The tubes are rapidly cooled in an ice-bath and transferred to a water-bath of 20 °C. Fluorescence is measured at 20 °C in thermostatted quartz cuvettes. Temperature control is essential because fluorescence sharply declines with increasing temperature. Standard dilutions of Na-ampicillin are made up in serum from the same subject, secured before administration of ampicillin, and are treated together with a blank (serum without ampicillin) in the same way.

In serum α-aminobenzylpenicilloic acid leads to the same product at a lower yield. This property allows one to distinguish ampicillin and α-aminobenzylpenicilloic acid and to determine ampicillin in the presence of α-aminobenzylpenicilloic acid by measuring fluorescence in one sample directly and in a second sample after enzymatic hydrolysis of ampicillin.

(b) *Indirect method in serum* (in the presence of ampicilloic acid). Unknown serum saples and standards in serum are divided into 2 portions. One portion receives 40 units of penicillinase (β-lactamase) per ml, dissolved in water (e.g. 0.02 ml/ml serum of a solution with 2,000 units/ml) and the other portion an equivalent amount of water. The penicillinase containing tubes are incubated for 20 min at 37 °C, the penicillinase-free samples are heated to 37 °C for a few min, 3.2% uranyl acetate dihydrate solution is added to all tubes, and the samples are processed as described under (a). For each standard concentration the difference in fluorescence reading between penicillinase-free and penicillinase-containing sample is formed, with these values a calibration curve is drawn and the corresponding differences of the unknowns are read on it.

Ampicillin was either commercial Na-ampicillin (Penbritin) or the trihydrate of the free acid dissolved by neutralizing with NaOH. Benzylpenicillin was the commercial Na salt (Penicillin NOVO). Uranyl acetate was the dihydrate ($UO_2(COOCH_3)_2 \cdot 2\ H_2O$) of analytical grade (Fluka). All other chemicals were a.g. material from Merck or Fluka. Penicillinase was a pure β-lactamase (Penase LEO). Ampicilloic acid was prepared by alkaline hydrolysis of an aqueous solution of 1 mg/ml Na-ampicillin (0.1 *N* NaOH, heating in boiling water bath for 30 min).

Blood was defibrinated by stirring with a wooden rod. Citrated plasma proved unsuitable because it requires higher uranyl acetate concentration for complete deproteinization.

A simple filter fluorimeter can be used with the mercury lines 313 + 366 nm for excitation and a filter passing emitted light above 420 nm.

A linear dependence of fluorescence intensity on ampicillin concentration is obtained between zero and 20 μg/ml serum with method (a).

Penicillinase reduces the fluorescence obtained with ampicillin alone and ampicillin in the presence of an equimolar amount of ampicilloic acid in serum by the same degree. This means that ampicilloic acid does not interfere with the action of penicillinase on ampicillin, and that method (b) indeed allows determination of ampicillin in the presence of ampicilloic acid. Calibration curves from method (b) are also linear.

Method (b) was applied 10 times to serum (from 2 horses) containing 0.1 μg/ml Na-ampicillin. The mean of the readings differed by 2 standard deviations from the blank value, which means that the odds of mistaking 0.1 μg/ml for zero are of the order of 2%. Consequently detectability in the indirect method is at least 0.1 μg/ml.

The indirect method (b) can be expected to have considerable specificity because 1. the colour development depends on the presence of the α-amino-group in the ampicillin molecule, and 2. the application of penicillinase imparts the high specificity of the enzyme for the β-lactam structure to the test. Its sensitivity is sufficient for the accurate assay of serum concentrations reached with therapeutic dosage schedules of ampicillin, which are above 1 μg/ml (WALTER and HEILMEYER[5]).

Zusammenfassung. Behandlung von Ampicillin oder Ampicilloylsäure mit Uranylacetat in der Wärme bei pH 5.2 ergibt ein stabiles fluoreszierendes Produkt. Ausnützung dieser Tatsache und der proteinfällenden Wirkung von Uranylacetat ergibt eine einfache Methode zur Ampicillinbestimmung im Serum mit einer Empfindlichkeit von etwa 0.1 μg/ml. Kombination mit Penicillinasebehandlung erlaubt, Ampicillin in Gegenwart von Ampicilloylsäure zu messen.

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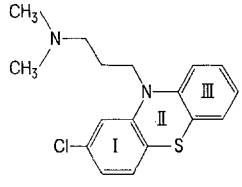
[5] A. M. WALTER and L. HEILMEYER, *Antibiotika-Fibel*, 2nd edn. (G. Thieme, Stuttgart 1965).

[6] We thank Messrs. Aldepha A.G. and Beecham for gifts of penicillin g and ampicillin.

Corrigenda

T. W. STONE: *On the Antagonism of Ergot Alkaloids and Dopamine by Phenothiazines*, Experientia *30*, 827 (1974). The formula for chlorpromazine in Figure 1 A has been printed incorrectly and illustrates chlorproethazine. The correct formula for chlorpromazine is shown in Figure 2 A. The correct formula is as follows:



Chlorpromazine

A. L. MISRA, P. K. NAYAK, M. N. PATEL, N. L. VADLAMANI and S. J. MULÉ: *Identification of Norcocaine as a Metabolite of [^3H]-Cocaine in Rat Brain*, Experientia *30*, 1312 (1974). In the Table on page 1312, column half-life (h), it should read **0.8** instead of 4.8 and **1.0** instead of 5.0.