absent in direct spectrum (figure 1). These peaks are probably the result of the binding of the porphyrin ring to the protein.

The pH of the solution had no effect on the behaviour of the absorption spectra. In the analysis of results account was taken of trypsin inactivation per se induced by Hp: the mean loss of trypsin activity in presence of Hp was 27%. Dose-response curves are shown in figure 2, where residual enzyme activity is plotted on a logarithmic scale against radiation doses (kR) in the 2 series of experiments: both curves are exponential with a D_{37} of 17,200 R in absence and 61,900 R in presence of Hp: the G values are respectively 0.19 and 0.05. Hp shows therefore a highly significant radioprotective action on trypsin irradiated in water solution ($p < 0.005$), with a dose reduction factor (DRF) of 3.6.

Discussion. This radioprotective action of Hp may depend partly on a radical scavenging mechanism by free Hp in solution, partly on a direct interference of the porphyrin ring in the dissipation of excitation energy of the protein following the binding of trypsin to Hp.

The true role of these mechanisms will be better defined by experiments performed using different molarity ratios of Hp versus trypsin and irradiating the solutions under vacuum or in pure oxygen.

The evidence of this radioprotective action suggests, as previously proposed by Cittadini[8], that the inconstant radiosensitizing effect of Hp observed in whole-body irradiated mice might be ascribed to an overlap of a Hp-induced radiodynamic disease. Finally, it is remarkable that the DRF obtained in our experiments is quite comparable to that known for the best radioprotective compounds.

1 Acknowledgments. The authors wish to acknowledge the invaluable suggestions and discussion offered by Prof. G. Cittadini.

2 A.B. Robins and J.A.V. Butler, Radiation Res. *16*, 7 (1962).
3 T. Sanner, Radiation Res. *26*, 95 (1965).
4 L. Weil and R.A. Buchert, Archs. Biochem. Biophys. *34*, 1 (1951).
5 A. Castellani, in: Progress in photobiology, p. 546. Elsevier, Amsterdam 1961.
6 G. Cittadini, unpublished data.
7 B.F. Erlanger, N. Kokowsky and W. Cohen, Archs. Biochem. Biophys. *95*, 271 (1961).
8 G. Cittadini, L. Lanfredini and G. Mancini, in: Radiation Protection and sensitization, p. 295. Ed. H.L. Moroson and M. Quintiliani. Taylor and Francis Ltd, London 1970.

The effect of dietary administration of aspartic acid on thymus weight in C57 black mice

Iva Pipalová and M. Pospíšil

Institute of Biophysics, Czechoslovak Academy of Sciences, CS-612 65 Brno 12 (Czechoslovakia), 19 September 1979

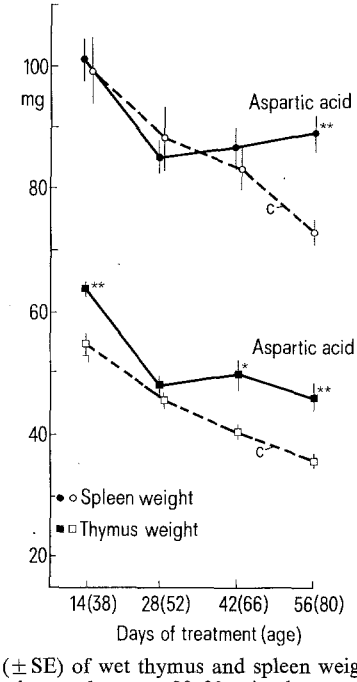
Summary. D,L aspartic acid incorporated at a level of 0.5% w/w into the diets of C57B1/10 male mice for 2–8 weeks increased significantly the thymus weight. Similar effects were observed after a 10 days' treatment of the mice with K- and Mg-salts of D,L aspartate (1%) in drinking water.

Aspartic acid deserves attention for its broad physiological and pharmacological properties. Of particular interest is its role in central nervous transmission[1] and its participation in metabolic pathways of citric acid cycle[2]. K- and Mg-salts of aspartic acid ameliorate some metabolic disturbances resulting from local as well as generalized tissue hypoxia[3]. Favourable effects of aspartates on hemopoietic cell renewal systems perturbed by radiation have been demonstrated[4]. In this communication the enhancing effect of dietary administration of aspartic acid on the weight of the thymus in mice is described.

Materials and methods. Male mice of the C57B1/10 strain were used. At the weaning age of 24 days the mice were matched according to body weight and caged in groups of 10. The experimental mice were fed with basal standard diet (20% protein) supplemented with D,L aspartic acid, which was incorporated into the pellets by food-drug trituration at a level of 5 mg/g of food (0.5%). The average daily ingestion of aspartic acid thus varied between 10 and 25 mg per mouse. The aspartic acid-containing diet, the

Mean values (± SE) of wet thymus and spleen weights in aspartate treated and control mice in the various experiments. 10–30 animals per group were used. Statistical significance as compared with controls: * $p < 0.01$

		Thymus weight (mg)	Spleen weight (mg)
Experiment 1	Aspartate	43.7 ± 1.6*	84.9 ± 2.2
	Control	37.5 ± 1.3	82.9 ± 2.7
Experiment 2	Aspartate	37.2 ± 1.9*	96.4 ± 6.5
	Control	29.5 ± 1.5	88.8 ± 5.4
Experiment 3	Aspartate	40.2 ± 1.4*	94.0 ± 2.9
	Control	29.0 ± 1.1	94.7 ± 4.3
Experiment 4	Aspartate	38.4 ± 0.6*	81.4 ± 4.1
	Control	31.7 ± 1.2	79.1 ± 3.2



Mean values (± SE) of wet thymus and spleen weights in aspartic acid treated and control groups. 20–30 animals per point were used. Statistical significance as compared with controls: ** $p < 0.001$, * $p < 0.01$.

control basal diet as well as tap water were given ad libitum. The mice were fed these diets for 14–56 days and killed at 14-day intervals to determine wet weights of the thymuses, spleens and adrenals.

Results and discussion. Body weights of the mice increased from the initial post-weaning mean value of 11–12 g to 25–26 g at the end of the experimental period. The weights of the thymuses and spleens in control groups follow the involution trend of the post-weaning period. No effects of aspartic acid treatment on body weight and on adrenals were noted. A statistically significant increase in thymus weight was observed after 2, 6 and 8 weeks of aspartic acid treatment; splenic weight increased significantly after 8 weeks (figure). In order to support these results, male mice of the same strain were treated at the age of 12 weeks with a 10-day administration of soluble K- and Mg-salts of aspartic acid in drinking water. Monokalium D,L asparagicum together with monomagnesium D,L asparagicum were dissolved in equal amounts in tap water at a level of 10 mg/ml (1%) and given ad libitum. Approximately similar daily intakes of aspartic acid as those used in the foregoing experiment were thus achieved. 4 experiments in various seasons of the year were performed and in all of them a significant increase in thymus weight was registered after aspartate treatment. No differences in the spleen weight were observed (table).

The results suggest that aspartic acid may play a role in the regulation of the size of the lymphatic tissue of the thymus, and that this function may be supported by dietary administration of this amino acid. The mechanism of the effect is not clear. K, Mg aspartate has been found to prevent the stress-induced disintegration of thymolymphatic tissue[5]. Ohnuma et al. emphasize the asparagine requirements of T-lymphocytes for cell growth[6]. In view of this evidence it could be hypothesized that, under the conditions of increased cellular level of aspartic acid, the enzymatically regulated balance between asparagine and aspartic acid is directed towards preservation of asparagine. Regardless of the mechanism involved, the importance of our findings for dietary attempts to modify cell-mediated immunity remains to be analyzed.

1 J. Bligh, A. Silver, C.A. Smith and M.J. Bacon, Experientia *34*, 1043 (1978).
2 H. Laborit, Aggressologie *7*, 515 (1966).
3 H.A. Nieper and Kj. Blumberger, Ärztl. Forsch. *15*, I/125, (1961).
4 M. Pospíšil, J. Vašků, J. Netíková and E. Urbánek, Strahlentherapie *155*, 67 (1979).
5 J. Vašků, E. Urbánek and S. Doležel, Drug Res. *16*, 559 (1966).
6 T. Ohnuma, J.F. Holland, H. Arkin and J. Minowada, J. natl Cancer Inst. *59*, 1061 (1977).

An improved electrophoretic method for a screening program for haemoglobinopathies[1]

P.C. Naoum, M.C.R. Moura Campos, M.F. Parenti and A.M. Szymanski

Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, UNESP, São José do Rio Preto, SP (Brasil), 6 August 1979

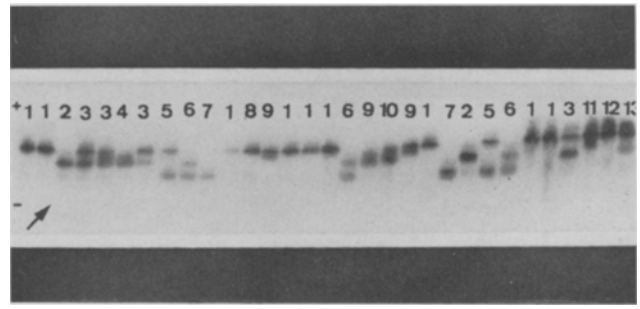
Summary. A method for a screening program for haemoglobinopathies in a starch agar gel mixed with saponin is presented. Normal and abnormal blood containing haemoglobins S, C, I, M Boston, D Punjab, beta thalassaemia major and beta thalassaemia minor, were applied, in a tray with the capacity for 100 samples. The electrophoresis was performed in 45 min using 300 V. This method offers special advantages for the examination of a large number of samples, using a small amount of whole blood and without the previous preparation of haemoglobin solution.

Millions of people throughout the world carry abnormal haemoglobins in various genetic combinations. Their identification is a public health problem of major importance[2,3]. It includes recognition of the relatively harmless mutants and of haemoglobins with known pathologic effects, particularly sickle cell anaemia and beta thalassaemia major[4]. This paper describes the application of an electrophoretic method for a screening program for haemoglobinopathies, and some of the advantages of this method are discussed.

Material and methods. Haemoglobin types used: In order to evaluate this procedure for human haemoglobins, whole blood samples were used, containing haemoglobins (Hb) A, S, C; fetal increased, from a patient with beta thalassaemia major; and Hb A_2 increased, from a patient with beta thalassaemia minor. All were identified by electrophoresis on cellulose acetate pH 8.6[5], starch gel pH 8.6, and agar gel pH 6.2[7]. Blood from patients with the rare abnormal haemoglobins M Boston. D Punjab and I, characterized by peptide maps and amino acid analysis[8], were also included in this study.

Electrolyte compartments and starch agar gel tray: The apparatus required for this study was made in our laboratory, and consisted of 2 electrolyte compartments separated by a support from the starch agar gel tray. Each electrolyte compartment measured $41 \times 5 \times 3$ cm in depth, and was constructed from acrylic plastic 0.3 cm thick. The tray, made from the same material, measured 38×8 cm. To its upper surface a frame of acrylic plastic 0.5 cm wide and 0.1 cm thick was fixed by adhesive.

Gel and electrode buffers: Discontinuous systems were used. For preparation of starch agar gel: 87 mM tris (hydroxy methyl) amino methane, 8.7 mM boric acid, 1 mM ethylene diamine tetracetic acid, pH 9.0[9], diluted 1:10 in water. For use in the electrolyte compartments: 300 mM boric acid and 60 mM sodium hydroxide pH 8.6[10], diluted 1:3 in water.



Electrophoresis of a part (1/3) of the agar gel tray. The arrow shows the line of application of whole blood. The number shows the haemoglobins types tested: 1 (AA), 2 (SS), 3 (AS), 4 (SF), 5 (AC), 6 (SC), 7 (CC), 8 (AA_2 increased), 9 (AF), 10 (mixture of ASF), 11 (mixture of AFI), 12 (AI) and 13 (mixture of ASI).