Table III. Pathogenicity and immunogenicity of the IUdR-resistant variant of *Herpes simplex* virus in rabbits

1st infection (IU i.c.s.)[a]		2nd infection (IU i.c.s.)[a]		No. rabbits affected from Conjunctivitis	Keratitis	Encephalitis	No. rabbits dead within 15 days from the 2nd infection
—		HS[b]	10^5	12/12	12/12	12/12	12/12
—		HS	10^4	12/12	11/12	11/12	11/12
—		HR	5×10^6	4/12[c]	2/12[c]	0/12	0/12
—		HR	5×10^5	0/12	0/12	0/12	0/12
HR[b]	2×10^6	HS (2 days apart)	10^5	1/12	1/12	0/12	0/12
HR	2×10^6	HS (30 days apart)	10^5	0/12	0/12	0/12	0/12

[a] Tissue culture infectious units, injected in the conjunctival sac. [b] See Table I. [c] Lesions reversed within 6 days.

that inhibitory for the parental virus. It is noteworthy that this IUdR-resistance is evident both in HEp 2 and in primary mouse embryo cells normally endowed with thymidine kinase activity[4].

Data in Table II show that on the basis of the lethal effect produced by intracerebral injection in mice, the IUdR-resistant strain is about 200 times less virulent than the parental virus. In addition, when injected i.p., the IUdR-resistant variant protects mice from lethal inocula of the parental strain, injected intracerebrally 45 days apart.

Data obtained in rabbits are of more interest, in view of the analogies which exist between rabbit and human herpetic keratitis. As shown in Table III, eye infection with as little as 10^4 IU of the parental sensitive strain of *Herpes simplex* virus are sufficient to trigger a sequence of pathologic events (conjunctivitis, keratitis, encephalitis) which, almost in all cases, results in death. Eye treatments with 2×10^6 IU of the IUdR-resistant variant are, on the contrary, almost deprived of any pathological effect and, moreover, protect rabbits from highly active inocula (10^4 IU) of the parental sensitive strain, introduced by the same way either 3 or 30 days apart.

Research is in progress to establish whether and which relationship exists between IUdR-resistance and avirulence of the virus strain under study, and to ascertain what kind of mechanism, either interferonic or immune specific, or both, is at the basis of the protection this virus induces in animals[5].

Résumé. Par passage en série sur des cellules en culture et en présence de 5-iodo-2-desoxyuridine (IUdR) on a obtenu une souche de virus herpétique résistant à l'IUdR. Cette souche est très peu virulente chez la souris et le lapin, mais est capable de protéger ces animaux contre le virus pathogène d'origine.

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[1] B. Loddo, M. L. Schivo and W. Ferrari, Lancet *2*, 914 (1963).
[2] W. Ferrari, B. Loddo and M. L. Schivo, Virology *26*, 154 (1965).
[3] V. I. Chernos, K. G. Apridonidze and Yu. Z. Ghendon, J. gen. Virol. *6*, 355 (1970).
[4] M. A. Marcialis, E. Biondi, A. Atzeni, M. L. Schivo, P. Ucchedu and B. Loddo, Experientia *29*, 733 (1973).
[5] This work has been supported by a Grant of Consiglio Nazionale delle Ricerche, Roma.

PRO EXPERIMENTIS

A Simple Fluorometric Assay for Ampicillin in Serum

A number of chemical methods for the determination of ampicillin are available. Some of them lack sensitivity (British Pharmacopoeia, 1963, addendum 1964, p. 2; Gravnetterova[1]; Bundgaard and Ilver[2]), or are not applicable to serum (Br. Pharmacopoeia; Smith et al.[3]; Bundgaard and Ilver[2]). Others are laborious because of an extraction step (Gravnetterova[1]; Jusko[4]) or titration procedures (Gravnetterova[1]). A fluorometric method (Jusko[4]) is sensitive and can be applied to serum but is not specific in that α-aminobenzylpenicilloic acid (ampicilloic acid) gives the same response as ampicillin itself.

A stable product with blue fluorescence is formed from ampicillin at pH 5.2 and 100 °C in the presence of low concentrations (< 1 m*M*) of uranyl acetate. The compound has an absorption maximum at 338 nm and an emission maximum at 415 nm at pH 5.2. Fluorescence intensity decreases below pH 3. Benzyl-penicillin does not give any fluorescence. An assay procedure for ampicillin in serum, based on these observations is proposed in the following. The method is simple because uranyl acetate achieves both deproteinization and colour development.

(a) *Direct method in serum* (in the absence of ampicilloic acid). In a polyethylene centrifuge tube, 9 parts of serum and 5 parts of a 3.2% (w/v) aqueous uranyl acetate (dihydrate) solution are mixed by vigorous shaking for 1 min. The precipitate is removed by centrifuging the tubes for 15 min at 4,500 rpm, the supernatant is filtered through paper (Schleicher and Schuell Nr. 589[3]) into glass test tubes and the clear and colourless filtrate is heated for 20 min to 100 °C, the tubes being covered with loosely fitting glass balls to minimize evaporation.

[1] J. Gravnetterová, Clin. chim. Acta *11*, 128 (1965).
[2] H. Bundgaard and K. Ilver, J. Pharm. Pharmac. *24*, 790 (1972).
[3] J. W. Smith, G. E. de Grey and V. J. Patel, Analyst *92*, 247 (1967).
[4] W. J. Jusko, J. Pharm. Sci. *60*, 728 (1971).

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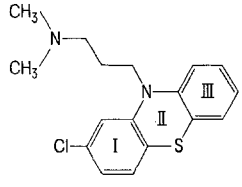
[5] A. M. WALTER and L. HEILMEYER, *Antibiotika-Fibel*, 2nd edn. (G. Thieme, Stuttgart 1965).

[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.