

Allergologic Department, II. Clinic of Internal Medicine, Medical Academy,
Cracow, Poland, Director of the Clinic: Assoc. Prof. dr. S. Kirchmayer.

Director of the Department: Prof. dr. M. Obtulowicz.

INDUCED HYPOPROTHROMBINEMIA AS AN INDICATOR OF LIVER FUNCTION IN ALLERGIC DISEASES

By

ADAM WIERNIKOWSKI & ANNA WEISS

In 1943 *v. Kaulla* (4) observed hypoprothrombinemia in persons with liver damage after small doses of dicumarol, whereas no similar reactions were observed in normal persons, or they were found to react with an increase of prothrombin level. This, when confirmed by *Deutch* (4), led to working out of tests based on observation of prothrombine time and/or some factors of prothrombin complex. Subsequently, *Sarachasy & Inceman* (14) in Turkey and independently of them *Kirchmayer & Bromowicz* (9) in Poland proposed in 1949 a new liver function test which they called hypoprothrombinemia test, known in Poland as the *Kirchmayer & Bromowicz* test (11). *Della Santa & v. Kaulla* (4) and *Kirchmayer & Bromowicz* (10, 11) published the results of their investigation on various liver disorders. Several authors confirmed the specific character and organ sensitiveness of the test based on the blood coagulation factor which can be additionally increased by anti-coagulant agents.

Giguari (5) observed in animals, changes in the behaviour in the coagulation factors in the liver experimentally produced by allergic reactions. Such allergic reactions can be of immediate type and the Arthus type, both leading to eosinophilic necrosis of the liver cells. The antigen-antibody complex

damages the hepatocyte, or through ischemia it causes the damage of some parts of the liver which are due to antigen-antibody precipitation. Such changes were confirmed especially in serum sickness (6, 12, 13).

The resulting changes directed our attention to the possibility of examining by means of the hypothyrombinemia test, liver disturbances in certain allergic diseases.

MATERIAL AND METHOD

Twenty patients aged between 14 to 34 hospitalized in Allergy Department were treated for: atopic dermatitis—6 cases, atopic dermatitis and bronchial asthma in remission—8 cases, urticaria and angioneurotic oedema—6 cases. In 11 patients with atopic dermatitis skin changes lasted from infancy. In 6 cases they were generalized. In 6 cases the food sensitivity (egg, fish, milk, wheat) lasted from infancy. Some patients with atopic dermatitis and bronchial asthma were sensitive to inhalants (house dust, epidermal allergens). In 6 cases of urticaria and angioneurotic oedema the disease had lasted from 2 to 5 years, among which there were 2 cases sensitive to food (egg, milk), and in 1 case an anaphylactic reaction to penicillin was observed. In 4 cases connection was confirmed between the exacerbation of the skin lesions and gastrointestinal disorders.

The results were compared with those obtained in 15 normal persons (control group). The subjects were given orally before breakfast a single dose of 0.3 Gm (children 0.15 Gm) of Pelentan (Spofa). Before using the drug the prothrombin time was recorded by means of Quick one stage method, and subsequently was checked after intervals of 24, 28, and 48 hours. Electrophoresis of the serum protein was performed. Protein content was estimated using a refractometric method, paper electrophoresis was performed in a barbiturate buffer with a pH 8.6, $u = 0.045$, $K = 0.00226$, $\Omega = 1.1$ cm⁻¹. The strips of filter paper were stained with bromphenol blue. The contents of each fraction was determined by means of a photo electric cell. Non protein nitrogen (NPN), bilirubin, alkaline phosphatase (AP), bromsulphalein test (BSP) and thymol turbidity were also determined.

In the induced hypoprothrombinemia test the following parameters were adopted: A) initial index of prothrombin complex *i.e.* before the administration of Pelentan. B) index of prothrombin complex after 24 hours. C) 28 hours. D) 48 hours after Pelentan administration. E) index of sensitivity phase *i.e.* the negative or positive difference between initial prothrombin index and the index after 24 hours from Pelentan administration. F) index of dispersion phase *i.e.* the negative or positive difference between initial index of prothrombin complex and similar index after an interval of 48 hours.

The results of electrophoresis investigations of serum proteins and the test of

TABLE I
Comparison of Paper Electrophoresis of Serum Proteins.

I Group - Normal Persons.

II Group - Patients with Allergic Diseases.

Parameter	Group	Number of cases	Mean value of index of prothrombin complex in per cent and its mean error	Standard deviation for sample	Standard deviation for population	Test F for the significance of difference of variance	Probability of "o" hypothesis
Serum Protein	I	15	7.65 $\pm$ 0.169	0.631	0.653	$P/F \geq 1.477 / > 0.10$ insignificant	$P/t \geq 1.890 / > 0.05$ insignificant
	II	20	8.00 $\pm$ 0.099	0.435	0.442		
Albumins	I	14	4.28 $\pm$ 0.118	0.426	0.442	$P/F \geq 1.303 / > 0.10$ insignificant	$P/t \geq 0.163 / > 0.8$ insignificant
	II	20	4.25 $\pm$ 0.129	0.562	0.576		
Globulin α 1	I	14	0.26 $\pm$ 0.030	0.110	0.115	$P/F \geq 1.322 / > 0.10$ insignificant	$P/t \geq 1.083 / > 0.2$ insignificant
	II	20	0.29 $\pm$ 0.020	0.086	0.087		
Globulin α 2	I	14	0.70 $\pm$ 0.068	0.246	0.255	$P/F \geq 1.795 / > 0.10$ insignificant	$P/t \geq 1.607 / > 0.1$ insignificant
	II	20	0.59 $\pm$ 0.032	0.139	0.142		
Globulin β 1+2	I	14	0.83 $\pm$ 0.042	0.151	0.157	$P/F \geq 1.495 / > 0.10$ insignificant	$P/t \geq 0.667 / > 0.5$ insignificant
	II	20	0.80 $\pm$ 0.023	0.104	0.105		
Globulin γ	I	14	1.58 $\pm$ 0.124	0.447	0.467	$P/F \geq 1.131 / > 0.10$ insignificant	$P/t \geq 3.251 / < 0.01$ significant
	II	20	2.07 $\pm$ 0.092	0.400	0.410		

induced hypothrombinemia were calculated statistically by means of *t* test student, *Fischer*, *Behrens*, methods, respectively (3).

RESULTS AND DISCUSSION

The group of allergic patients differed only in the serum protein amount from the control group. A higher arithmetical value of gamma globulins was noted. Allergic patients 2.07 Gm%, control 1.58 Gm% (Table 1). In 3 allergic patients and in 2 normal people a raised thymol turbidity was observed. The respective values of bilirubin, NPN, AP, in both groups were normal. Among 16 BSP tests performed in allergic patients, 5 had pathological retention of bromsulphalein.

In the control group the mean arithmetical values of prothrombin complex amounted to: initially 91.1 per cent, at 24 hours 85.4 per cent, at 28 hours 86.4 per cent, at 48 hours 91.3 per cent, index of sensitivity phase being —5.7 per cent,

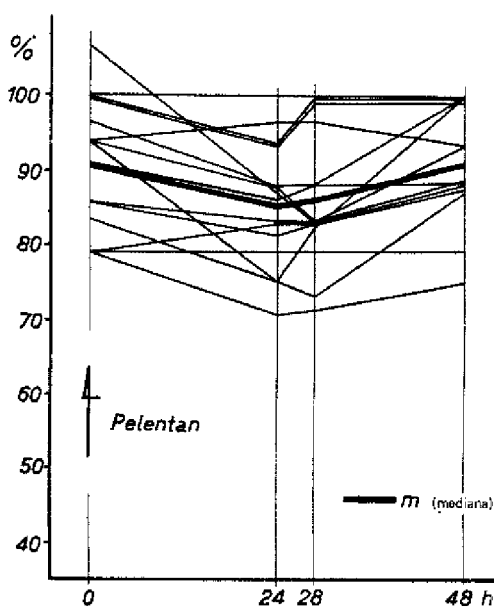


DIAGRAM 1

Index of the Prothrombin Complex in Normal Persons.

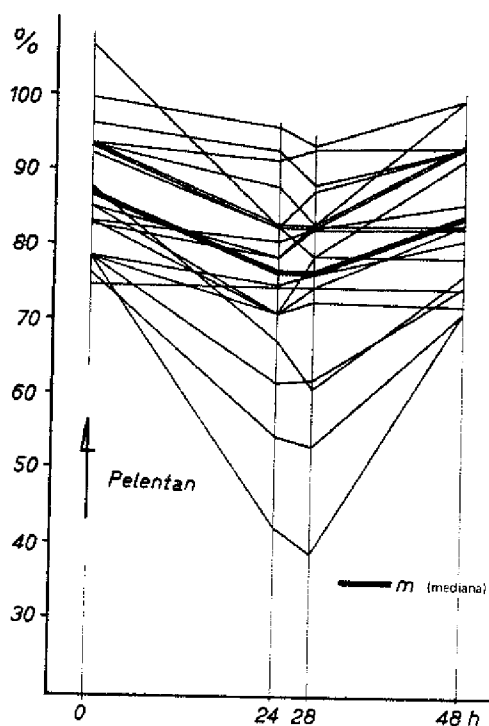


DIAGRAM 2

Index of the Prothrombin Complex in Allergy Diseases.

and index of dispersion phase $+0.2$ per cent (Diagram I). In the group of allergic persons the index of prothrombin complex amounted to: initially 87.4 per cent, at 24 hours 76.8 per cent, at 28 hours 76.9 per cent, at 48 hours 84.6 per cent, index of sensitivity phase -10.7 per cent and index of dispersion phase -2.2 per cent (Diagram II).

Diagram III represents the mean arithmetical values of prothrombin complex index of the control group, the allergic group and the group of 27 patients with cirrhosis of the liver (16). It will be noted that those suffering from allergic diseases occupied the medium position between normals and patients suffering from cirrhosis of the liver.

TABLE 2
Comparison of Results of the Test of Induced Hypothrombinemia.

I Group - Normal Persons.
 II Group - Patients with Allergic Diseases.

Parameter	Group	Number of cases	Mean value of index of prothrombin complex in per cent and its mean error	Standard deviation for sample	Standard deviation for population	Test F for the significance of difference of variance	Probability of "0" hypothesis
Initial index of the prothrombin complex	I	15	91.1 ± 2.3	8.60	8.91	P/F ≥ 1.18 / < 0.10	P/t ≥ 1.157 / > 0.10
	II	20	87.4 ± 2.2	9.43	9.68	insignificant	insignificant
Index of the prothrombin compl. in 24 h.	I	15	85.4 ± 2.2	8.10	8.38	P/F ≥ 3.046 / < 0.05	P/d ≥ 2.230 / < 0.05
	II	20	76.7 ± 3.2	14.16	14.53	significant	θ = 56° 23' signific.
Index of the prothrombin compl. in 28 h.	I	15	86.4 ± 2.4	8.87	9.18	P/F ≥ 2.05 / > 0.05	P/t ≥ 2.399 / < 0.05
	II	20	76.9 ± 2.9	12.76	13.08	insignificant	significant
Index of the prothrombin compl. in 48 h.	I	15	91.3 ± 2.0	7.60	7.87	/F ≥ 1.42 / > 0.10	P/t ≥ 2.238 / < 0.05
	II	20	84.6 ± 2.1	9.14	9.37	insignificant	significant
Index of the phase of sensitiveness	I	15	-5.7 ± 1.8	6.59	6.89	P/F ≥ 2.03 / > 0.05	P/t ≥ 1.708 / > 0.05
	II	20	-10.7 ± 2.2	9.41	9.66	insignificant	insignificant
Index of the phase of the dispersion	I	15	+0.2 ± 1.4	5.21	5.39	P/F ≥ 1.674 / > 0.10	P/t ≥ 1.819 / > 0.05
	II	20	-2.8 ± 0.9	4.06	4.17	insignificant	insignificant

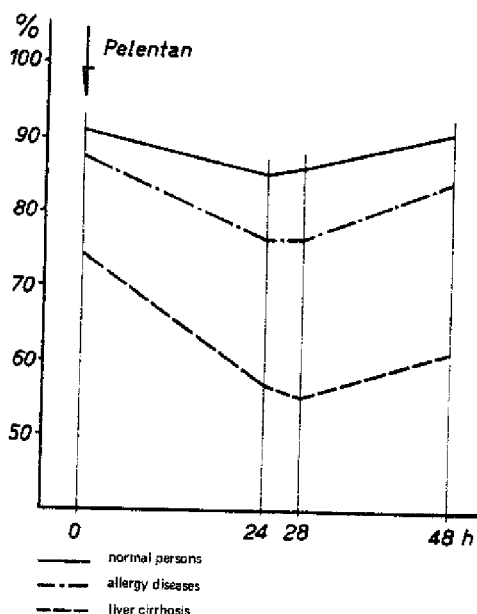


DIAGRAM 3

Mean Value of Index of the Prothrombin Complex.

Patients with allergic diseases differed significantly from normals by showing higher value of standard deviation of the index of prothrombin complex at 24 hours, a lower mean arithmetical value of the index of prothrombin complex at 24, 28 and 48 hours after administration of Pelentan (Table 2). This may indicate a state of functional damage of the liver in patients with allergic diseases, no other abnormalities were found except the pathological BSP which was noted in 5 cases.

In the group of allergic patients when contrasted to the group of liver cirrhosis patients no connection between the behaviour of serum protein fractions and the index of prothrombin complex was noted.

The induced hypoprothrombinemia test tended to be more positive in patients with food sensitivity than among those who had no such sensitivity. Liver damage also occurred in food

sensitivity in several forms of atopy (skin, respiratory system).

The liver can become a shock organ if the allergen reaches it through the portal vein or through the hepatic artery (8, 13).

Thus the liver performs a function similar to the spleen and the lungs when removing antigen-antibody complexes from the circulation. Hence it is easier to sensitize animals by means of an antigen when given orally than rectally. In human material the allergic reactions in the liver caused a temporary increase of bilirubin level in the serum or in some enzymatic tests (7, 8). This acute form of allergic jaundice was noted by *Massionon* in cases of food sensitivity (8). It was not known whether this liver damage due to an allergic reaction would lead to chronic inflammation of the liver or to cirrhosis. It is also possible that autoimmune processes in the liver against the liver cells may occur. *Blackwell* (1, 2) who experimented with rats has shown that 5 minutes after intravenous administration of human albumin, changes in the liver cell cytoplasm occurred. Injections of human serum with Freund adjuvants when repeated for a period of 4 to 32 weeks led to the liver cirrhosis in 70 per cent of the rats. Antibodies against cytoplasm, mitochondria and nuclei of liver cells in sensitized rabbits were demonstrated by means of electronic microscopy and by the immunofluorescent technique. Animals sensitized with the cytoplasmatic fraction showed chronic hepatitis, whereas animals sensitized with nuclear fraction showed no such changes (12). *Tsuji* (15) confirmed antibodies against cytoplasm of liver cells in 65 per cent of 20 patients suffering from various liver disorders.

The data obtained together with the results of experimental investigations emphasizes the importance of cytoplasmatic antibodies in the development of chronic diseases of the liver parenchyma.

By means of induced hypothermia it was found that in atopic dermatitis, urticaria and angioneurotic oedema especially giving food allergens over many years tended to affect the state of prothrombin production in the liver.

SUMMARY

The authors using the method of induced hypoprothrombinemia examined a group consisting of 20 patients suffering from allergic diseases (atopic dermatitis, atopic dermatitis and asthma bronchiale in remission, urticaria and angioneurotic oedema) and compared the results with those obtained in 14 normal persons. A significant difference in the standard deviation of the index of prothrombin complex at 24 hours and a lower mean arithmetical value of the index of prothrombin complex at 24, 28 and 48 hours was found after Pelentan administration. Prolonged exposure to food allergens and increased severity of the diseases add to the change in prothrombin production of the liver.

REFERENCES

1. *Blackwell, J. B.*: Cirrhosis resulting from repeated injection of antigen. *J. Path. Bact.* 90, 245, 1965.
2. *Blackwell, J. B.*: The effects on the liver of sensitised rats of intravenous injection of antigen. *J. Path. Bact.* 90, 259, 1965.
3. *Cramér, H.*: Metody matematyczne w statystyce. P.W.N., Warszawa, 1958.
4. *Della Santa, R. & v. Kaulla K. N.*: L'épreuve de l'hypoprothrombinémie provoquée chez les hépatiques. *Le Sang.* 22, 11, 1951.
5. *Gigauri, V. S.*: Protein function of the liver in sensitization. *ZH Mikrobiol. Immunobiol.* 42, 27, 1965. *Cit. Rev. Allergy.* 20, 43, 1966.
6. *Gresham, G. A. & Jennings, A. R.*: An introduction to comparative pathology. Academic Press, London-New York, 1962.
7. *Hanashiro, P. K. & Weil, M. H.*: Anaphylactic shock in man. *Arch. Int. Med.* 119, 129, 1967.
8. *Hansen, K. & Weber, M.*: Lehrbuch der Klinischen Allergie. G. Thieme, Stuttgart, 1967.
9. *Kirchmayer, S. & Bromowiczowa, K.*: Różnica "czasu protrombiny" przed i po podaniu małych dawek "pelentanu-rapid" jako nowa próba czynnościowa wątroby. *Przegl. Lek.* 6/II, 60, 1950.
10. *Kirchmayer, S. & Bromowiczowa, K.*: Nowa próba czynnościowa wątroby oparta na pomiarach czasu protrombiny. *Pol. Tyg. Lek.* 8, 873, 1953.
11. *Orłowski, W.*: Choroby wątroby, układu żółciowego i trzustki. P.Z.W.L., Warszawa, 1961.
12. *Popper, H., Paronetto, F. & Rubin, E.*: Piecemeal necrosis as a reflection of immunologic hepatic injury. *Aktuelle Probleme der Hapatologie.* G. Thieme, Stuttgart, 1962.

13. *Rajka, E.*: Allergie und Allergische Erkrankungen. Akadémiai Kiadó, Budapest, 1959.
14. *Saracbası, Z. & Inceman, S.*: Un nouveau test des fonction hépatiques: le test de la dicoumarine. Presse Méd. 74, 1089, 1949.
15. *Tsuji, T., Obawa, K. & Kosaka, K.*: An immunofluorescent study of circulating anti-liver antibodies in chronic liver diseases. Acta Path. Jap. 15, 273, 1965. Cit. Rev. Allergy. 20, 196, 1966.
16. *Wiernikowski, A.*: Wartość kliniczna próby hipoprotrombinemii spowodowanej. VIII Zjazd Polskiego Towarzystwa Hematologicznego, Łódź, 1966.

Requests for reprints:

Dr. *Anna Weiss*,
2nd Clinic of Internal Medicine,
Department of Allergy,
Kopernika 15 str.
Cracow, Poland.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.