Table III
Anaphylaxis in Passively Sensitized Mice

Pre-Shock Treatment (IP Injection)	Pre-Shock Temperature (°C) Mean S. E.	Temperature 30 min after shock Mean* S. E.	Difference of Means	Deaths	Shock/ total	(% Shock)	P**
Exp. 9 ♂ *and* ♀ *Mice*							
None	36·4 ± 0·2	33·8 ± 0·8	−2·6	0	4/5	(80)	—
Cysteine 30 min.	35·0 ± 0·2	33·2 ± 0·6	−1·8	1	3/4	(75)	0·50
Cysteine 60 min.	35·0 ± 0·6	33·6 ± 1·2	−1·4	0	1/5	(20)	0·25
Cysteine 120 min.	35·0 + 0·5	34·2 ± 0·7	−0·8	0	2/5	(40)	0·70
Tee 30 min.	34·0 ± 0·6	32·0 ± 0·7	−2·0	1	4/5	(80)	—
Tee 60 min.	36·4 ± 0·5	35·4 ± 0·8	−1·0	0	3/5	(60)	—
Tee 120 min.	37·2 ± 0·2	34.0 ± 0·3	−3·2	0	5/5	(100)	—
Lee 30 min.	36·0 ± 0·3	33·6 ± 0·8	−2·4	0	3/5	(60)	—
Lee 60 min.	36·2 ± 0·5	36·5 ± 0·4	+0·3	0	0/5	(0)	0·10
Lee 120 min.	36·0 ± 0·3	33·8 ± 0·7	−2·2	1	4/5	(80)	—
Saline 30 min.	36·5 ± 0·4	34·9 ± 0·7	−1·6	0	4/5	(80)	—
Saline 60 min.	36·7 ± 0·2	32·4 ± 0·6	−4·3	3	5/5	(100)	—
Saline 120 min.	37·0 ± 0·3	34·7 ± 0·9	−2·3	1	4/5	(80)	—

* Deaths considered as — 5·0 °C temperature drop.

** Determined from Chi-square values using Yates correction.

exist, however, between preshock temperature and susceptibility to anaphylaxis. It was noted, furthermore, that the usual clinical indications of anaphylaxis in mice (e.g. lethargy and paralysis) were absent except in those animals showing a depression of body temperature following injection of the shocking material.

A definitive explanation of the modifying effect of these drugs cannot now be offered for lack of direct proof. It is possible that their action may be concerned with their capacity to inhibit $C^1$1, which is activated by antigen-antibody complexes[2]. It is tempting to speculate that the shocked animals may have been refractory to further attempts to produce an anaphylactic response within 24 h in part because of depletion of C^1 1.

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W. M. MEYERS[6] and K. L. BURDON

Department of Microbiology, Baylor University College of Medicine, Houston (Texas), August 18, 1959.

Résumé

L'injection intrapéritonéale chez la souris de cystéine, tyrosine-éthylester ou lysine-éthyl-ester, 1 h avant la production du choc anaphylactique diminue d'une façon significative la sensibilité des animaux à la réaction. Il est possible que cela soit dû à une modification de l'effet du premier composé actif du complément.

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Intracellular Peptides of Escherichia coli

One of the ways which could contribute to the elucidation of the mechanism of protein synthesis consists in finding intermediate products of peptide nature. Although free peptides have been detected in various organisms, their relationship to protein synthesis has not been demonstrated conclusively[1–5]. Gram-negative bacteria contain much less intracellular free ninhydrin-positive substances than Gram-positive ones, and glutathione is the only substance of peptidic character which has definitely been detected so far[6]. Further, non-identified ninhydrin-positive substances were described from *E. coli* cells[6] and from the nutrient medium remaining after the growth of these bacteria[7]. Recently, two pure dipeptides were found and identified which accumulate in *E. coli* cells particularly during growth in the presence of chloramphenicol[8].

In further work, other ninhydrin-positive substances from *E. coli B* extract prepared in cold with 5% trichloracetic acid were investigated. This extract of bacteria from 20 l of synthetic medium was treated with ethyl ether to remove trichloroacetic acid, freeze-dried and oxidized with performic acid[9], so that glutathione and possibly other cysteine-containing peptides were present in a single oxidized form. After de-salting on Dowex-50 in the hydrogen form, amino acids and peptides were eluted with ammonia, volume reduced by evaporation and chromatography carried out in a mixture of butanol-acetic acid. In this way free amino acids were separated from other ninhydrin-positive substances which remained at the start of the chromatogram.

After elution, substances from the chromatogram start were separated by high-voltage paper electrophoresis in a pyridine-acetate buffer at pH 5·6 (2·5 h, 29 V/cm)[10].

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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.

D. Grünberger, Jiřina Černá and F. Šorm

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.

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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).

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