This separation produced three weakly basic, one neutral and five acid ninhydrin-positive components. Chromatography in the butanol-acetic acid-pyridine mixture made it possible to resolve the individual components, yielding 30 different ninhydrin-positive substances. These substances proved to be homogeneous on further chromatography in a different solvent system. Hydrolysis in 6 *N* HCl for 18 h at 105°C and chromatography in the butanol-acetic acid mixture provided evidence of the peptide character of these substances.

The isolated peptides contain 3–8 different amino acids, the following occurring most frequently: glutamic acid, cysteine, glycine (i. e. glutathione components), lysine, alanine, and aspartic acid. In isolated cases also arginine, valine, leucine, and threonine were identified. Only with 10 peptides was a sufficient amount obtained to determine the N-terminal amino acids by the dinitrophenyl method[11]. In 5 cases, glutamic acid was found to be the N-terminal amino acid; in other cases aspartic acid, lysine, serine, and alanine (Table).

Table

Intracellular Peptides of *E. coli*

glu. (cys, gly, lys)
glu. (ala, cys, gly, lys)
asp. (cys, gly, lys)
lys. (ala, arg, asp, cys, gly, glu, ser)
asp. (arg, gly, glu, γ-NH_2but, lys, val)
ser. (asp, gly, lys)
ala. (asp, lys)
glu. (ala, asp, cys, gly, lys, leu, val)
glu. (ala, asp, lys, cys, gly)
glu. (cys, gly)

During *E. coli* growth in the presence of chloramphenicol, these peptides can be found intracellularly in larger amounts. The chloramphenicol-resistant strain also contains these ninhydrin-positive substances in a greater degree than the sensitive one.

Metabolic activity and possible participation of these peptides in protein synthesis is being further investigated with the aid of C^{14}-labelled amino acids.

A complete presentation of the work will be published in the Collection of Czechoslovak Chemical Communications.

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Cechoslovac Academy of Sciences, Institute of Chemistry, Prague, September 3, 1959.

Zusammenfassung

Mittels Papierchromatographie und Hochspannungselektrophorese konnten in Trichloressigsäureextrakten von *E. coli* 3–8 Aminosäuren enthaltende Peptide isoliert werden, in denen am häufigsten Glutaminsäure, Glycin, Cystein, Lysin, Asparagin und Alanin vorkommt.

[11] F. Sanger and E. O. P. Thompson, Biochem. J. *53*, 353 (1953).

The Rate of Cleavage of β-Mercaptopyruvate by Rapidly Dividing Cells[1]

The enzyme which cleaves β-mercaptopyruvate to pyruvate and atomic sulfur has recently been purified in this laboratory[2]. The same enzyme catalyzes not only the cleavage of the C-S bond but also the transfer of S to an acceptor molecule[3]. The 'physiological' S acceptor as well as the biological importance of this enzyme is unknown. It appears, however, certain that the transsulfurase is one of the key enzymes involved in the anaerobic metabolism of cysteine. The role of thiol compounds in cell division has been often considered although an actual biochemical reaction where SH compounds play a specific role remains to be discovered. A probable exception is glutathione or some acid soluble SH group containing substance which shows quantitative correlation with the rate of mitosis as described by Rapkine[4] and Mazia[5].

We attempted to perform experiments in order to ascertain whether or not the metabolism of β-mercaptopyruvate is correlated with rapid cell division. Quantitative enzyme analyses were made on certain tissues of normal and cancer bearing mice as well as on cancer cells. The choice of the type of cancer cells in such studies is of considerable importance as pointed out by Furth[6] and Klein[7] who suggest that it is preferable to use newly induced tumors. Recently a rapidly growing tumor was induced in pregnant mice by the injection of a suspension of human lung cancer tissue[8,9]. The enzyme content of this tumor and its effect on the host were determined. As shown in the Table, the enzyme content of liver and kidney of normal and tumor bearing animals does not differ significantly. However, the activity of the tumor cells is in every case markedly lower than that of 'normal' tissues. Since the tumors analyzed did not contain necrotic

Table

Rate of pyruvate formation from β-mercaptopyruvate by normal and tumor tissues of mice

No. of Exp.	Liver		Kidney		Tumor
	Normal	Tumor bearing	Normal	Tumor bearing	(Ref. [8,9])
1	6·4	6·0	8·7	15·8	1·3
2	14·5	7·6	17·6	9·0	1·0
3	10·2	8·8	10·7	18·0	1·4
4	7·1	7·4	7·0	8·4	4·2
5	10·1	5·4	11·1	14·6	3·6
6	7·7	7·1	6·7	9·4	2·8
7	12·6	8·9	14·1	13·5	3·2
8	8·7	8·5	—	12·8	1·0
9	6·2	9·4	—	12·6	2·9
10	9·3	11·1	—	17·0	—
11	8·3	7·0	—	8·8	—
	9·2 (± 2·5)	7·9 (± 1·5)	10·8 (± 2·5)	12·7 (± 3·2)	

The results are expressed as 'specific activity' (S.A.) i.e. μmoles of pyruvate formed per 1mg protein per 10 min at 30°C in the presence of 2-mercapto-ethanol. The assay method has been described earlier[2]

[1] Supported by U.S. Public Health Grants H-2897, C-3211 and by a grant of the American Cancer Society (70-4612-24).

[2] E. Kun and D. W. Fanshier, Biochem. biophys. Acta *32*, 338 (1959).

[3] E. Kun and D. W. Fanshier, Biochem. biophys. Acta *33*, 26 (1959).

[4] L. Rapkine, Ann. physiol. physiochim. Biol. *9*, 383 (1931).

[5] D. Mazia, *SH and Growth* in *Glutathione* (A Symposium) (Acad. Press, N. Y.), 1954, p. 209; *The Role of Thiol Groups in the Structure and Functions of the Mitotic Apparatus* in *Sulfur in Proteins* (Acad. Press, N. Y. 1959).

[6] J. Furth, Cancer Res. *19*, 241 (1959).

[7] G. Klein, Cancer Res. *19*, 343 (1959).

[8] C. Klausner and V. Richards, Extr. Bull. internat. Chirurgie *17*, 174 (1958).

[9] V. Richards and C. Klausner, Surgery *44*, 181 (1958).

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.

[10] S. D. BELL, JR. and R. E. JOHNSON, Proc. Soc. exp. Biol. Med. *92*, 46 (1956).
[11] A. W. FRISCH, V. JENTOFT, R. BERGER, and E. J. LOOSLI, Amer. J. clin. Pathol. *25*, 1107 (1955).
* Established Investigator of the American Heart Association. Inc., New York.

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.

[1] A. MINGANTI and E. VASSEUR, Acta Embryol. Morphol. exper. *2*, 195 (1959).
[2] J. RUNNSTRÖM and J. IMMERS, Exper. Cell Res. *10*, 354 (1956).