Investigation of the N and P Losses in Different Media Used for Inert Dehydration

The inert dehydration (ID) technique described by PEASE[1] in 1966 is claimed as one of the preferable methods for ultrastructure research. It was proved in our former experiments[2-5] that serious losses of different substances may result in the course of dehydration of biological material. We therefore tried to find out the effect of different media used in ID on the losses of N and P.

Material and method. The liver of Wistar strain male rats weighing 150–200 g was used in all experiments. Fragments of 17–40 mg wet weight taken from the same lobe were divided into ca. 10 parts to enable better contact with the medium. For each medium 26 fragments were used. 6 of them were used as control, i.e. that after drying their total N and P was determined and taken as 100%, and the remainder used for the experiments. 10 fragments were put into chosen medium at room temperature for 3 min, the other 10 for 30 min dehydration. The fragments were then rinsed in ethanol, dried, and their N and P determined. As in the original paper of PEASE, the following media were used: anhydrous ethylene glycol, its 50% solution in water, glycerol, 80% dextrose and DMSO (dimethylsulphoxide) N and P were determined by methods described elsewhere[3]. Statistical analysis was performed after HILL[6].

Results. The results are summarized in the Table and the Figure. The total N and P content of rat liver incubated in different media is given. No changes are observed in tissues dehydrated in anhydrous ethylene glycol, glycerol and DMSO for 3 min. A period of 30 min treatment in these media causes statistically significant losses, e.g. 6.5–11.6% for N and 8.6–17.8% for P. One would like to stress the relatively high losses of P after 3 min incubation in 50% ethylene glycol and 80% dextrose solution, reaching 10.6 and 14.0% respectively. No losses of N were found in any other of the media used after 3 min incubation. The prolongation to 30 min dehydration of tissues results in high losses of P and N into media used for ID. The highest loss for N (reaching 16.9%) was found in 50% ethylene glycol. The generally low losses of N probably signify that proteins are not removed from tissues by ID media. P losses are much higher. The highest observed is 27.7% loss of P after 30 min incubation in 50% ethylene glycol and 80% dextrose. Considerable losses of P as a result of quick replacement of water and water soluble substances may induce unpre-

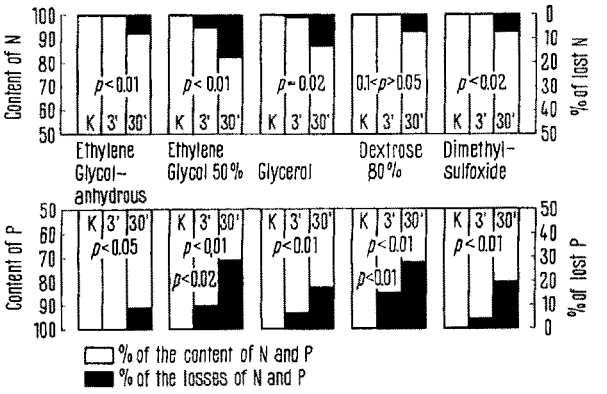


Diagram representing the content and losses of total N and P in rat liver treated in different ways.

[1] D. C. PEASE, J. Ultrastruct. Res. *14*, 356 (1966).
[2] K. OSTROWSKI, J. KOMENDER and K. KWARECKI, Acta biochim. pol. *8*, 83 (1961).
[3] K. OSTROWSKI, J. KOMENDER, H. KOŚCIANEK and K. KWARECKI, Experientia *18*, 142 (1962).
[4] K. OSTROWSKI, J. KOMENDER, H. KOŚCIANEK and K. KWARECKI, Experientia *18*, 227 (1962).
[5] J. KOMENDER, H. KOŚCIANEK-MALCZEWSKA and K. OSTROWSKI, Experientia *21*, 249 (1965).
[6] A. B. HILL, *Statystyka dla lekarzy* (PWN, 1962).

Total nitrogen and total phosphorus content in rat liver processed in different ways

Different procedures		No. of experiments	Nitrogen content, % of fresh weight				Phosphorus content, μg% of fresh weight			
			Mean value	Standard deviation	Standard error	P^a	Mean value	Standard deviation	Standard error	P^a
Control		5	3.30	0.07	0.03	–	601.7	28.1	12.8	–
Anhydrous glycol	3 min	5	3.33	0.06	0.03	–	603.2	34.8	15.8	–
Anhydrous glycol	30 min	5	3.07	0.05	0.02	0.01	549.9	27.9	12.7	0.05
Control		6	3.38	0.12	0.05	–	650.6	47.4	19.8	–
Glycol 50%	3 min	10	3.24	0.18	0.05	–	582.0	33.7	10.1	0.02
Glycol 50%	30 min	10	2.81	0.14	0.04	0.01	470.0	37.5	12.5	0.01
Control		6	3.52	0.25	0.10	–	608.1	41.8	17.4	–
Glycerol	3 min	10	3.50	0.10	0.03	–	569.9	60.5	18.3	–
Glycerol	30 min	10	3.11	0.29	0.09	0.02	506.9	28.5	8.6	0.01
Control		6	3.25	0.11	0.05	–	637.1	31.6	13.2	–
Dextrose 80%	3 min	10	3.25	0.15	0.05	–	548.0	22.1	6.7	0.01
Dextrose 80%	30 min	10	3.06	0.29	0.09	0.1, 0.05	465.3	34.8	10.5	0.01
Control		6	3.24	0.10	0.04	–	590.0	36.0	15.0	–
DMSO	3 min	10	3.25	0.19	0.06	–	568.4	40.7	12.3	–
DMSO	30 min	10	3.03	0.20	0.06	0.02	485.2	36.5	11.1	0.01

[a] Significant P values in relation to the control.

dictable changes in ultrastructure. Taking into account that the losses of P could be found already after 3 min of incubation in the last mentioned media, one would not recommend them for ID.

Conclusions. (1) No changes in N and P content are found after 3 min dehydration in anhydrous ethylene glycol, glycerol and DMSO; (2) one can find considerably high losses of N and P after 30 min incubation in 50% and anhydrous ethylene glycol, 80% dextrose and DMSO; (3) especially high losses of P in 50% ethylene glycol and 80% dextrose show that these media could not be recommended for ID technique.

Résumé. Les auteurs ont examiné les possibilités de diminution du contenu en azote et phosphore dans le foie du Rat au cours de l'«inert dehydration». La diminution la plus marquée a été observée après l'incubation dans une solution de glycol éthylène à 50% et de dextrose à 80%.

H. KOŠCIANEK-MALCZEWSKA, J. KOMENDER and K. OSTROWSKI

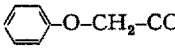
Department of Histology and Embryology, Medical Academy, Warsaw (Poland), 12th September 1966.

Photochemical Transformations of 6-Aminopenicillanic Acid and Phenoxymethylpenicillin

As part of an investigation concerning the influence of UV-irradiation on various penicillins, an aqueous solution of the potassium salt of 6-aminopenicillanic acid (6-APA; Ia) was irradiated in a quartz container with UV-light from a medium pressure mercury lamp (Hanovia, Type 509) at 15 °C. Bioautography of paper chromatograms of the irradiated solution on agar plates inoculated with *Staph. aureus* revealed that a new antibiotically active compound, somewhat more polar than 6-APA had been formed during the irradiation (Figure 1). The zone of inhibition due to the new compound, as well as that due to 6-APA, was considerably increased when the paper chromatograms before incubation were sprayed with aqueous $NaHCO_3$ followed by phenoxyacetylchloride or phenylacetylchloride in acetone according to BATCHELOR et al.[1].

The phenoxyacetyl derivative of the new compound, in the following called WG 942, was prepared and isolated as follows: after extraction with ether the irradiated solution was adjusted to pH 4.0 to precipitate most of the unreacted 6-APA, whereafter the filtrate was treated at pH 8 and 5–10 °C with excess of phenoxyacetylchloride in acetone. The resulting solution was washed with ether and, after acidification to pH 2.5, extracted with ethyl acetate. The extract, which in addition to WG 942 contained phenoxymethylpenicillin (Ib), phenoxyacetic acid, and other compounds, was subjected to a counter-current distribution (solvent system: citrate buffer (pH 5.0)-ethyl acetate) to separate the components. As revealed by bioautography of paper chromatograms, a complete separation of WG 942 from phenoxymethylpencillin was achieved after 40 upperphase transfers, and the compound could finally be isolated in the form of a crystalline potassium salt.

The elementary analysis of this salt corresponds well with the formula $C_{18}H_{20}N_3O_6SK$, $2H_2O$; its UV-spectrum is essentially the same as that of the potassium salt of Ib, and a strong band at 1773 cm^{-1} in the IR-spectrum (KBr) indicates the presence of a β-lactam ring. The fact that the compound is rapidly inactivated by *Bacillus cereus* penicillinase suggests that the fused thiazolidine-β-lactam ring system is intact. This assumption is supported by the NMR-spectrum[2] (D_2O) which contains signals closely corresponding to those present in the spectrum of the potassium salt of Ib: Singlets at $\delta = 1.78$ and 1.87 due to the methyl groups at C-2, a singlet at $\delta = 4.51$ due to the proton at C-3, a two-proton singlet at $\delta = 4.97$ arising from the methylene group in the grouping



an AB-system consisting of doublets centred at $\delta = 5.78$ and 5.83 ($J_{AB} = 4$ c/s) due to the protons at C-5 and C-6, and signals corresponding to 5 aromatic protons. In addition, a two-proton singlet at $\delta = 4.33$, not present in the spectrum of Ib, consistent with the presence of the grouping $-CONDCH_2CO-$[3] could be detected. The presence of latter signal suggested that WG 942 and its progenitor are represented by the structures Ic and Id, respectively. This assumption was verified by comparison of WG 942 with an authentic sample of Ic, prepared by acylation of 6-APA with phenoxyaceturic acid by the mixed anhydride method[4]. The 2 samples were identical

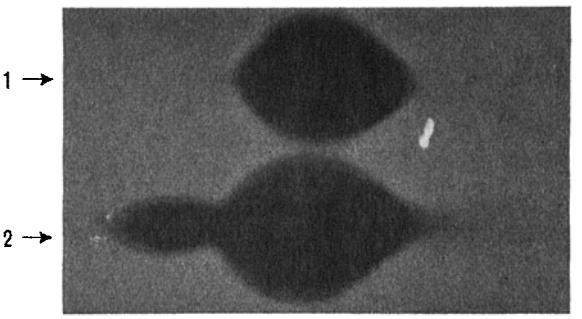


Fig. 1. Paper chromatography of an irradiated solution of 6-APA. Solvent system: *n*-Butanol-ethanol-water (5:1:4). Whatman No. 1 paper. Test organism: *Staph. aureus*. Before the incubation the paperchromatogram was sprayed with aqueous $NaHCO_3$ followed by 1% phenoxyacetylchloride in acetone. 1) 6-APA. 2) Irradiated solution of 6-APA.

[1] F. R. BATCHELOR, F. P. DOYLE, J. H. C. NAYLOR and G. N. ROLINSON, Nature, *183*, 257 (1959).

[2] The NMR-spectra were obtained with Varian HR-100 (100 Mc) and Varian A-60 (60 Mc) spectrometers, D_2O and $CDCl_3$ being used as solvents. In the former case hexamethyldisiloxane was used as external Lock signal whereas in the latter case tetramethylsilane was used as internal reference. We are indebted to Dr. J. R. ANDERSEN, University of Copenhagen and Dr. A. MELERA, Varian AG, Zurich, for the spectra.

[3] NMR-spectra Catalog (Varian Associated, Palo Alto. Calif., USA 1963) vol. 2, No. 413.

[4] Belg. Pat. No. 593,295.