plasma, prothrombin time (QUICK), fibrinogen[5], 'true prothrombin', factor V, factors VII and X[6], plasminogen and antiplasmin[7]. Factors VIII, IX, XI and XII were determined by the one-stage slightly modified methods of SOULIER and LARRIEU[8]. Plasmas of patients with congenital deficiency of the above factors were used as substrates for these determinations. The so-called 'exhausted plasma' (plasma artificially deprived of factors XI and XII, prepared according to WAALER[9]) has also been used to determine the level of both total factor XI and total factor XII together. The level of blood clotting factors in the tested blood plasma was calculated by means of interpolation from standard dilution curves.

Samples of human blood plasma were shaken with various amounts of collagen for 10 min at room temperature. Platelet count and clotting factors were determined in supernatant after centrifugation of collagen fibres.

Experimental results are presented in the Table. It can be seen that collagen, shaken with platelet-rich plasma, adsorbs about 30–45% of Hageman factor. The adsorption of platelet-poor plasma on collagen causes a decrease of Hageman factor to 20% and of factor IX to 72% of their initial values. This procedure did not significantly affect other clotting factors, including PTA (factor XI).

It has been noticed that a sample of human plasma, adsorbed six times with collagen (20 mg/ml), contains about 5–7% of Hageman factor.

The following experiment was performed in order to elute Hageman factor from collagen. 10 cm^3 of plasma were shaken with 200 mg of collagen. After centrifugation, the precipitate was twice washed with distilled water and with 0.9% NaCl. Then 0.9% NaCl was added and the pH was adjusted to 10.5. The eluate contained about 0.7% of protein; the yield of Hageman factor being 25%. Its specific activity increased about 10 times as compared with human plasma.

The conclusion may be drawn that Hageman factor is almost selectively adsorbed from human plasma by collagen and that it is possible to elute this factor from collagen fibres in alkaline medium.

The adsorption of Hageman factor is greater in platelet-poor plasma. It is suggested that platelets, adhering to the collagen fibres, interfere with the adsorption of Hageman factor.

It is possible that the adsorption of Hageman factor by collagen also occurs *in vivo* and that this phenomenon is of some significance in haemostasis. It seems that certain preparative procedures, e.g. separation of PTA and Hageman factor, purification of Hageman factor, and preparation of Hageman-deficient plasma, could be elaborated on the basis of the experimental facts presented above[10].

The influence of collagen on platelet count and blood clotting factors

Clotting test	Platelet-rich plasma		Platelet-poor plasma	
	Before adsorption	After adsorption	Before adsorption	After adsorption
Platelet count	410000	158500	30000	22500
Protrombin time	15.0 sec	15.0 sec	15.6 sec	15.6 sec
Clotting time of recalcified plasma	125.0 sec	132.5 sec	139.0 sec	159.0 sec
Fibrinogen	280 mg%	250 mg%	380 mg%	390 mg%
Prothrombin-Factor II	100%	87.9 %	100%	93.7 %
Factor V	100%	100 %	100%	100 %
Factor VII + X	100%	100 %	100%	100 %
Factor VIII	100%	87.6 %	100%	91.6 %
Factor IX	100%	81.25%	100%	72.11%
Factor XI	100%	100 %	100%	100 %
Factor XI + XII[a]	100%	54.6 %	100%	21 %
Factor XII	100%	73 %	100%	20 %
Plasminogen	100%	100 %	100%	100 %
Antiplasmin	100%	100 %	100%	100 %

[a] Tested on exhausted plasma.

Résumé. Nous avons trouvé que le facteur Hageman (factor XII), contenu dans le plasma humain citraté, pauvre en plaquettes, est adsorbé presque sélectivement par le collagène. Il est également possible d'éluer ce facteur au pH alcalin.

S. NIEWIAROWSKI, E. BAŃKOWSKI, and T. FIEDORUK

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Photodynamic Effect of Dye on Frog Muscle Fibre Using Microelectrodes

ROSENBLUM[1] reported that frog sartorii, when stained by Rose Bengal and illuminated by an artificial source of light from tungsten lamp, developed twitch contractions. We were interested in studying the effect of light on the resting potentials of stained muscle fibres and examining whether the photodynamic effect of the dye is exerted on the fibre membrane.

Frog sartorii from *Rana pipiens* were stained by Rose Bengal (1:25,000) for 2 h in the dark; then light from a 300 watt tungsten lamp at a distance of 18 inches was focused on the muscle for varying periods of time. An IR-glass filter was interposed to exclude the effect of heat. The membrane resting potentials were recorded by the method of NASTUK and HODGKIN[2]. The resting membrane potentials of stained fibres in frog Ringer did not differ significantly from unstained fibres. There was no

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change in the resting potentials in the unstained fibres on being exposed to light for several minutes. On being exposed to light the stained fibres showed varying degrees of depolarization depending on the duration of exposure and the depth of the situation of the fibres. The superficial fibres were depolarized more easily. In 50% of the exposures (65 observations) there was repetitive discharge during the exposure time (1 min) and the fibres irreversibly depolarized from the resting membrane values of 85.5 ± 1.8 mV to 54.5 ± 1.5 mV. In 20% of the fibres the repetitive discharge ensued immediately after switching off the light and the fibres were irreversibly depolarized. Repolarization was observed in those fibres that did not attain threshold firing level. After a number of exposures the muscle went into a state of contracture and the fibres were difficult to impale. The phenomenon was reproducible in curarized preparations ($1 \cdot 10^{-4}$ g/ml of *d*-tubocurarine). In choline Ringer solution and under lack of oxygen the photodynamic effect was not demonstrable.

Zusammenfassung. Kurarisierte und nichtkurarisierte Frosch-Sartorii zeigten einen veränderlichen Grad der Depolarisation, wenn sie mit Bengalrot (1:25000) gefärbt und durch scharf zentriertes Licht einer 300-Watt-Tungsten-Lampe beleuchtet wurden. Die Depolarisation wurde niemals im äusseren Natrium und ohne Sauerstoff beobachtet.

R. P. Kohli and S. H. Bryant

Department of Pharmacology, K.G. Medical College, Lucknow (India) and Department of Pharmacology, University of Cincinnati (Cincinnati USA),
April 1, 1964.

The Study of the Cysteine Content of the Chicken Erythrocyte Histone by Polarography

The cysteine content of the histones is not well known; only some contradictory information is available[1-7]. Since the polarographic method is a sensitive test for the –SH groups[8], we studied the polarographic activity of the chicken erythrocyte histones.

Experimental. The chicken erythrocyte nuclei were isolated with a citrate method[9] and were homogenized in saline-EDTA solution[10]. The whole histone (WH) from the washed homogenate was extracted with 0.25 *N* HCl and precipitated with 6 vol acetone. The precipitate was washed and dried *in vacuo*. The WH was fractionated by using a carboxymethyl-cellulose column with the method of Johns et al.[11]. The protein content of fractions was followed turbidimetrically[5]. The polarographic assay was carried out according to our previous report[12].

Results. As previously indicated[13] the WH was polarographically inactive at a concentration of 10 µg/ml. However, a mild catalytic wave appeared when the WH concentration was increased in the polarographic test-solution. The height of the wave reached its maximum at a concentration of 100 µg/ml (Figure 2, curves a–d). The height of the protein waves decreased with the increase of histone concentrations, until estimation of the polarographic curve became impossible (Figure 2, curves e–g).

The WH chromatographed on a carboxymethyl-cellulose column gave four fractions, similar to thymus histone[11] and to a tumour histone[6]. From these four fractions only one histone (F3) proved to be active polarographically (Figures 1 and 2, curves i–k).

Discussion. The polarographic analysis of the chicken erythrocyte histones showed in all cases that one of them contains –SH groups. This consideration was substantiated by the following facts: that the observed catalytic waves of the WH and F3 fraction are characteristic protein waves, and that the oxidized histones gave no cata-

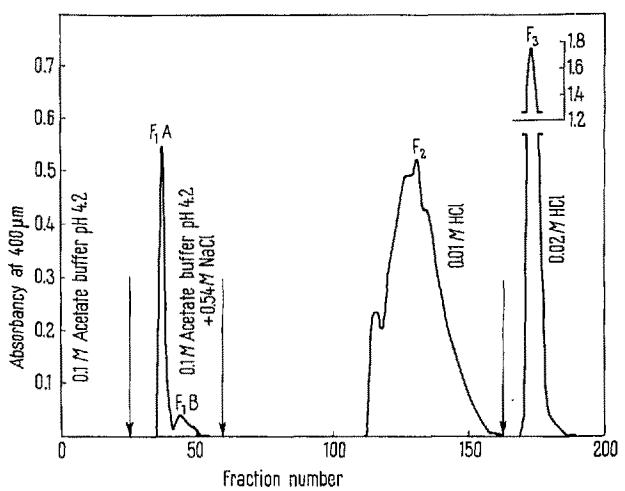

Fig. 1. Elution pattern of chicken erythrocyte histones (117 mg) on a carboxymethyl-cellulose column (14 × 2.3 cm).

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