exception of serum No. 6 which gave non-specific precipitation (Figure 1). The results read before and after staining were always in agreement.

RIEOP revealed far more details. Apart from the already recorded precipitation of rabbit hyperimmune serum, also human sera Nos. 2, 4, 5, 6, 7 gave positive reaction and some of them showed even 2 lines of precipitation (Figure 2). The results of RIEOP were always unequivocal and enabled easy differentiation from non-specific precipitation seen in counterelectrophoresis. However, it was not until 7th day of exposition that every detail of the test could be identified. It seems to be the main drawback of the method, in comparison with counterelectrophoresis which is completed within 1–2 h, and which is much less expensive.

We think that counterelectrophoresis, such as recently described by DESPOMMIER et al.[3] will be most suitable for rapid and large-scale application, while RIEOP will remain on invaluable diagnostic tool in some selected cases being extremely sensitive and specific. In our hands the method proved to be at least as sensitive as IHA and IFA tests detecting microprecipitates not discernible to the naked eye.

Zusammenfassung. Serum *Trichinella-spiralis*-infizierter Menschen wurde mittels Radioimmunoelektro-Osmophorese (RIEOP) bei Verwendung von mit radioaktivem Jod gezeichnetem Antigen untersucht. Die RIEOP-Technik erwies sich als mindestens so empfindlich wie die passive Haemagglutinationsreaktion und die Immunofluorescenz.

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[8] We wish to thank Dr K. MADALIŃSKI and Dr A. GAJEWSKI of the National Institute of Hygiene for their help in labelling the antigen.

Flambamycin, a New Antibiotic from *Streptomyces hygroscopicus* DS 23 230

In the course of a study on the production of antimicrobial agents by microorganisms, a new antibiotic, flambamycin (also known as 21 190 RP), was discovered in the culture broths of *Streptomyces hygroscopicus* DS 23230.

The strain was isolated from a sample of soil collected in Great Britain and its antibiotic properties were demonstrated by classical methods[1]. It exhibits all the main morphological and biochemical characteristics of the species *Streptomyces hygroscopicus*, as described by TRESNER and BACKUS[2] and WAKSMAN[3], especially the tight spiral sporophores and their clustered insertion upon the main filament, the dark grey colour of the normally sporulated mycelium, and also in ageing cultures the production of dark patches and of an exudate on the surface of the colonies on agar medium.

Streptomyces hygroscopicus DS 23230 thrives on aerated and stirred media while producing the antibiotic. The preparation of a substantial quantity of flambamycin is carried out as follows: the strain, stored as a spore-soil mixture, is grown in test tubes on PRIDHAM's starch plus minerals agar medium[4] for 15 days at 26 °C. It is brought to a suitable state of development by 2 successive transfers, first into 250 ml of liquid medium (composition in g/l: hydrated glucose 10, peptone 10, meat extract 5) in a 2 l flask, incubated for 48 h at 26 °C on a rotary shaker, then into 40 l of the same medium in a 75 l fer-

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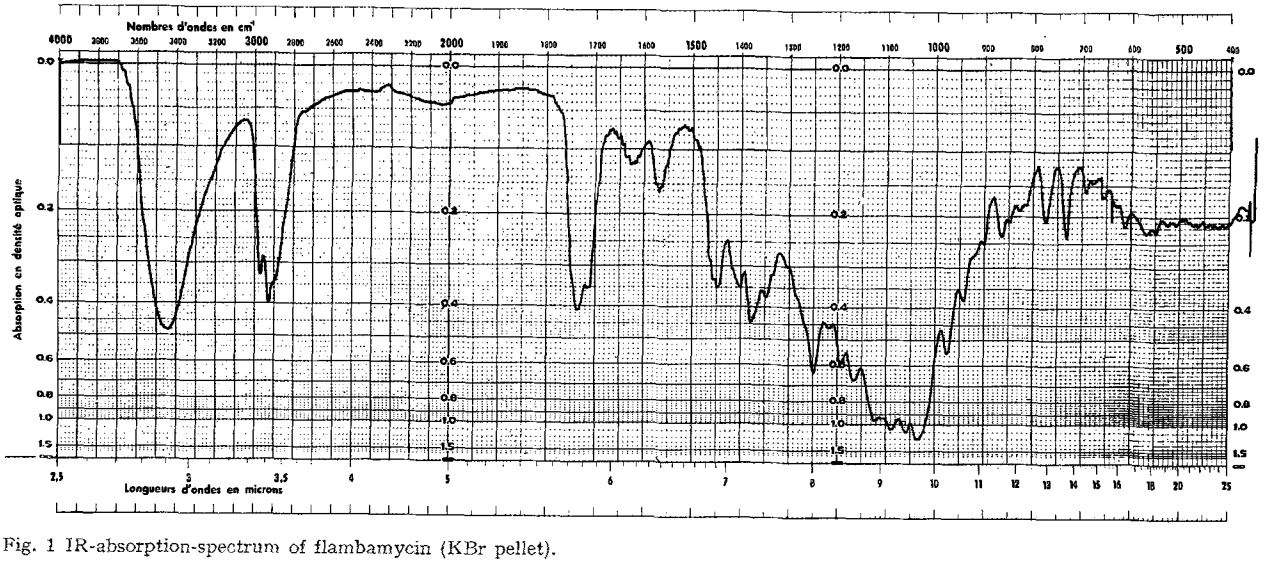

Fig. 1 IR-absorption-spectrum of flambamycin (KBr pellet).

menter in which the culture is agitated and aerated for 30 h at 26 °C. The final culture is obtained by seeding the previous culture as a whole into a 800 l stainless steel fermenter containing 400 l of the following medium (in g/l): soybean meal 15, distillers' solubles 25, glucose monohydrate 20, calcium carbonate 5, ammonium sulphate 2, hydrated cobalt chloride 0.02. The culture is agitated, aerated and kept at 26 °C. 2 further additions of glucose each of 2 g/l are made after 24 and 48 h. The production of flambymacin reaches its peak after 60 h at about 300 µg/ml.

Most of the antibiotic is in the broth filtrate. The cell mass is removed by filtration at pH 7 with a filtration adjuvant, Clarcel Dic (CD). Flambamycin is then extracted from the filtrate by an equal volume of ethyl acetate without changing the pH. The organic layer is separated and concentrated to 1/100 th of its volume by distillation under reduced pressure and with progressive addition of butanol. The butanol solution is allowed to stand at 4 °C for 16 h, which causes the precipitation of a crude product containing about 70% of the antibiotic.

Flambamycin is crystallised under nitrogen from acetone, methanol or ethanol, after treating the hot solution with Darco G 60 active charcoal. The antibiotic may also be crystallised by dissolving the crude product in pyridine or dimethylformamide and precipitating by dilution with water. It yields a fine colourless crystalline solid, m.p. 226–228 (Kofler); $[\alpha]_D^{20} = -11.4° \pm 0.4°$ ($c = 1$ in chloroform). It is soluble in chloroform, dimethylformamide and pyridine, moderately soluble in water, acetone and ethanol, sparingly soluble in n-hexane. $\lambda_{max} = 288$ nm (E 1%/1 cm = 12) (ethanol). The IR-spectrum (KBr pellet) is given in the Figure.

Elemental composition; C 50.5%; H 6.6%; O 38.4%; Cl 4.5% agrees with the hypothetical molecular formula $C_{61}H_{90}Cl_2O_{35}$. The molecular weight (1454) is in good agreement with the acid equivalent (1400) titrated in dimethylformamide with tetrabutylammonium hydroxide.

From its physicochemical and antibacterial properties, flambamycin is very similar to 3 other antibiotics avilamycin[5], curamycin[6], and everninomycin[7]. It is readily, however, differentiated from the latter, which contains nitrogen in its molecule, and from the 2 former compounds by thin layer chromatography as shown by the Rf values in Table I.

Structural studies carried out by W. D. Ollis, C. Smith and D. E. Wright[8] showed that, by acid hydrolysis, flambamycin yields 4-*O*-dichloroisoeverninyl-2-desoxy-D-rhamnose (curacin), 2,6-*O*-dimethyl-D-mannose (curamicose) and 4-*O*-methyl-D-fucose. All these com-

Table I. Chromatographic comparison of antibiotics

Substances	Rf [a]
Avilamycin	0.54
Curamycin	0.50
Flambamycin	0.32

[a] Conditions: Silicagel Merck No. 7 731 plate, activated at 130 °C for 2 h; 25 µg spots in 25 µl of a chloroform-methanol mixture (50:50 v/v); development at 20 °C in a tank equilibrated with a chloroform-methanol (92-8) mixture; drying of the plate; the antibiotic activity is located autobiographically on agar medium plate seeded with *Bacillus subtilis* or colorimetrically by spraying the plate with concentrated sulphuric acid and heating for 10 min at 110 °C.

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Table II. Bacteriostatic activity of flambamycin

Test organism	Minimum inhibitory concentration (µg/ml)
Staphylococcus aureus (strain 209 P – ATCC 6 538 P)	0.85
Staphylococcus aureus (strain 133, Institut Pasteur)	1.1
Staphylococcus aureus (strain Smith)	1.2
Sarcina lutea (ATCC 9 341)	0.2
Streptococcus faecalis (ATCC 9 790)	2
Streptococcus viridans (Institut Pasteur)	15
Streptococcus pyogenes hemolyticus (strain Dig 7, Institut Pasteur)	0.25
Diplococcus pneumoniae (strain Til, Institut Pasteur)	0.1
Neisseria catarrhalis (A 152, Institut Pasteur)	1.25
Neisseria meningitidis (5 813, Institut Pasteur)	0.6
Neisseria gonorrhoeae (A 50, Institut Pasteur)	1.25
Bacillus subtilis (ATCC 6 633)	15
Bacillus cereus (ATCC 6 630)	9
Mycobacterium species (ATCC 607)	> 150
Escherichia coli (ATCC 9 637)	> 150
Shigella dysenteriae (Shiga L, Institut Pasteur)	> 150
Salmonella paratyphi A (strain Lacasse, Institut Pasteur)	> 150
Salmonella schottmuelleri (paratyphi B; strain Fougenc, Institut Pasteur)	> 150
Proteus vulgaris	> 150
Klebsiella pneumoniae (ATCC 10 031)	> 150
Pseudomonas aeruginosa (strain Bass, Institut Pasteur)	> 150
Brucella bronchiseptica (CN 387, Wellcome Institute)	35
Pasteurella multocida (A 125, Institut Pasteur)	0.2
Mycoplasma gallisepticum (A 514, Institut Pasteur)	3

ponents have been found in avilamycin, curamycin, and everninomycin, which confirms the close relationship between those 4 antibiotics[9].

Flambamycin exhibits a very low toxicity. The aqueous suspension is practically non-toxic orally in the mouse and its LD_{50} is 2,500 mg/kg by the s.c. route.

The bacteriostatic activity of flambamycin against some organisms is shown in Table II. The minimum inhibitory concentration (MIC) determinations were carried out by the dilution method in the appropriate medium for each organism and after incubation for 18 h at 37 °C. Flambamycin is mainly active in vitro against gram-positive or gram-negative cocci and some gram-positive bacilli. It is practically inactive against gram-negative bacilli, yeasts and filamentous fungi.

In vivo it retains its activity against the organisms already shown to be sensitive in vitro. It therefore has an excellent therapeutic activity in mice infected experimentally with staphylococcus, streptococcus and meningococcus. However, as shown in Table III, flambamycin is active only by the s.c. route, since it is not assimilated through the intestinal tract.

Given by the s.c. route, flambamycin is inactive against several animal parasitic infections: e.g. chickens infected with *Eimeria tenella* or *Plasmodium gallinaceum* and mice infected with *Plasmodium berghei*.

In conclusion, flambamycin, a new member of the heterosidic antibiotics derived from dichloroisoeverninic acid, exhibits in vitro excellent activity against gram-positive or gram-negative cocci. The same activity is found in vivo when the antibiotic is given parenterally.

Résumé. La flambamycine, nouvel antibiotique produit par *Streptomyces hygroscopicus* DS 23 230, est un hétéroside dont l'aglycone est l'acide dichloroisoéverninique, précédemment mis en évidence dans l'avilamycine, la curamycine et l'éverninomycine. Tant in vitro qu'in vivo elle inhibe fortement la croissance des cocci gram-positifs ou -négatifs, mais thérapeutiquement elle n'est utilisable que par la voie parentérale.

Table III. Curative doses of flambamycin in the mouse[a]

Infecting organism	CD_{50} (mg/kg/day)
Staphylococcus aureus (strain Smith)	13
Streptococcus pyogenes hemolyticus (strain Dig 7)	18
Neisseria meningitidis (strain IP 5 813)	2

[a] s.c. route for 2 days.

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In vitro and in vivo Inhibitory Action of 2-Amino-4,6-Dichloropyrimidine on Polio and Herpes Virus

2-Amino-4, 6-dichloropyrimidine (Py 11) prevents the growth of poliovirus in aminoacid-free medium, by impairing the assembly of virus RNA and proteins into complete particles[1]. Due to the antagonism exerted by glutamine and cysteine[2] Py 11 has been found to be scarcely active, in complete medium, on poliovirus and other unrelated, viral agents, which require complete medium for growth. Data herein referred to show that Py 11 is able to inhibit the growth of both polio and *Herpes simplex* virus in complete, glutamine and cysteine containing medium, provided that mercaptoethanolamine is also present in that medium. Evidence is also given that combined treatment with Py 11 and mercaptoethanolamine has a protective effect against herpes keratitis in rabbits.

Material and methods. Colchicine (Simes); β-mercaptoethanolamine HCl (Sigma); 2-amino-4, 6-dichloropyrimidine (Py 11, Istituto Chemioterapico Italiano); 5-fluoro-

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Table I. Potentiating effect of non-inhibitory doses of mercaptoethanolamine on the antiviral action of Py 11 in HEp 2 cell cultures

Culture medium	Drugs in the medium (μg/ml)		Virus yield at 24 h (input: 10 infectious units/cell)		
			Polio	Herpes	Vaccinia
AFE		—	2.6×10^7	—	—
AFE	Py 11	30	3×10^4	—	—
MEM		—	3.4×10^7	1.2×10^7	1.5×10^7
MEM	Py 11	90	7.8×10^6	9.5×10^6	8.6×10^6
MEM	Mercapt.	30	2.1×10^7	8.9×10^6	1.2×10^7
MEM	Mercapt.	15	1.6×10^7	1.3×10^7	2.1×10^7
MEM	Py 11	30			
	+ Mercapt.	15	6.6×10^4	1.1×10^5	6.2×10^6