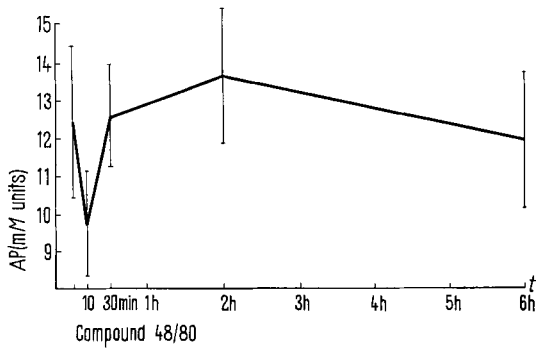
Ratten) wurde durch intraperitoneale Injektion von 1,25 mg/kg Compound 48/80 ein anaphylaktoider Schock ausgelöst. Compound 48/80 ist eine synthetische mastzelldegranulierende Substanz[4]. Chemisch handelt es sich um ein Gemisch von Dimeren, Trimeren und Tetrameren, die bei Kondensation von *p*-Methoxyphenyläthylmethylamin mit Formaldehyd entstehen. 10 und 30 min, 2, 6, 9, 15 und 24 h nach Injektion von Compound 48/80 erfolgte bei je 20 Tieren eine Bestimmung der AP im Serum. Die Methodik der Enzymbestimmung wurde bereits an anderer Stelle beschrieben[1,2].

Ergebnisse. Die normale Aktivität der alkalischen Phosphatase im Serum des verwendeten Rattenstammes betrug 12,4 ± 2,0 m*M*U. 10 min nach Auslösung des anaphylaktoiden Schocks sank die Aktivität auf 9,7 ± 1,4 m*M*U (Figur). 30 min nach der Injektion des Compound 48/80 fanden sich praktisch normale Aktivitäten (12,5 ± 1,3 m*M*U). 2 h nach der Auslösung des anaphylaktoiden Schocks war eine geringe Erhöhung der AP-Aktivität im Serum auf 13,6 ± 1,8 m*M*U zu beobachten. Die AP-Aktivitäten 6, 9, 15 und 24 h nach dem Schock lagen im Bereich der Norm (11,9, 11,8, 12,6 bzw. 12,4 m*M*U).



Die alkalische Phosphatase-Aktivität (AP) des Rattenserums nach anaphylaktoidem Schock. m*M*U = milliMol Unit.

Schlussfolgerungen und Zusammenfassung. Bei toxischen Nierenveränderungen legen alle histochemischen und biochemischen Befunde über die Abnahme der AP in der Niere und dem hierzu parallel gehenden Anstieg der AP-Aktivität im Harn übereinstimmend eine renale Herkunft der erhöhten AP-Ausscheidung nahe[2]. Für den anaphylaktoiden Schock ist ein gleicher Mechanismus anzunehmen. Da weder eine pathologische Erhöhung der AP-Aktivität des Serums nachzuweisen ist, noch eine pathologische Proteinurie vorliegt, muss auf eine renale Herkunft der erhöhten AP-Aktivität im Harn nach anaphylaktoiden und nach anaphylaktischen Reaktionen geschlossen werden. Die Differenzierung der AP des Harnes in Isoenzyme[5] könnte den letzten Beweis für die rein renale Herkunft der gesteigerten AP-Aktivität im Harn nach Schockreaktionen bringen.

Der Abfall der AP-Aktivität im Serum 10 min nach Injektion von Compound 48/80 dürfte keinen spezifischen Befund darstellen, sondern auf hämodynamischen Faktoren beruhen[6].

Summary. At seven different intervals after the elicitation of anaphylactoid shock (compound 48/80), determinations of alkaline phosphatase (AP) activity were carried out in rat serum. No significant increase could be detected. These findings exclude extrarenal factors as the cause for increased AP-activity in urine following anaphylactoid shock.

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Reduced Excretion of Urinary Procoagulant in Uremic Patients

Normal human urine when added in vitro to hemophilic blood corrects completely the clotting abnormality[1,2]. The responsible urine component is prepared from urine by adsorption onto $BaSO_4$[3], porcelain thimbles[4], or extensive dialysis of the urine and then subsequently purified by isoelectric precipitation at pH 3.5[3,4], precipitation with 0.57 *M* NaCl followed by chromatography on Sephadex G-200, and other steps[5]. The resulting white amorphous material is soluble in 0.005 *M* glycine buffer pH 8.6. One outstanding feature is the marked heat resistance of the procoagulant in solution, which can be brought to 98 °C for 10 min with little loss of activity. A few micrograms of the purified material normalize the clotting of hemophilic plasma (A, B, C) in the test tube (clotting time, prothrombin consumption, thrombelastogram). The urinary procoagulant also normalizes the clotting of plasma from patients with circulating 'antithromboplastins'[4] but not of thrombocytopenic plasma. The procoagulant converts purified prothrombin into thrombin in the presence of factor V, $CaCl_2$ and either whole platelets, platelet factor 3[6] or brain lipid[5]. The conversion of prothrombin to thrombin by the procoagulant is a concentration dependent time reaction. The yield of thrombin can be made

[1] W. GRUNKE, Z. ges. exp. Med. *96*, 512 (1935).

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[5] N. AOKI and K. N. VON KAULLA, (a) Thromb. Diath. haemorrh. *13*, 583 (1965); (b) in preparation.

[6] M. J. CALDWELL, K. N. VON KAULLA, E. VON KAULLA, and W. H. SEEGERS, Thromb. Diath. haemorrh. *9*, 53 (1963).

dependent in a linear relationship upon the concentration of the procoagulant.

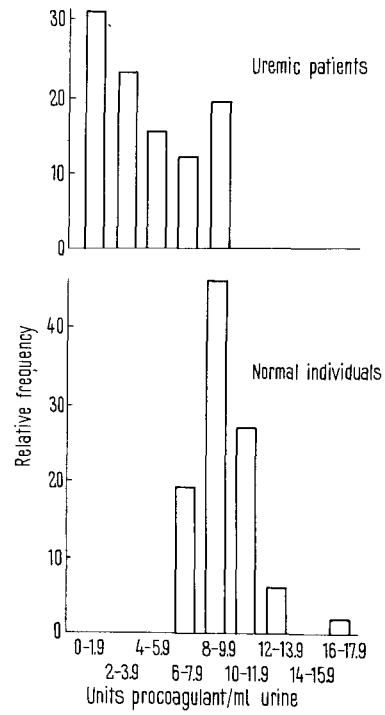
Based on this reaction mechanism and the linear relationship, a method for quantitative determination of the procoagulant in urine was developed. With such determination it is hoped to elucidate the origin of the procoagulant and to investigate its role (if any) in various diseases. The reagents for the determination are the following: 0.1 ml partially purified prothrombin (2000 units/ml), 0.05 ml brain lipid (30 mg/ml diluted 1:100 with saline before use), 0.2 ml $BaSO_4$-adsorbed, diluted bovine serum (1:75 with imidazol buffer pH 7.2 before use), 0.2 ml fresh urine previously dialyzed in the cold against distilled water for 4 h, 0.4 ml buffered saline pH 7.42 and finally 0.05 ml calcium chloride 0.5 *M*. They are mixed in the order given and the mixture is incubated for 90 min at 28 °C, at which time maximum thrombin yield is reached. The amount of thrombin generated is measured in an aliquot (0.4 ml) of the mixture with 0.1 ml of a 1% bovine fibrinogen solution as substrate and expressed in arbitrary laboratory procoagulant units according to the formula (thrombin units[7]/ml divided by volume test sample) = procoagulant units/ml test sample.

The average excretion (first morning specimen) of 63 normal individuals was studied. There was a moderately but significantly higher ($P < 0.01$) excretion of procoagulant in (a) males under 30 than in females under 30 and (b) males under 30 as compared to males over 50 years of age.

The origin of the urinary procoagulant is not established. Based on studies with material prepared in our laboratory, it was suggested that the procoagulant represents a moderately active tissue thromboplastin[8]. However, this view needs reconciliation with the clear-cut variation in excretion patterns depending on the group of persons investigated and also with the marked heat stability of the procoagulant. If the material is a tissue thromboplastin, it can only derive from the urogenital tract. Urine obtained from the pelvis of human kidneys in vivo contains essentially normal amounts of procoagulant. Therefore, the procoagulant derives either from the kidneys themselves as a tissue thromboplastin or possibly from the blood as product of coagulation. With either possibility, the excretion pattern of the procoagulant could be expected to be dependent on some kidney function. This is indeed the case: uremic patients (primarily with chronic glomerulonephritis) excrete considerably less procoagulant than normal individuals. The relative frequency distribution of the excretion values of the urinary procoagulant in 26 uremic patients is grossly different from the one of 63 normal individuals. This is shown in the Figure.

The average excretion (4.08 units/ml) of 26 uremic patients was significantly lower ($P < 0.01$) than in healthy individuals (9.47 units/ml). Most startling, however, was the total absence of the procoagulant in urine of 27% of the uremic patients. Also, 70% of the uremic values were below the lowest normal values. These findings suggest that the low excretion of the procoagulant in uremic patients reflects an impaired kidney function. No relation has been observed so far between procoagulant excretion and blood urea nitrogen, blood creatinine, creatinine clearance, specific gravity, or protein content of the urine. The low or absent excretion is a real one and is not simulated by the appearance of a clotting inhibitor in the uremic urine: adding of zero-excretion uremic urine to normal urine reduces its activity only in relation to the dilution of this urine with the urine free of procoagulant (normal urine contains at times such an inhibitor which contaminates the procoagulant preparation and which is removed during further purification[5]).

The observation of low excretion of urinary procoagulant in uremic patients does not settle the question of its origin (it might, however, indicate that the excretion could be related to a metabolic function of the kidney). Preliminary rabbit experiments show that tubular damage induced by $HgCl_2$-poisoning results in a complete loss of procoagulant excretion in the urine. With recovery the procoagulant excretion returns gradually to normal. Further studies with this poorly explored urine component are indicated[8,9].



Distribution of relative frequency of the excretion of the urinary procoagulant in uremic patients (upper histograms) and normal individuals (lower histograms). Note the prevalence of low (or totally absent) excretion values in uremic patients.

Zusammenfassung. Ein hochaktiver, gerinnungsfördernder Faktor wird normalerweise im menschlichen Urin ausgeschieden. Die Ausscheidung ist quantitativ bei gesunden Erwachsenen altersabhängig und bei Urämie wesentlich vermindert.

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[9] The work reported here was supported by grants-in-aid from the Belle Bonfils Memorial Blood Bank, Denver, and the National Heart Institute USPHS (HE 5538).