der GZ. bei weiterer Steigerung der Protaminsulfatkonzentration nur noch geringgradig zunahm, verwendeten wir die angegebene Dosierung für alle folgenden Versuche.

Die Thrombininaktivierung der Antibiotika konnte durch Protaminsulfat beträchtlich vermindert werden (Abb. 3). So findet sich vor allem bei Penicillinkonzentrationen, die sonst das Plasma bei Zusatz der verwendeten Thrombinmenge praktisch ungerinnbar machen, eine kaum verlängerte GZ.

Aureomycin bildet hier eine Ausnahme, da es bei Zusatz von Protaminsulfat nicht zur normalen Gerinnselbildung, sondern zur Ausfällung kleinster, nicht zusammenhängender Flocken kommt (strichlierte Kurve).

Das Protaminsulfat hat also, neben seiner Eigenschaft, Heparin zu inaktivieren, auch einen Einfluss auf die thrombinhemmende Wirkung der Antibiotika, die es innerhalb einer gewissen Konzentration aufzuheben vermag.

Diese Tatsache wirft eine Reihe von Problemen auf:

1. Es handelt sich bei der verkürzenden Wirkung des Streptomycins und des Protaminsulfats auf die GZ. um zwei verschiedene Wirkungsmechanismen. Beide zusammen haben eine stärkere Wirkung auf die Antithrombine als jedes für sich allein (siehe Abb. 2). Dementsprechend wird die Gerinnung bei Zusatz von Streptomycin plus Protaminsulfat zu Plasma stärker verkürzt als bei Zusatz der für die Gerinnungsverkürzung optimalen Protaminsulfatkonzentration.

2. Der eigenartig wellenförmige Verlauf der Penicillinkurve, der nach einem anfänglichen Anstieg keine Änderung der GZ. zwischen 30000 und 60000 Einheiten Penicillin/cm³ erkennen lässt, spricht ebenfalls für zwei getrennt zur Wirkung kommende Faktoren der Thrombininaktivierung.

3. Völlig ungeklärt ist die Tatsache, dass Aureomycin in höherer Dosierung eine kompakte Gerinnselbildung verhindert.

F. MLCZOCH und H. VINAZZER

II. medizinische Klinik der Universität Wien, den 15. Oktober 1951.

Summary

The influence of penicillin, streptomycin and aureomycin on the second phase of coagulation has been examined. The following results have been found:

(1) Doses which are adequate to the therapeutic blood level do not influence the thrombin-fibrinogen reaction *in vitro*.

(2) Doses which highly exceed the therapeutic blood level considerably prolong the thrombin-fibrinogen reaction by blocking thrombin.

(3) This thrombin-blocking effect of antibiotics is suppressed by protamin sulphate up to a certain concentration.

Antidiuretic Action of Small Doses of Enteramine Extracts in the Rat

II. Extracts of *Discoglossus pictus*' skin

In a previous report[1] the antidiuretic action displayed in rats by small doses of acetone extracts of posterior salivary glands of *Octopus vulgaris* was described, and the mechanism of this action discussed.

In the present researches, which complete the preceding ones, acetone extracts of *Discoglossus pictus*' skin were used. This material is very rich in enteramine; just as rich, or nearly so, as the salivary extracts of *Octopus vulgaris*[1], over which it has the great advantage of being free of tyramine, of octopamine, and, apparently, also of other substances interfering with the antidiuretic action of enteramine.

The methods here employed correspond to those previously described: to the study of PAI (*p*-aminohippuric acid) and thiosulphate renal excretion, we have added that of creatinine excretion.

Our experimental procedure allows us to ascertain, with satisfactory accuracy, the absolute and relative values of urine flow, and also to obtain reliable information on the relative changes of glomerular filtration rate and renal plasma flow in animals treated with *Discoglossus* extract, as compared to controls.

The following doses of *Discoglossus* extract (expressed in fresh tissue) were injected subcutaneously, immediately after the second water load (5 cm³/100 g) was given by stomach tube: 0·001 g, 0·01 g, and 0·1 g per 100 g rat.

At the same time, still subcutaneously, the following test substances were administered: PAI 50 mg/kg (effective renal plasma flow), sodium thiosulphate 500 mg/kg or creatinine 200 mg/kg (glomerular filtration rate).

Diuresis was closely followed for 7 h. The concentration of test substances was estimated in samples of urine collected after 60 min, 90 min, 2 h, 3 h, and 7 h. For the smallest dose of *Discoglossus* extract only the urine flow was recorded.

The *Discoglossus* material we investigated, obtained from about 1000 animals (Sicily, March 1951), leaves a total dry residue of 17·4 mg per gram fresh tissue. Its acute toxicity in rats is very low: a subcutaneous injection of the extract corresponding to 10 g fresh tissue/100 g body weight allows all treated animals to survive.

The table shows the percent excretion of water and test substances in the with *Discoglossus* extract treated groups, when compared to the excretion in the control groups, arbitrarily considered = 100. Absolute excretion values, from which these data are calculated, will be reported in the work *in extenso*.

The conclusions we can draw from the results set down in the table are quite similar to those reported in our preceding communication[2]:

(1) The extract of *Discoglossus pictus*' skin constantly causes, in hydrated rats, a remarkable reduction of the urine flow. The antidiuretic action is roughly proportional to the amount of extract administered. Using our experimental methods, the smallest dose of extract still active on the diuresis corresponds to 1 mg fresh skin/100 g body weight.

In the work *in extenso* we shall demonstrate that the antidiuretic action of the *Discoglossus* extract is exclusively, or nearly so, due to its enteramine content. Probably such content is about 1/1000, when referred to fresh tissue: this means that a quantity of pure enteramine as little as 1 µg/100 g body weight is sufficient to reduce, in a significant manner, diuresis in rats.

Experiments carried out with pure enteramine (= 5-hydroxytryptamine) and with synthetic enteramine-like derivatives, have confirmed these data of sensitiveness and have also confirmed that, in rats, the smallest active dose of enteramine is at least 5000–10000 times lower than the letal dose.

(2) The reduction of urine flow is invariably accompanied by a conspicuous reduction of renal excretion of

[1] V. ERSPAMER and A. OTTOLENGHI, Exper. *8*, 31 (1952).

[1] V. ERSPAMER and M. VIALLI, Nature *167*, 1033 (1951).

[2] V. ERSPAMER and A. OTTOLENGHI, Exper. *8*, 31 (1952).

	Relative percent excretion (controls = 100) after					
	1	1½	2	3	4	7 hours
Water						
Discoglossus 0·001 g/100 g (*C*)	85·9	85·9	90·9	97·2	96·8	—
Discoglossus 0·01 g/100 g (*A*)	33·1	53·8	69·5	76·1	81·2	86·4
Discoglossus 0·01 g/100 g (*B*)	31·7	55·2	66·8	81·6	89·6	90·3
Discoglossus 0·1 g/100 g (*A*)	13·8	21·3	35·1	68·1	75·7	86·8
Discoglossus 0·1 g/100 g (*B*)	16·7	13·6	27·8	64·3	82·1	86·6
Thiosulphate						
Discoglossus 0·01 g/100 g (*A*)	25·3	54·1	70·1	75·5	—	78·6
Discoglossus 0·1 g/100 g (*A*)	7·3	22·6	33·7	48·3	—	53·2
Creatinine						
Discoglossus 0·01 g/100 g (*B*)	41·5	68·4	86·8	97·7	—	101·1
Discoglossus 0·1 g/100 g (*B*)	7·5	19·4	50·0	97·6	—	107·7
PAI						
Discoglossus 0·01 g/100 g (*A*)	38·2	68·8	91·6	96·3	—	102·0
Discoglossus 0·01 g/100 g (*B*)	51·4	75·9	92·7	100·8	—	103·1
Discoglossus 0·1 g/100 g (*A*)	10·4	36·9	56·5	81·6	—	92·1
Discoglossus 0·1 g/100 g (*B*)	14·0	15·5	45·5	80·2	—	86·5

Groups *A* = 48 rats, each. Groups *B* = 64 rats, each. Group *C* = 48 rats.

thiosulphate and creatinine, that is by a decrease in glomerular filtration rate.

Since at our dosage the *Discoglossus* extract is wholly ineffective on the systemic blood pressure of the rat (extract doses exceeding 0·2–0·3 g fresh tissue/100 g body weight are required to cause hypotension), only a spasm of the contractile structures of the *afferent* vascular bed of the glomerulus may explain the decrease of the glomerular filtration rate, as a consequence of a drop in the intraglomerular hydrostatic pressure.

Of course, also the blood flow in the intertubular capillary network will be compromised: this fact is confirmed by the reduction of the tubular excretion of PAI.

(3) After administration of the *Discoglossus* extract, the reduction of urine flow nearly always exceeds that of glomerular filtration, as inferred from creatinine excretion.

In our opinion this is not due to a stimulation of the tubular reabsorptive activity, but simply represents the consequence of a slowing in the passage of the glomerular filtrate through the tubulus; a slowing brought about by a reduction in the quantity of the filtrate.

Chemical and pharmacological researches carried out on pure enteramine, obtained by extraction and synthetically, and on natural and synthetic enteramine-like derivatives, will be published shortly.

These researches will allow us to indicate with greater accuracy, besides the pharmacological actions, also the biological significance of enteramine, substance which represents the specific secretion or deposit product of the typical enterochromaffin cells and of similar chromaffin cellular elements.

The working hypothesis that grows ever stronger as our researches go on, is the one that considers enteramine as a regulator, probably an hormonal regulator, of the flow of circulating fluids (blood, hemolymph) through the kidney, and therefore as a regulator of the function itself of the latter.

It may be that other hypotheses on the physiological significance of enteramine will be put forward in the future. It is quite evident that none of them will be acceptable when neglecting the numerous data we have already collected on the distribution of enteramine and of the enterochromaffin cell system in Vertebrates and Invertebrates.

V. Erspamer and A. Ottolenghi

Pharmacological Institute, University of Bari, October 2, 1951.

Zusammenfassung

Enteraminhaltige Hautextrakte von *Discoglossus pictus* wirken deutlich diuresehemmend noch in einer Menge, die 1 mg frischen Gewebes/100 g Ratte entspricht. Bei höheren Dosen ist die antidiuretische Wirkung sehr ausgeprägt und viel besser wahrnehmbar als mit Speicheldrüsenextrakten von *Octopus vulgaris*.

Der Mechanismus der Enteramin-Antidiurese wird nochmals in einer Drosselung des afferenten Gefässsystems des Glomerulus identifiziert.

Enteramin (5-Hydroxytryptamin) ist als spezifischer, hormonaler Regulator der intrarenalen Vasomotorik zu betrachten.

Über die Ausscheidung und Speicherung von radioaktiv indizierten Xylanschwefelsäureestern

In einer früheren Arbeit[1] wurde gezeigt, dass sowohl die blutgerinnungshemmende Wirksamkeit als auch besonders die Toxizität der Polysaccharidschwefelsäureester von der Grösse und Gestalt der Moleküle abhängen. Es ergab sich ferner, dass Xylanschwefelsäureester vermutlich infolge der Abwesenheit von veresterten primären Alkoholgruppen bei geringer Toxizität besonders gute blutgerinnungshemmende Wirkungen besitzen. Sie haben bereits therapeutische Bedeutung erlangt.

Zur eindeutigen Klärung der Frage, ob derartige Substanzen ohne Bedenken an Stelle von Heparin therapeutisch verwendet werden können, schien es notwendig, ihr Schicksal im Organismus, das heisst das Ausmass von Ausscheidung im Harn und eventuelle spezifische Speicherung in einzelnen Organen, zu untersuchen. Da es nicht möglich ist, Xylanschwefelsäureester in ge-

[1] E. Husemann, K. N. von Kaulla und R. Kappesser, Z. Naturforsch. *1*, 584 (1946).