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SHORT COMMUNICATIONS

APPROXIMATIVE MOLECULAR WEIGHT OF THE ACTIVE COMPONENT IN TOXOPLASMIN

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Abstract. The approximative molecular weight of toxoplasmin — a skin reactive extract from *Toxoplasma gondii* — was estimated by ultrafiltration through different Amicon membranes. The activity of the filtered and unfiltered product was compared with the aid of intradermal test on humans. The results indicate that the molecular weight of an active component in toxoplasmin is in the range from 10 000 to 50 000.

Toxoplasmin Sevac — the highly purified extract from *Toxoplasma gondii* (Frenkel 1948) has been employed for many years for the detection of sensitivity to this protozoan using an intradermal test (Jírovec and Jíra 1961, Jírovec 1971). This study was directed to the approximative determination of the molecular weight of a component responsible for the skin reactivity of toxoplasmin.

MATERIAL AND METHODS

Toxoplasmin Sevac. This diagnostic product represents heat stable toxoplasma antigens (Jíra and Jírovec 1966) prepared from purified extract of *Toxoplasma gondii*. The parasites were obtained from the peritoneal exudate of infected mice.
Ultrafiltration. For the ultrafiltration Diaflo membranes XM 100, XM 50 and UM 10 were used. (Manufacturer: Amicon, Holland).
Activity of toxoplasmin. The tested person was given strictly intradermally 0.1 ml of toxoplasmin in the outer side of the left arm. The control solution, i. e., the unfiltered product was administered in the same manner at the distance of 6 cm. The skin reaction of a delayed hypersensitivity type was read after 48 hours. The size of the erythema and induration was measured and evaluated according to the table of Jíra:

Erythema diameter	Induration	Evaluation
0—5 mm	0	negative
5—15 mm	slightly palpable to 5 mm	weakly positive
15—30 mm	palpable 5—10 mm	positive
larger than 30 mm	palpable larger than 10 mm	strongly positive

RESULTS

In the first experimental series toxoplasmin Sevac was filtered through an Amicon membrane XM 100 which retains molecules larger than 100 000, the filtered and the

unfiltered samples gave the same results in tests on patients. The membrane XM 100 did not reduce the activity of the toxoplasmin.

Table 1. Activity of toxoplasmin in intradermal test

Skin test reaction	Strongly positive	Positive	Weakly positive	Negative
Filtered XM 100	3/25	4/25	3/25	15/25
Unifiltered (Control)	3/25	4/25	3/25	15/25
Filtered XM 50	9/25	1/25	0/25	15/25
Control	9/25	1/25	0/25	15/25
Filtered UM 10	0/57	0/57	0/57	57/57
Control	16/57	4/57	0/57	37/57

In a further step we tried the XM 50 membrane removing molecules larger than 50 000. In this case the filtered sample also retained its full activity.

Finally the UM 50 membrane was tried. Filtration through this membrane completely removed the activity of toxoplasmin. (Table 1.) These results indicate that the molecular weight of active component or components of toxoplasmin is smaller than 50 000 and larger than 10 000, under the conditions of these experiments. As the retention of molecules by Diaflo membranes is also a function of molecular configuration and charge, the determination of molecular weight by this method is only approximative.

DISCUSSION

This result is of practical importance. The ultrafiltration offers a simple way for further purification and standardization of toxoplasmin, which is widely used in epidemiological studies and as a supplement to serological examination.

The *Toxoplasma* infection is a very suitable model for diseases in which delayed hypersensitivity is involved. The use of the better defined antigen will be advantageous also for studies in this field.

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ПРИБЛИЗИТЕЛЬНЫЙ МОЛЕКУЛЯРНЫЙ ВЕС АКТИВНЫХ КОМПОНЕНТОВ ТОКСОПЛАЗМИНА

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Резюме. Путем ультрафильтрации через разные мембраны Амикон определяли приблизительный молекулярный вес токсоплазмина — экстракта из *Toxoplasma gondii*, применяемого для кожной реакции. Активность фильтруемых и не фильтруемых продуктов сравнивали с помощью интрадермального теста на человеку. По полученным результатам молекулярный вес активной компоненты токсоплазмина от 10 000 до 50 000.

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V. M. Leshchenko: Laboratornaya diagnostika gribkovykh zabolevaniy. (Laboratory diagnostics of fungal diseases).

Publ. House Meditsina, Moscow 1977, 126 pp., 71 Figs. Price 50 kop.

The soft-cover booklet is the work of one of the prominent Soviet medical mycologists — head of the Mycological Laboratory, Central Research Institute of Dermatovenerology, the USSR Ministry of Health, Moscow. It is a practical handbook intended for physicians working in laboratories, mycologists and dermatologists. The number of copies (8000 issues) is a good evidence of a need for this work offering in a brief and illustrative manner instructions for laboratory diagnosis of mycoses. In the introductory section the author gives detailed accounts of morphology, biology, systematics of the pathogenous fungi and a classification of the diseases caused by these organisms. In a chapter devoted to methods of mycological examinations, the author recommends easy and special procedures which have been tested in his long-lasting practice. For the selection of cultivation media he rightly stresses their division into isolation, differentiation and conservation media. However, the metodological section does not cover instructions for the induction of sexual stages of dermatophytes, on the distinction of which a contemporary concept of the species diagnosis of this group of mycotic agents is based. In the chapter concerned with classification of mycotic diseases the division of pseudomycoses into surface (erythrasma, trichomycosis axillaris) and deep (actinomycosis, nocardiosis) is rather unusual. A special section of the book is devoted primarily to the causative agents of dermatomycoses. The author uses current mode of providing information — a name of the agent and its synonyms are followed by descriptions of the host range, mode of parasitism and a detailed characterization of the morphology of paratrophical and saprotrophical stage of the fungus. Data on causative agents of visceral mycoses are complemented with basic ecological data, schematic drawings of life cycles of causative agents and with information about possibilities of a special diagnosis (experiments on animal, immunological tests). The illustrations in the book include primarily line drawings in which the author not only tries to depict the most important morphological characters of mycotic parasites, but also makes the instructions for laboratory techniques more understandable (mode of inoculation of material for cultivation media, re-inoculation of isolates, preparation of slides from cultures etc.). Among the photomicrographs even those taken by means of a scanning electron microscope are not lacking. Small errors occurring primarily in the general section of the book — (incorrect placing of *Microsporum vanbreuseghemii* in anthropophilic dermatophytes, using obsolete generic names *Trichosporum*, *Sporotrichum* etc.) do not reduce the total value of the work, whose main merit is that it can promptly provide most important information, given in a very lucid and illustrative manner. The book will be helpful to beginners providing an easy orientation in the fundamentals of the laboratory diagnosis of mycoses.

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