Table II

Dose of CPZ mg/kg	Optical density of the plasma				
	before admin.	10 min after of protamine	15 min	25 min	35 min
			after administration of CPZ		
7·0	0·833	0·497	1·041	1·033	0·892
7·5	1·440	1·473	1·723	1·926	1·577
7·5	2·886	2·858	2·862	3·312	3·102
10·0	1·383	1·193	2·033	1·850	1·440
10·0	1·200	1·630	1·657	2·070	2·017

derivatives, and he also suggests that these drugs increase the heparin content of the blood. According to COURVOISIER[5], the coagulation time of blood is prolonged by administration of chlorpromazine.

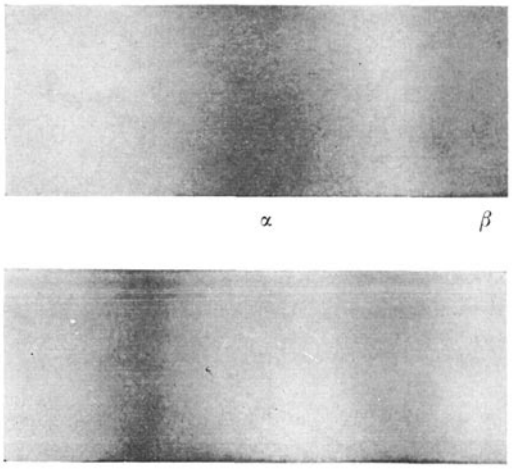

Fig. 2. – Changes in lipoprotein pattern caused by 10 mg/kg chlorpromazine shown by paper electrophoresis. *1:* before, *2:* 25 min after chlorpromazine administration (180 V, 10 mA, 20 h, Sudan Black stain).

Since the phenothiazine derivatives are widely used in therapy, several observations suggested that thromboembolic complications frequently occur[6] following the use of these drugs. Our clinical observations also support these findings. On the basis of our experiments, it may be assumed that owing to the increased release of heparin during phenothiazine-treatment the heparin-stores throughout the body become diminished; thus the interruption of continuous administration may be followed by a decrease of the heparin blood-level. The possibility that such a mechanism may be involved in post-phenothiazine thromboembolic conditions might also be taken into consideration.

B. M. KOVÁCS, G. S. KOVÁCS, and G. PETRI.

Institute of Experimental and Operative Surgery, University Medical School, Szeged, Hungary, June 19, 1956.

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[6] H. LABORIT and P. HUGUENARD, *Pratique de l'hibernothérapie en chirurgie et en médecine* (Masson et Cie, Paris 1954). – R. S. LAMBIE, L. G. JOSEPH, and G. WILSON, Brit. med. J. *1*, 840 (1956). – W. OSTEN, Ärztl. Wschr. *11*, 152 (1956).

Zusammenfassung

Bei Hunden ergibt Chlorpromazin *in vivo* eine Klärung des lipämischen Plasmas. Dieser Effekt des Präparates wird durch vorausgehende, intravenöse Protaminsulfatinjektionen aufgehoben. Es darf angenommen werden, dass diese Wirkung durch Mobilisierung von endogenem Heparin vermittelt wird. Möglicherweise ist dieser Mechanismus an den thromboembolischen Komplikationen nach Chlorpromazinbehandlung beteiligt.

The Oxidation of Cystamine and Other Sulfur-Diamines by Diamine-Oxidase Preparations

Although it is known that cystamine is oxidized by the rat *in vivo* to taurine[1], hypotaurine[2] and sulfate[3], enzymatic systems capable of carrying oxidative reaction on cystamine *in vitro* are at present unknown. Only recently, SALVADOR and BRADY[4] have reported the oxidation of cysteamine to cystamine disulfoxide by a pigeon liver preparation. No experimental data are however reported in their paper.

The incubation of cystamine with a rat liver homogenate in a Warburg system results in a slight increase of the O_2 uptake over the endogenous respiration only in the first period of incubation, followed by a depression in the second part (unpublished experiments). This result may possibly be interpreted by a partial oxidation of cystamine by the diamine-oxidase, which is contained only in small amounts in the rat liver[5], to compounds which are toxic to the liver enzymes.

In order to test the ability of diamine-oxidase to catalyze the oxidation of cystamine, and to gain evidence on the possible role of diamine-oxidase in the biological degradation of cystamine and other sulfur-diamines, a comparative study has been carried out by incubating cadaverine and sulfur-containing diamines with diamine-oxidase preparations extracted from traditional sources.

Pig-kidney diamine-oxidase.–The enzyme system was prepared in a crude form according to ZELLER[6], with minor modifications. As it is shown in Figure 1, cystamine is an excellent substrate for this enzyme, the rate of oxidation being comparable to that of cadaverine. Using cadaverine as substrate, the final O_2 uptake is close to the theory of 0·5 *M* per mole substrate; using cystamine we invariably found a higher O_2 consumption which was more than double the theoretical amount.

The higher O_2 uptake in the case of cystamine is not caused by the accumulation of H_2O_2 in the incubation mixture. This has been ruled out by tipping a solution of catalase at the end of one of these reactions: no evolution of O_2 was noticed after the catalase addition. Furthermore the higher O_2 consumption is also not attributable to the presence in the enzymatic preparation of a system capable of oxidizing the disulfide group contained in the initial substrate. This possibility has been eliminated by using N-diacetylcystamine, which was left unoxidized by the same preparation.

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Analytical data obtained on the deproteinized incubation mixture at the end of the O_2 uptake are reported below:

O_2 uptake	Theory: 5 μM	Found: 12 μM
NH_3	10 μM	9·6 μM
Cystamine. . .	0 μM	0 μM
Taurine	?	0 μM
Hypotaurine. .	?	0 μM
–SS-groups . .	100%	21 %
Sulfate	0 μM	0 μM
H_2S	0 μM	0 μM

Analysis of a deproteinized incubation mixture of pig-kidney diamine-oxidase with cystamine. Time: at exhaustion O_2 uptake (6 h). Substrate 10 μM. Other conditions as in Figure 1.

The inspection of the table shows that one mole of ammonia is produced per mole substrate at the end of the oxidation. This is indicative of a typical reaction of the diamine-oxidase on cystamine as follows:

$$NH_2{\cdot}CH_2{-}CH_2{-}SS{-}CH_2{-}CH_2{\cdot}NH_2 \xrightarrow{O} NH_2{\cdot}CH_2{-}CH_2{-}SS{-}CH_2{-}CHO + NH_3.$$

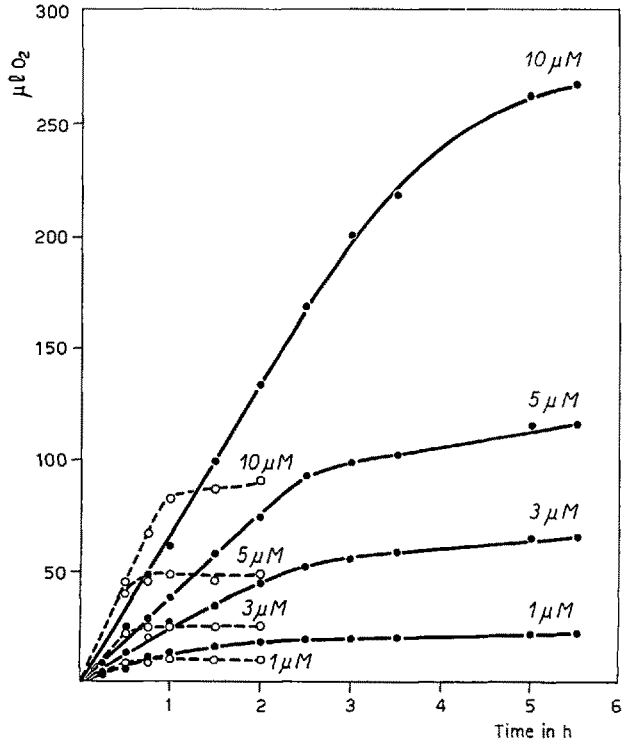

Fig. 1. – Oxidation of cadaverine and cystamine by diamine-oxidase. Each vessel contained: 0·2 g pig-kidney aceton powder[6], stirred in 2 ml phosphate 0·1 M, pH 7·4 for 30 min, and centrifuged. Substrates in the specified amount. Water to 3 ml. 0·2 ml NaOH 25% in the center well. Temperature 38°. Gas, air. Full line, cystamine; dotted line, cadaverine.

We have a preliminary indication that, also with cystamine, as in the case of cadaverine and putrescine[7], the compound formed exists in the form of a cyclized internal Schiff-base; this will be referred later.

The discrepancy between the ammonia production, in agreement with the theory, and the higher O_2 uptake may possibly be imputed to the further oxidation of the reaction product. Nevertheless taurine and hypotaurine are not detectable in the deproteinizate by paper or ion-exchange chromatography. Though –SS-groups are notably decreased at the end of incubation, this finding is only apparent since it is better explained by the binding properties of the reaction product with the proteins of the preparation, which have been removed by the deproteinizer. That the oxidation of cystamine is complicated by side-reactions will be shown later; it is also indicated by the intense brown color assumed by the mixtures incubated with cystamine in contrast with those incubated with cadaverine.

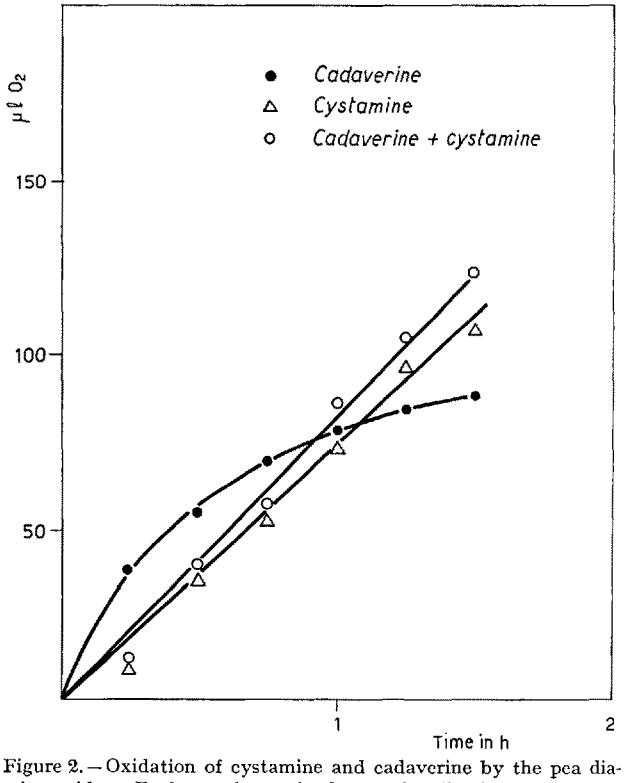

Figure 2. – Oxidation of cystamine and cadaverine by the pea diamine-oxidase. Each vessel contained 5 mg dry diamine-oxidase preparation[8], stirred in 2 ml phosphate 0·1 M, pH 7·4, and filtered. Substrates, 10 μM. Temperature 25°C. Other conditions as in Figure 1.

Pea seedlings diamine-oxidase. – With pea diamine-oxidase extracted according to KENTEN and MANN[8], the results are comparable to those obtained with the animal oxidase.

Figure 2 reports the result of an experiment performed by incubating with the enzyme preparation cystamine and cadaverine either separately or together. The initial rate of O_2 consumption when both the substrates are incubated together with the preparation is of the order found for cystamine alone and lower than that found for cadaverine. This shows that cystamine is also a good substrate for the plant oxidase and suggests that the enzyme involved is the same for the two substrates.

In order to test the specificity of diamine-oxidase towards sulfur-containing diamines, the following dia-

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mines have been compared for their ability to be oxidized by the extracted enzyme:

$NH_2{\cdot}CH_2{-}CH_2{-}CH_2{-}CH_2{-}CH_2{\cdot}NH_2$	cadaverine
$NH_2{\cdot}CH_2{-}CH_2{-}SS{-}CH_2{-}CH_2{\cdot}NH_2$	cystamine
$NH_2{\cdot}CH_2{-}CH_2{-}S{-}CH_2{-}CH_2{\cdot}NH_2$	lanthionamine
$NH_2{\cdot}CH_2{-}CH_2{-}SO{\cdot}SO{-}CH_2{-}CH_2{\cdot}NH_2$	cystamine disulfoxide
$NH_2{\cdot}CH_2{-}CH_2{-}SO_2{-}CH_2{-}CH_2{\cdot}NH_2$	lanthionamine sulfone

The reaction was followed at pH 5·6 in order to avoid dismutation of cystamine disulfoxide.

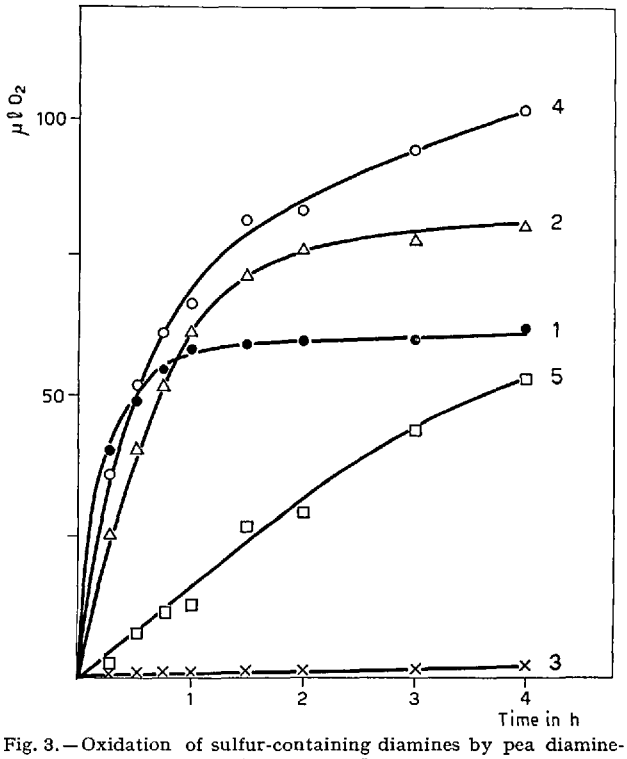

Fig. 3. – Oxidation of sulfur-containing diamines by pea diamine-oxidase. Enzyme preparation 20 mg. pH 5·6. Substrates 5 μM. Temperature 25° C. Other conditions as in Figure 1. *1* Cadaverine; *2* cystamine; *3* cystamine disulfoxide; *4* lanthionamine; *5* lanthionamine sulfone.

As reported in Figure 3, lanthionamine represents the best substrate among the sulfur-diamines. The presence of oxidized sulfur in the molecule results in a depression of activity, in the case of lanthionamine sulfone, and in a complete abolition of activity, in the case of cystamine disulfoxide.

Experiments are continued along this line in order to detect the final products and to relate these findings with the metabolism of cystamine *in vivo*.

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Riassunto

Le diamine solforate cistamina e lantionamina sono ossidate con velocità comparabile a quella della cadaverina dalla diamino-ossidasi. Il solfone della lantionamina è ossidato più lentamente ed il disolfossido della cistamina non è ossidato affatto. Benchè dall'ossidazione della cistamina si produca una mole di ammoniaca per mole di substrato, il consumo di ossigeno è due volte più elevato del teorico, indicante una ulteriore ossidazione del prodotto metabolico della reazione. Tuttavia taurina, ipotaurina e solfati, non sono determinabili nel deproteinizzato finale.

The Influence of Pyridoxine on the Free Amino Acids of Decotylized Pea Seedlings

In other (unpublished) investigations, it was established that cotyledon-less pea seedlings (if the excision of cotyledons carried out at the 3rd day of germination) are deficient in free glutamic acid, at least for a few days.

According to Burkholder and McVeigh[1] and Cheldelin and Lane[2], an accumulation of vitamin B_6, pyridoxine, is observable in the germinating seeds. The pyridoxine and its several derivatives function as cotransaminases, and consequently play an important role in the intensive transamination of the seedlings[3]. Wilson *et al.*[4] have prepared from 6–10 day old seedlings an enzyme fraction, which, in the presence of pyridoxamine or pyridoxal-5-phosphate, transfers the amino groups to α-ketoglutaric acid.

Fries[5] showed that cotyledon-less pea seedlings require in embryo culture a significantly high amount of pyridoxine.

I have confirmed experimentally in the following manner. Seedlings of "Folger" pea were deprived of their cotyledons after 3 days of germination (at room temperature) and exposed on light in Knop solution. 3 days after, one group of the plants was infiltrated in vacuum (two times during 24 h) with 0·1% pyridoxine dissolved in water. The other group (control) was given only distilled water (also by vacuuminfiltration) at the same time. 24 h later I analyzed the seedlings of the two variants with respect to their free amino acids, using the method of Awapara[6]. The solvent of the (25 cm diameter) paper chromatograms was butylic alcohol–water–acetic acid (4:1:5). The fresh weight of the extracted plant material was of 0·5 g.*

The results of the investigation showed that while the water-infiltrated plants were deficient of free glutamic acid, in the plants provided with pyridoxine glutamic acid appeared also among the other free amino acids (arginine, serine, tyrosine, leucine),

It is probable that in cotyledon-less pea seedlings there is no sufficient amount of pyridoxine.

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* The reproducibility of these results was good and free of controversy. The investigation was carried out three times, each of them contained 8 repetitions uniformly showing the results presented above.