *Paracentrotus* is fucose (MINGANTI and VASSEUR[1]). The following amino acids have also been identified by two dimensional paper chromatography of the acid hydrolysate of the fluff: cyst-(e)ine, arginine, lysine, histidine, aspartic acid, glutamic acid, glycine, serine, alanine, proline, methionine, valine, threonine, phenylalanine, leucine, iso-leucine.

In a second series of experiments the total polysaccharides present in the TCA soluble fraction of unfertilized and newly fertilized eggs were precipitated with alcohol. Equal amounts of eggs were used in each one of three parallel sets of experiments. The results given in the Table indicate that the amount of alcohol precipitable material has significantly decreased after fertilization and that an even greater decrease in the *N* content of the precipitate takes place.

Here again the only carbohydrate present was glucose and it made up almost the total dry weight of the precipitate.

On the other hand determinations of the total (free + bound) carbohydrates present in the TCA soluble fraction failed to show any significant change as a result of fertilization. The average of six determinations was (as % of the total carbohydrates of the eggs), 30 ± 1·47 in the unfertilized eggs and 27·2 ± 1·29 in those newly fertilized, $t = 0{\cdot}89$, $P = 0{\cdot}4$. It must be added that in four different determinations we have also been unable to detect any change in the total carbohydrate content of the eggs following fertilization.

Table

Amount of alcohol-precipitable material from the TCA-soluble fraction from equal amounts of unfertilized and newly fertilized eggs of *Paracentrotus lividus*

Exp. No.	mg (dry weight) of precipitate		% total N	
	Unfertilized	Fertilized	Unfertilized	Fertilized
1	12·0	8·8	1·35	0·62
2	10·4	8·5	2·20	1·35
3	15·9	12·5	1·93	1·21

The most likely interpretation of these results seems to be that upon fertilization some change takes place in a glycoprotein fraction present in the unfertilized egg as a result of which it becomes no longer precipitable either by the treatment with ether or alcohol.

By applying the Hotchkis method, RUNNSTRÖM and IMMERS[2] have described a staining of the outer rim of the cortex of the unfertilized egg. 5 min after fertilization this continuous layer is broken up into a great number of small granules which a little later become incorporated into the hyaline layer. This observation agrees well with our own results as it gives histological evidence of a change of some kind in a cortical glycoprotein of the egg upon fertilization.

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[2] J. RUNNSTRÖM and J. IMMERS, Exper. Cell Res. *10*, 354 (1956).

Experimental. Eggs of *Paracentrotus lividus* were washed several times with sea water, made up to an exact volume and then divided into two equal portions, one of which was fertilized and collected 5 min after the beginning of the elevation of the fertilization membrane while the other one was processed unfertilized. The jelly coat was removed by the usual treatment with acidified (pH 5·0) sea water, the eggs packed by a quick centrifugation and then suspended and homogenized in cold 10% TCA. Samples were withdrawn for the determination of total nitrogen and carbohydrates and then the homogenate was centrifuged at 12000 *g* in the cold for 10 min. The supernatant was collected and samples taken for the determination of total carbohydrates. The TCA-soluble fraction was then shaken 3 to 5 times with an equal volume of ether in a glass-stoppered cylinder. After the last shaking, the ether was withdrawn and the water phase with the fluffy layer at its surface was decanted to a centrifuge tube and left overnight in the refrigerator. Upon centrifugation, the fluffy layer collected at the surface: using a thin stemmed pipette which reached the bottom of the tube and by carefully regulating the suction of a water pump connected to it, the water phase could be withdrawn almost entirely. The fluff was then suspended in 50% alcohol and sedimented by centrifugation; this washing was repeated five times. The precipitate was then washed three more times with 95% alcohol, twice with 3:1 alcohol-ether, ether-alcohol (3:1), ether and dried.

For the preparation of the alcohol-precipitable components, two volumes of cold 95% ethanol were slowly added, with continual stirring, to one volume of the TCA soluble fraction in a centrifuge tube. After standing overnight in the refrigerator, the precipitate was centrifuged, the supernatant pipetted off, the precipitate resuspended in a small amount of 50% alcohol and transferred quantitatively to a smaller tube in which it was washed and dried as described above.

For the chromatographic analysis of carbohydrates, the material was hydrolyzed in 0·5 *N* H_2SO_4 at 110°C for about 12 h and the hydrolysate neutralized, desalted (by treatment with IR-B 4 and IR-120 Amberlites) dried, redissolved in a very small amount of water and chromatographed on Whatman No. 1 paper. The chromatogram was irrigated for 24 h with butanol-acetic acid-water (3:1:1) and developed with anilin-phtalate (PARTRIDGE[3]). For the quantitative determination of carbohydrates, a known amount of material was hydrolyzed as indicated above and the hydrolysate was neutralized, brought to volume and aliquotes were used for the analysis according to PARK and JOHNSON[4] using glucose as a reference. The anthrone method (HEWITT[5]) has been used in some cases. The chromatographic analysis of the amino acids was done on material hydrolysed with 6 *N* HCl and desalted by passage through a Dowex-50 column in the acid cycle. The two dimensional paper chromatography was run according to REDFIELD[6]. Glucosamine was determined according to GARDELL[7]. Nitrogen was determined by Nesslerization after combustion and phosphorus according to ALLEN[8].

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Riassunto

Sbattendo con etere la frazione solubile in acido tricloroacetico di uova vergini di *Paracentrotus lividus* si forma un precipitato all'interfacie tra etere e fase acquosa. L'analisi di questo precipitato ha dimostrato che si tratta di una glicoproteina. Il precipitato non si forma da estratti di uova fecondate.

Étude immunologique des Haptoglobines humaines individuelles

Les Haptoglobines individuelles[1] se manifestent dans le sérum humain sous forme de trois phenotypes principaux (Hp 1–1, Hp 2–2 et Hp 2–1) correspondant à deux types I et II d'haptoglobine.

Ceux-ci ont pu être identifiés à une forme monomère ou dimère de l'haptoglobine[2] et présentent à l'ultracentrifugation des constantes de sédimentation différentes[3]. L'étude immunologique de ces constituants (BEARN et FRANKLIN[3]) par les courbes de précipitation spécifique entre antisérums anti-haptoglobine de chaque type et ces haptoglobines a conclu à une identé antigénique de ces constituants.

Cette note a pour objet de rapporter les résultats de nos propres expériences concernant l'étude antigénique des types d'haptoglobine.

Protocole expérimental

L'étude du comportement immunoélectrophorétique de chaque type de sérum a été réalisée suivant les modalités décrites par GRABAR et WILLIAMS[4], soit avec un sérum de cheval anti-sérum humain total, soit avec des sérums de lapins préalablement immunisés par des sérums humains de différents types (anti-Hp 1–1, anti-Hp 2–1, anti-Hp 2–2).

La coloration spécifique du complexe haptoglobine-hémoglobine dans les lignes de précipitation était réalisée par la technique que nous avons décrite avec URIEL[5].

Enfin, les expériences de double diffusion en gélose ont été réalisées, soit par la méthode d'OUCHTERLONY[6] soit par la variante de KAMINSKI[7].

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