−65.9% in the inactivated samples of sera. Sera of Hp 2–2 phenotype: We found a loss of peroxydase activity ranging from −49.1 to −73.5 % in the inactivated samples of sera.

The difference of the peroxydase activity in native and inactivated sera seems to be dependent upon the Hp phenotype of the sera. Therefore it is not probable that a third heat-sensitive-substance, e.g. the complement, is involved in the formation of the complex, or that a yet unknown heat-sensitive factor is required for exerting the peroxydase activity of the complex. The assumption seems obvious that it is the Hp molecule that is altered by the action of heat. At present we have no satisfactory explanation for this phenomenon.

It has to be considered, however, that the haptoglobins of Hp 1–1 phenotype may differ in their chemical composition. CONNELL et al.[3] proved that the Hp 1–1 phenotype is divided into 3 subtypes which are controlled by the 2 genes: Hp^{1F} and Hp^{1S}. The α-chains of the Hp molecules, determined by these 2 genes, differ from each other in only one amino acid. It may be supposed that the decrease in peroxydase activity appearing as a consequence of inactivation can be attributed to this minor chemical dissimilarity. SMITHIES et al.[4] pointed out, and later NAUCE and SMITHIES[5] proved conclusively that the Hp^2 gene is a result of a partial gene duplication, and the consequence of the non-homologous crossing over of Hp^{1F} and Hp^{1S}. This would mean that complete heat-resistant α-chains could only occur purely in the subtype of some Hp 1–1 homozygote – Hp 1F–1F or Hp 1S–1S. Depending on the subtype of Hp^1 gene, certain sera of Hp 2–1 phenotype might partly contain complete heat-resistant α-chains. Sera of Hp 2–2 phenotype could no longer contain complete, heat-resistant α-chains. In accordance with this, we found more distinct loss of peroxydase activity in sera of Hp 2–1 phenotypes, and an even more distinct loss in sera of Hp 2–2 phenotypes. This is only a hypothesis, and further studies on subtypes of haptoglobins are required to solve the problem.

Zusammenfassung. Nachweis, dass in nativen und inaktivierten identischen Serumproben, gemessen an der Peroxydaseaktivität des Haptoglobin-Hämoglobin-Komplexes, die Hitzeresistenz der Haptoglobin-Typen in der Reihenfolge Hp 1–1, Hp 2–1, Hp 2–2 abnimmt.

Ö. HEVÉR

State Institute Fodor József, H–1528 Budapest 12 (Hungary), 16 February 1973.

[3] G. E. CONNELL, G. H. DIXON and O. SMITHIES, Nature, Lond. *193*, 505 (1962).
[4] O. SMITHIES, G. E. CONNELL and G. H. DIXON, Nature, Lond. *196*, 232 (1962).
[5] W. E. NAUCE and O. SMITHIES, Nature, Lond. *198*, 896 (1963).

Antibodies to Venous Tissue in Phlebothrombosis

The increasing number of surgical interventions by the use of homologous venous grafts in the arterial system suggested the significance of studies on the antigenicity of venous tissue[1]. In addition, in a certain number of cases, phlebothrombosis is a chronic disease of a cyclic course, characterized by the alternation of active and inactive phases. This course exhibits similarity with allergic events. Based on the assumption that allergic and autoimmune processes may play a role in the pathology of phlebothrombosis, we attempted to demonstrate antibodies against venous tissue in the sera of patients with phlebothrombosis.

Material and methods. Patients. Sera of 35 patients with phlebothrombosis of the limb and of 22 control subjects were investigated. The patients showed different clinical features predisposing to the development of the phlebothrombosis. The venous obstruction was not a consequence of surgical intervention. The severity of the classical symptoms depended on the size and the length of the veins involved.

Antigen. An extract was prepared from human vena cava tissue with 1 *M* calcium chloride-*Tris*-citrate buffer, pH 7.5 (CTC-extract), according to ROBERT et al.[2–4]. The CTC-extract contained water-soluble proteins (partly of plasmatic origin), and proteoglycans.

Immunologic tests. a) Linear immunodiffusion (Oudin)-method. Agar (1% w/v) was dissolved in 0.15 *M* phosphate buffer containing 0.85 saline (pH 7.0). The antigen concentration was 2 mg protein/ml. b) Passive haemagglutination test. The CTC-extract was coated with tannine-treated human red cells of blood group O.

Results and discussion. Using the Oudin-test, precipitation was obtained in the case of 1:16–1:32 serum dilution. Passive haemagglutination test was accepted as positive when titres showed 1:32–1:64 values. As seen in the Table, the presence of auto-antibodies against the CTC-extract of human veins could be established in 70% of the cases so far investigated. Both tests gave negative results in the control subjects.

The question whether the presence of vena-autoantibodies in phlebothrombosis is the cause or the consequence of the pathological process cannot be answered as yet, similarly to several other autoimmune diseases. Probably our further investigations on the specificity of these autoantibodies will give more information on this subject.

Immunological tests with vein CTC-extract

Methods / Patients	Lincar immunodiff. (Oudin)			Passive haemagglutination			Total		
	n	pos	neg	*n*	pos	neg	*n*	pos	neg
Phlebothrombosis	24	19	5	11	7	4	35	26	9
Controls	22	0	22	22	0	22	44	0	44

[1] S. I. SCHWARTZ, F. R. KUTNER, A. NEISTADT, H. BARNER, S. RESNICOFF and J. VAUGHAN, Surgery *61*, 471 (1967).
[2] L. ROBERT, J. PARLEBAS, P. OUDEA, A. ZWEIBAUM and B. ROBERT, in *Structure and function of connective and skeletal tissue* (Butterworth, London 1965), p. 406.
[3] F. STEIN, M. P. PEZESS, N. POULLAIN and L. ROBERT, Nature, Lond. *207*, 312 (1965).
[4] L. ROBERT, M. ROBERT, M. MOCZAR and E. MOCZAR, in *Le role de la paroi artérielle dans l'athérogénèse* (C.N.R.S., Paris 1968), p. 395.

Our preliminary findings reported above support the theory that auto-antigen formation through modification by any aggression of the constituents of the venous tissue would result in antibody production indicating the aggression. Increased production of these auto-antibodies (by repeated aggressions and/or dysfunction of the antibody-forming systems) could render these antibodies pathogenic to the vascular tissue, thus possibly perpetuating the course of the venous disease.

Résumé. Dans 70% des cas examinés, les auteurs ont montré l'existence d'anticorps antiveines dans le plasma des malades atteints de thrombophlébithe. Il est à présumer que des facteurs auto-immunes jouent quelque rôle dans la pathogenèse des thrombophlébites.

S. Gero, Judit Székely, Eva Szondy, A. Jobbágy, A. Orosz and Eva Seregélyi

Semmelweis University of Medicine, IIIrd Department of Medicine, Mezo Imre ut. 17, Budapest VIII (Hungary), 9 February 1973.

Effect of Synthetic Luteinizing Hormone Releasing Hormone on Pituitary and Serum Levels of Luteinizing Hormone of Intact and Median Eminence Lesioned Male Rats

Various reports have indicated that the hypothalamus may control both the synthesis and release of pituitary follicle stimulating hormone (FSH)[1-4]. The availability of synthetic luteinizing hormone (LH) releasing hormone (LRF), which is known to stimulate the secretion of both hypophysial FSH and LH[5], provided us with the opportunity to test the hypothesis that this specific hypothalamic neurohormone may regulate both the synthesis and release of pituitary LH.

Methods and materials. Synthetic LRF[6] was injected into the jugular vein of mature male rats (Sprague-Dawley, 225–250 g; 5–10 rats/group), both intact animals and those that had borne ME lesions for approximately 10 days, at which time pituitary LH was significantly depressed. The lesions had been made by applying a direct current of 5 mA/15 sec. A study was carried out to determine optimal post-injection time to detect major changes in pituitary and serum LH in both intact and ME-lesioned animals.

For the dose-response studies, 45 min after injection of LRF blood was withdrawn by cardiac puncture and the animals were immediately decapitated. Anterior pituitaries were removed, pooled, weighed, homogenized and diluted with physiological saline for use in the Parlow ovarian ascorbic acid depletion assay for LH[7]. The standard NIH-LH-S17 was tested at 2 or 3 levels, with the total doses ranging from 0.4 μg to 6.4 μg (4-fold interval). Pituitary homogenates were assayed at total doses of 0.5 mg and 2.0 mg. Five assay rats were employed at each dose level. Estimates of pituitary LH concentration were calculated by the method of Bliss[8]. Results are expressed in terms of NIH-LH-S1. Serum LH levels were determined by the double antibody radioimmunoassay technique of Niswender et al.[9], and are expressed in terms of NIAMD-Rat LH-RP-1.

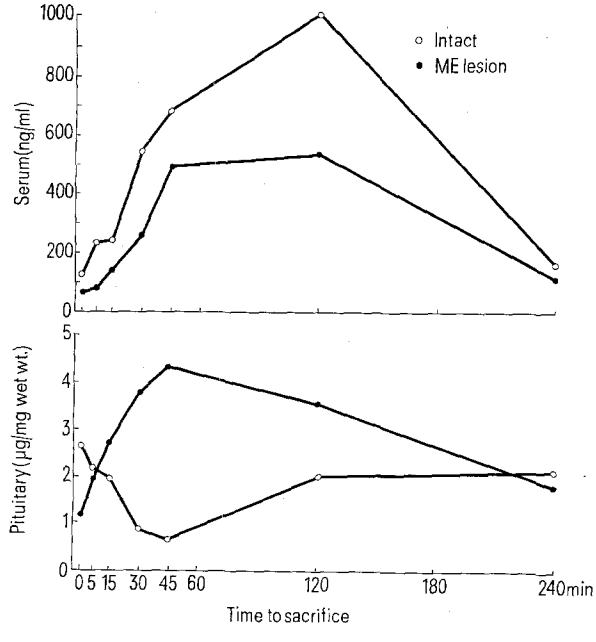

Fig. 1. Time study: Effect of synthetic LRF (100 μg/rat, i.v.) on pituitary and serum LH of intact and ME-lesioned mature male rats.

Results and discussion. Figure 1 represents the effect of a single i.v. injection of 100 μg LRF on pituitary and serum LH of intact and ME-lesioned rats in relation to time. In the intact rat, as pituitary LH was being significantly depleted ($P \leq 0.05$ vs. intact + saline), serum LH rose significantly ($P \leq 0.05$ vs. intact + saline). Maximal pituitary LH depletion occurred at 45 min whereas serum LH continued to rise, reaching its apex at 120 min post-injection. Animals with ME lesions, whose pituitary and serum LH levels were significantly depressed ($P \leq 0.05$ and $P \leq 0.01$ vs. sham lesion, respectively), exhibited maximal and significant pituitary LH repletion at 45 min ($P \leq 0.01$ vs. ME lesion + saline) associated with corresponding maximal and significant increments in serum LH ($P \leq 0.01$ vs. ME lesion + saline.) Both pituitary and serum LH levels in both types of recipients returned to baseline values by 4 h after LRF administration.

Figure 2 represents combined means of LRF stimulation studies performed individually but in triplicate and clearly

[1] J. S. Evans and M. B. Nikitovitch-Winer, Neuroendocrinology *4*, 83 (1969).
[2] A. Corbin, J. E. Milmore and E. L. Daniels, Experientia *26*, 1010 (1970).
[3] A. Corbin and J. E. Milmore, Endocrinology *89*, 426 (1971).
[4] A. Corbin, in *The Regulation of Mammalian Reproduction* (Eds. S. J. Segal, R. Crozier, P. A. Corfman and P. G. Condliffe; C. C. Thomas, Springfield 1973), p. 45.
[5] A. V. Schally, A. Arimura and A. J. Kastin, Science *179*, 341 (1973).
[6] Wyeth Compound No. 16,558.
[7] A. F. Parlow, in *Human Pituitary Gonadotropins* (Ed. A. Albert; C. C. Thomas, Springfield 1961), p. 300.
[8] C. I. Bliss, *The Statistics of Bioassay* (Academic Press, New York 1952).
[9] G. N. Niswender, A. R. Midgley, jr., S. E. Monroe and L. E. Reichert jr., Proc. Soc. exp. Biol. Med. *128*, 807 (1968).