Use of Radioimmunoelectro-Osmophoresis for the Diagnosis of Human Trichinosis

Most sensitive methods used in the diagnosis of *Trichinella spiralis* infection include immuno-fluorescent antibody test (IFA), indirect haemagglutination (IHA), bentonite flocculation and complement fixation. The precipitation tests, once very popular, have been found less suitable owing to their lesser sensitivity, e.g. precipitation in the agar gel is 1,000 times less sensitive than indirect haemagglutination test[1]. However, the sensitivity of the precipitation technique has been considerably increased, particularly by the recently developed counter-electrophoresis[2,3] and radioimmunoelectro-osmophoresis (RIEOP)[4].

The purpose of the experiments described below was to ascertain the usefulness of the RIEOP test for the diagnosis of human infections with *T. spiralis*.

Material and method. The antigen used in the experiments was prepared from muscle larvae as described previously[5] and kept in the lyophylized condition at +4 °C. Prior to being labelled with ^{125}I, it was dissolved with distilled water to the concentration of 2 mg/ml. Labelling by iodination was accomplished according to the method of McConahey and Dixon[6], and the labelled antigen was diluted with Hanks balanced salt solution to the concentration of 0.28 mg of the antigen protein/ml of the diluent as determined by the method of Lowry et al.[7].

The immune sera were obtained from persons suspected of having recent infection with *T. spiralis*. The sera were examined for the presence of antibodies with the aid of IFA and IHA[5] tests, then stored at −20 °C. Just before being used in the experiments they were re-examined by both of the methods mentioned above and in addition by the double diffusion in the agar gel. Detailed results of the examination are set out in the Table.

RIEOP was carried out on the glass photographic plates (8.2 × 8.2 cm) covered with 8.5 ml of 1% agarose in barbital buffer of pH 8.2. Wells of 5 mm in diameter were cut in pairs 7 mm apart. The row of wells near the cathode was filled with the antigen while that near the anode was filled with undiluted sera. The plates were then connected with the electrophoresis tanks containing barbital buffer at pH 8.2 and submitted to an electric potential of 6 V/cm for 2 h. After completion of electrophoresis the plates were photographed and washed for 24 h in several changes of 0.85% NaCl solution and distilled water, and finally they were dried. Dried plates were placed with the gel surface in direct contact with medical X-ray film, 9 × 12 cm (Kodak Support Bleu, France) and kept in a light-tight box for 2, 4 and 7 days. The plates were finally stained with Amido black and photographed again.

Results and discussion. Counterelectrophoresis showed the presence of 3 lines of precipitation between the pair of wells filled with the antigen and control serum of hyperimmunized rabbit (Figure 1). None of the human sera tested produced visible lines of precipitation, with the

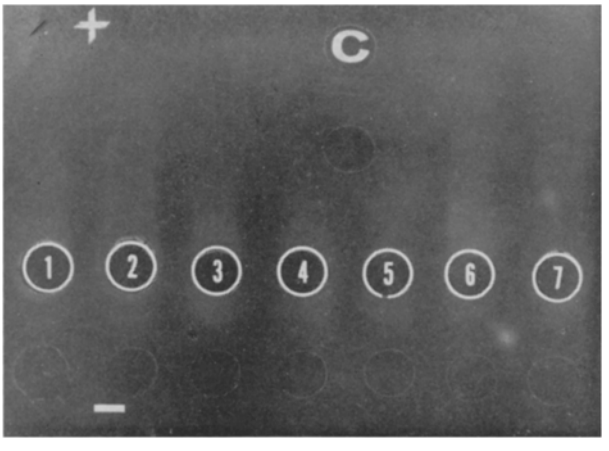

Fig. 1. Results of counterelectrophoretic examination of 7 human sera with the aid of *T. spiralis* antigen. Amido black staining. c, control rabbit serum.

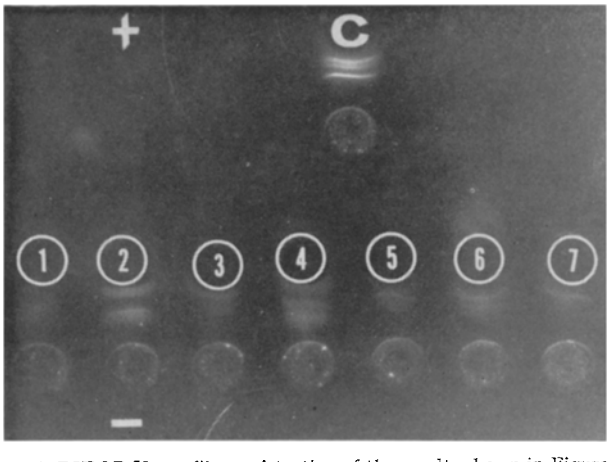

Fig. 2. RIEOP-X-ray film registration of the results shown in Figure 1. Exposure time 7 days.

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Results of examination of human sera for the presence of anti-*T. spiralis* antibodies with the aid of 5 serological tests

Sera No.	IFA		IHA		DDG	CE	RIEOP
	I	II	I	II			
1	—	—	160	10	—	—	—
2	400	100	2560	320	—	—	+
3	—	—	—	—	—	—	—
4		50		20	—	—	+
5	—	—	40	10	—	—	+
6	100	50	640	20	—	NS	+
7	50	—	160	10	—	—	+

DDG, double diffusion in the agar gel; CE, counterelectrophoresis; NS, non-specific precipitation; I, before storage; II, after storage; +, positive. Numbers denote titres given as reciprocals of the highest serum dilution producing positive reaction.

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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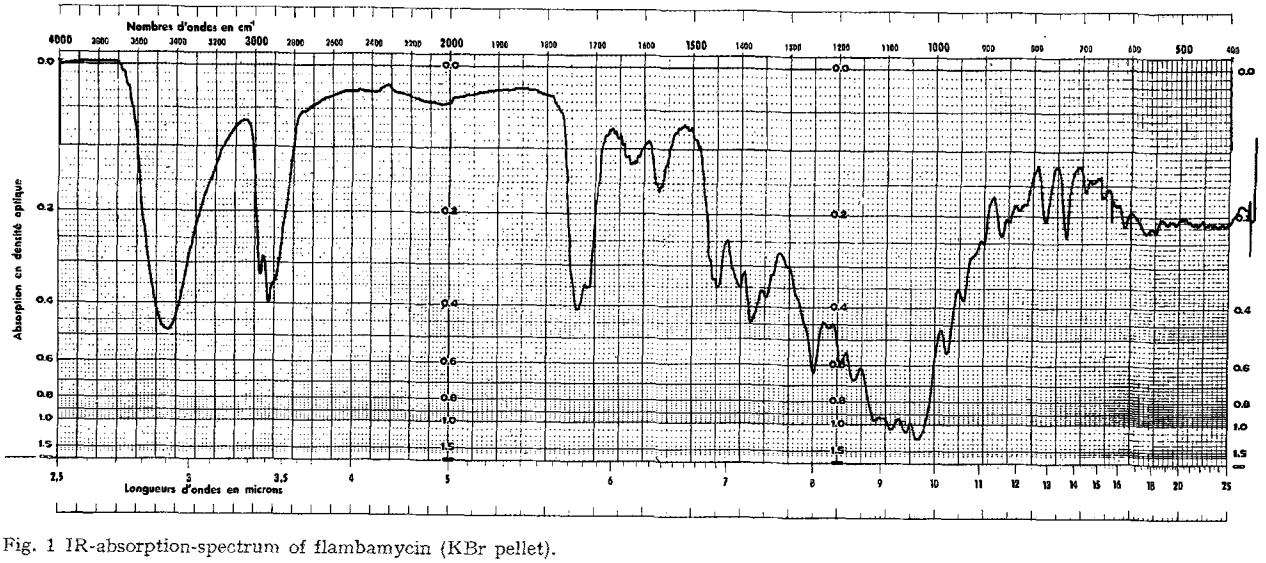

Fig. 1 IR-absorption-spectrum of flambamycin (KBr pellet).