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HEPARIN-PRECIPITABLE FRACTION (HPF) IN AN UNSELECTED MATERIAL OF DERMATOLOGICAL INPATIENTS

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Abstract. Four hundred and forty-two dermatological in-patients, 10 patients with rheumatoid arthritis and a control group of 48 healthy persons were examined for heparin-precipitable fraction (HPF) in plasma, cryoglobulin in serum, and blood sedimentation rate. Pathological values were seen in different dermatosis-like infectious diseases and disorders combined with arthritis, such as psoriasis arthropathica and Reiter's disease. The importance of arthritis was confirmed in the rheumatoid arthritis group. Treatment and consequent improved clinical condition was followed by a rapid reduction of HPF in plasma, whereas a deterioration of the clinical condition caused a rapid rise in HPF.

In 1933 Wintrobe & Bull (21) described a cold precipitate of the serum of a patient with multiple myeloma. Lerner & Watson (7) characterized this in 1947 as a globulin and named it cryoglobulin. In 1946 Morrison (8) discovered a cryoprecipitate in the serum of pregnant women, which she called "contractinogen" as she found that the substance belonged to a fibrinogen complex. Later Morrison et al. (9) found certain components in normal pooled plasma that were insoluble at low temperatures and had fibrinogen-like properties. This cryoprecipitate was soluble upon rewarming.

In 1954 Thomas et al. (18) observed a fibrinogen-cryoprecipitate in plasma in connection with injection of bacterial endotoxin in rabbits which induced the Shwartzman-Sanarelli reaction. In 1955 the same type of plasma-cryoprecipitate was found by Korst & Kratochwil (6), who named it cryofibrinogen. In the same year Thomas et al. (19) found the same substance in plasma of patients with diseases such as rheumatoid arthritis, acute rheumatic fever, Hodgkin's disease, bacterial endocarditis, and meningitis. It was also observed, in 1960, by Kalbfleisch & Bird (4) in patients with malignant tumours and haemorrhagic diathesis and in 1961 by Kessel (5) in patients with cold sensitivity. Since then, cryofibrinogen has been reported in many other diseases. Cryoprecipitation of this substance in heparinized plasma is called heparin-precipitable fraction (HPF). The present work investigates the incidence of HPF in an unselected material of in-patients with dermatological disorders.

MATERIAL

Four hundred and forty-two in-patients from the Dermatologic Department, Rikshospitalet, University of Oslo, were examined for HPF, cryoglobulin, and blood sedimentation rate (BSR). Ten in-patients from Oslo Sanitetsforenings Revmatismesykehus, University of Oslo, were examined for HPF, BSR and rheuma serology (Rose-Waaler and Latex tests).

Forty-eight healthy individuals of all ages, from the staff of Rikshospitalet, including medical students, served as a control group. They were examined for HPF, cryoglobulin and BSR.

All persons investigated were Caucasians.

Reagents

Vacutainer	Ref. cat 4710, 3200 without additive.
Vacutainer	Ref. cat 4770, 3206N, containing 0.5 mg of 3.8% sol. sodium citrate and 0.2 mg/ml potassium sorbate.
Vacutainer	Ref. cat 4716, 3200KA containing 143 units U.S.P. heparin sodium, corresponding to 1.2 mg.
Thrombin: Topostasin®	F. Hoffman-La Roche & Co, Basel, Switzerland.

METHODS

Blood samples were drawn by venepuncture of the cubital vein and collected using the evacuated glass container technique of Vacutainer B.D. system (see reagents). Serum was obtained by drawing whole blood into a Vacutainer without additive, followed by incubation of the Vacutainer at 37°C for 2 hours. For preparation of citrated plasma 4.5 ml whole blood was drawn into a Vacutainer containing 0.5 mg of 3.8% sol. sodium citrate and 0.2 mg/ml potassium sorbate.

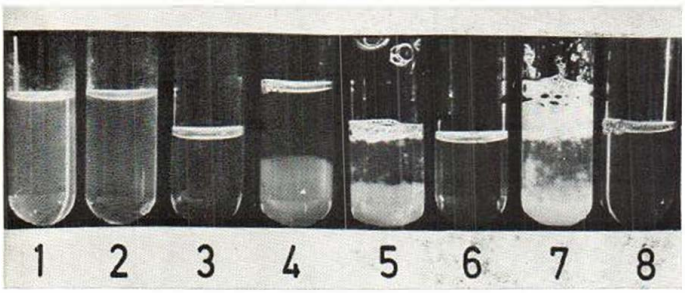

Fig. 1. Reaction in plasma and serum at various temperatures from a single patient. *1*, Serum at 37°C. *2*, Plasma at 37°C. *3*, Serum at 4°C. *4*, Plasma at 4°C with cryoprecipitation (HPF). *5*, Isolated HPF in physiological saline solution at 4°C. *6*, HPF redissolved in physiological saline solution at 37°C. *7*, Reprecipitation of HPF from tube 6 at 4°C. *8*, Supernatant plasma from tube 4 after isolation of HPF showing no further cryoprecipitation at 4°C.

For preparation of heparinized plasma, 10 ml blood was drawn into a Vacutainer containing 143 Units U.S.P. heparin sodium, corresponding to 1.2 mg heparin. The blood samples were centrifuged at 22°C in a MSD Super Medium centrifuge for 10 min at 900 *g*. The plasma or serum was removed and placed in a refrigerator at 4°C for 48 hours. The plasma specimens containing a cryoprecipitate were centrifuged in a MSD Mistral 6L centrifuge for 10 min at 1 300 *g* at 4°C. The supernatant was discarded and the precipitate was washed twice, adding 10 ml of 0.9 % NaCl solution at 4°C and shaken by hand, and recentrifuged each time as above. Three ml 0.9 % NaCl was added to the isolated cryoprecipitate and the suspension was incubated at 37°C for about 12 hours, or left in a 37°C water bath for 3 hours until the precipitate was dissolved in the solution. Sometimes, a small insoluble precipitate was left at the bottom of the tube and in such cases the solution was centrifuged once more at room temperature for 10 min at 900 *g* and the supernatant containing the redissolved cryoprecipitate was used for further investigations.

The cryoprecipitate was then quantitated according to the tyrosine method of Ratnoff & Menzie (11); in the present study 60 IU thrombin (Topastasin®) was used for the conversion of fibrinogen into fibrin.

A Hitachi 101 spectrophotometer operated at 650 nm, with a light path of 10 mm, was used for quantitation. Rose-Waaler and Latex tests were performed after routine methods at the Institute of Immunology and Rheumatology, Rikshospitalet, Oslo. BSR was measured after routine methods at the Central Laboratory, Rikshospitalet, Oslo.

RESULTS

Three different types of cryoprecipitates were found.

Type 1. The cryoprecipitate formed a thin layer at the bottom of the glass tube. If the tube was shaken, the precipitate floated like a thin veil in the plasma.

Type 2. A cloud-like precipitate occupied varying amounts of the plasma. It was easily dispersed in the plasma by slightly shaking the tube.

Type 3. A jelification of the plasma. When the tube was tilted, the gel slid along the sides of the tube. When shaken, the lump dissolved into smaller lumps in the plasma.

When heated to 37°C, the cryoprecipitate dissolved in the plasma and disappeared. When this plasma was cooled to 4°C, the cryoprecipitate reappeared (Fig. 1), and this process could be repeated. Sometimes, a small quantity of insoluble precipitate would remain at the bottom of the tube, especially in the type 1 precipitate, but generally the amount of insoluble precipitation increased with the time the plasma was left standing before it was quantitated.

In the investigation of HPF in the plasma of healthy individuals, 18 out of 48 (37 %) had a cryoprecipitation in plasma. 16 of these had type 1 precipitation (Table I). In this study, type 1 of the normal group represented low HPF values with an average of 0.16 mg/ml, whereas type 2 showed an average of 0.33 mg/ml. Owing to its low values and frequent occurrence, type 1 precipitation is not regarded as pathological. Low values in the patient material correlated well with those found in the control group. Precipitation types 2 and 3 only have therefore been included in the calculations of HPF in patient plasmas in the present study. The frequency of these precipitates in the control group is 4 %, i.e. 2 of type 2 and none of type 3 out of 48 plasmas (Table I). No cryoglobulin occurred in the sera from the 48 persons in the control group.

Table II shows the results of HPF in the plasmas of 442 dermatological in-patients examined. Compared with the control group, there are significantly

Table I. *Control group: type of precipitation, HPF and BSR*

Cryoprecipitation in plasma	Persons showing precipitate		Mean values of	
	n	%	HPF (mg/ml)	BSR
Type 1	16	33	0.16	9
Type 2	2	4	0.33	12
Type 3	0	0	—	—

Total number of persons: 48.

higher HPF values in the plasmas of patients suffering from psoriasis arthropathica, Reiter's disease, dermatitis herpetiformis, acne conglobata, and "miscellaneous", with a significance level of $p \leq 0.05$. At the significance level $p \leq 0.01$, only psoriasis arthropathica and Reiter's disease show significant differences. No cryoglobulin could be detected in serum.

There is a marked difference in the content of HPF in uncomplicated psoriasis vulgaris and psoriasis arthropathica. Out of 114 patients with psoriasis vulgaris only 4 (3.5%) had HPF in plasma, whereas 20 out of 25 patients (80%) with psoriasis arthropathica had HPF in plasma.

Elevated HPF values were also found in Reiter's disease. All our patients with this disease had high HPF values in plasma (Table III). Salicylates, phenyl-butazone derivates and steroids per os caused improvement of the clinical condition, and the HPF values decreased rapidly (Fig. 2). If this treatment was interrupted, the HPF values rose parallel with the clinical deterioration. This is demonstrated in Fig. 2 by the curve ●—● where oxiphenbutazon (Tanderil®) treatment had to be stopped because of dyspepsia, and the initial clinical improvement with corresponding HPF reductions in plasma, was followed by clinical deterioration and HPF increase. On the administration of steroids, the patient's condition improved, with a reduction of HPF in plasma.

In psoriasis arthropathica the incidence of increased HPF was generally less pronounced than in Reiter's disease. The arthritis was subacute to chronic, and did not respond well to therapy. A reduction of HPF was observed in a few patients in this group during their hospitalization, but most patients showed a fluctuating course of HPF formation.

Table II. *442 in-patients examined for HPF*

Diagnosis	No. of patients Total	With HPF	%	Mean values of HPF (mg/ml)	BSR
Psoriasis arthropathica	25	20	80	0.49	50
Psoriasis vulgaris	115	4	4	0.55	18
Reiters disease	7	7	100	1.30	59
Eczema contactum	43	8	18	0.40	27
Eczema atopicum	55	2	4	0.42	30
Dermatitis herpatiformis	8	2	25	0.42	30
Acne conglobata	7	2	30	0.42	30
Miscellaneous	182	19	10	0.30	30

Table III. *HPF in Reiter's disease*

Patient	HPF (mg/ml)	BSR	Symptoms
T. K.	2.38	60	Severe hydrops
B. S.	0.39	20	Moderate hydrops
T. S.	1.60	69	Severe hydrops
L. K.	0.32	19	No hydrops
T. W.	2.10	50	Severe hydrops
S. K.	1.50	92	Severe hydrops
S. R.	0.76	102	Moderate hydrops

The patients with rheumatoid arthritis all had positive Rose-Waaler reactions and Latex tests, and showed HPF in plasma (Table IV). All had regular arthritis treatment. The patient HH (Table IV) had cryoglobulin in serum. He had received prednisolone 10 mg daily for 4 days prior to investigation. In this group no statistical significant connection could be demonstrated between quantitation of HPF on the one hand, and BSR, Rose-Waaler reaction and Latex test on the other hand.

High values of HPF were demonstrated in cases of acne conglobata, furunculosis, trichophytia profunda barbae and complicated gonorrhoea (epididymitis). These cases are registered in Table III under miscellaneous. From the commencement of treatment, especially in cases where therapy with systemic antibiotics was started, the HPF-values in plasma fell rapidly, and in some cases could no longer be

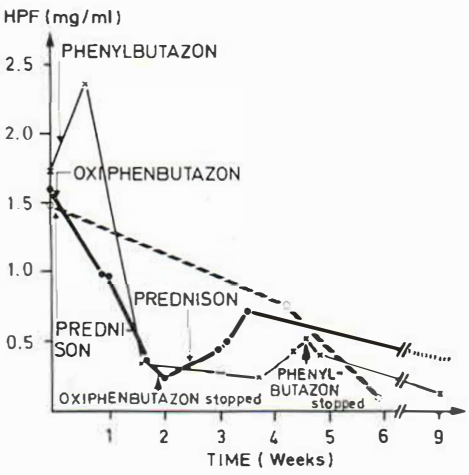

Fig. 2. Alterations in HPF values in plasma in 3 patients with Reiter's disease during treatment.

Table IV. *HPF in patients with rheumatoid arthritis*

Patients	Therapy	Rose-Waaler reaction	Latex test	HPF (mg/ml)	BSR
R. H.	Salicylates	256	2+	0.68	51
A. L.	Salicylates; indomethacin	256	2–3+	1.51	65
I. F.	Salicylates	256	3–4+	0.61	65
K. O.	Gold	256	2–3+	0.46	15
L. C.	Gold; Indomethacin	128	1+	1.40	26
B. T.	Brufen	256	3+	0.32	21
B. S.	Salicylates	64	—	0.50	16
T. L.	Indomethacin	512	3+	0.35	65
B. F.	Salicylates	128	2+	0.26	18
H. H.	Salicylates; 4 days prednisone 10 mg daily	1 024	4+	0.73	92

Brufen® = Iboprofen.

detected after a few days. On recurrence of the infection, the HPF-values increased parallel with the clinical deterioration.

By using different types of anticoagulants during collection of the blood specimens, the importance of the type of anticoagulant upon the yield of the cryoprecipitate in plasma could be demonstrated. At the beginning of this investigation, 3.8% citrate was added to the plasma as anticoagulant. Later this was replaced by heparin at 0.12 mg/ml whole blood. There was no cryoprecipitation in the citrated plasmas from 139 patients, whereas in the heparinized plasmas from the same number of patients, cryoprecipitation occurred in 22 patients (Table V). For the evaluation of cryoprecipitation in the present study, only heparinized plasmas have been used.

In this study, all patient groups showed statistically significant increased BSR compared with the control group ($p \leq 0.001$). However, there was no direct quantitative correlation between BSR and HPF.

DISCUSSION

In most cases of cryoprecipitation in plasma, the condition is clinically asymptomatic. Heparinized plasma must be kept in a cold state for several hours to induce the formation of a cryoprecipitate. Precipitation at room temperature has been observed. Cryoprecipitates in the blood and intravascular precipitation in cold-exposed parts of the body with circulatory and trophic disturbances have been described in a few cases (20).

Table V. *Number of plasmas showing cryoprecipitation*

No. of patients	Type of anticoagulant	
	Citrate	Heparin
139	0	22

Three different types of cryoprecipitation in heparinized plasma have been observed, and may represent different constituents or quantities of HPF. In the present study and in other investigations (13, 14, 15, 16), small amounts of cryoprecipitates could be found in the plasma of healthy persons. This type 1 precipitation (see above) may be explained by the possible admixture of tissue factors to whole blood by the venepuncture, thus inducing changes in the blood, most probably in the fibrinogen-fibrin system. This also explains the relatively high content of cryoprecipitation in type I that does not dissolve upon rewarming.

Plasma cryoprecipitation changes with the type of anticoagulant in plasma. If citrate is used, the number of positive tests falls and the amount of cryoprecipitation is smaller, compared with heparin. The optimal addition of heparin to whole blood for HPF investigations is 0.1–0.15 mg/ml (13, 17, 18, 22). Other heparin-related substances, such as dextran, alginic acid sulfate, chitin sulfate 47A and polymannuronic sulfate, have the same effect as heparin. The substances inducing cryoprecipitation may to a certain extent possibly be kept in a soluble state in plasma, even in the cold. At higher concentrations or by the addition of heparin or related substances, cryoprecipitation may occur. Tissue inflammation seems to be most important in the formation of HPF, as in arthritis and in microbial inflammations, especially in cases of tissue destruction. Furthermore, neutrophil leucocytes "digest" fibrin (1), and the eosinophilic leucocytes in the bone marrow and in the tissue contain profibrinolysin and induce fibrinolysis (12). Deposition of fibrin is also an important factor in tumour biology, in the expansion and spreading of the malignant process (2).

Although N-terminal amino acid analysis of cryofibrinogen (10) shows results that are in agreement with the end groups reported for human fibrinogen, it cannot be conclusively stated that this is solely a fibrinogen. Together with ultracentrifugal and immunoelectrophoretic analysis, this may indicate

that other protein moieties are present, differing in solubility, clottability, sedimentation coefficients, and N-terminal amino acids (3, 10, 13, 22, 23). This cryoprecipitate may represent a combination of small amounts of fibrin monomer or fibrinogen devoid of peptide A, fibrinogen, fibrinogen degradation products and possibly a nonclottable globulin.

Further investigations in this field are needed.

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