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INTRADERMAL TEST IN THE DIAGNOSIS OF BOVINE CYSTICERCOSIS (CYSTICERCUS BOVIS)

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Dedicated to Academician B. Ryšavý on the occasion of his 60th birthday

Abstract. The skin test was performed in cattle infected with *C. bovis*. The examinations were carried out on naturally (23 head) and artificially infected animals (20 head) at different times after infection. Antigens prepared from *T. saginata* (B_1, $N_{0.1}$, C, C_{alk}) and antigens extracted from activated oncospheres of *T. saginata* (O_I and O_{II}) were inoculated intradermally. The most active were the antigens B_1, $N_{0.1}$, and C prepared from *T. saginata*. In the majority of infected animals the antigens produced the immediate type of reaction developing the maximum intensity during 2 h post injection. It is worth mentioning that Arthus-type responses have often been observed. Uninfected animals when tested with the same antigens developed mild reaction, not exceeding 4 mm skin thickness. The second testing of the same animals after one or five months resulted in a more intensive reaction slightly resembling that one observed in infected animals. There is no correlation between titre of passive haemagglutination test and the skin test reaction in naturally infected animals. Neither the number of cysticerci with which the animal is infected has an influence on the degree of the skin edema.

During the last 20 years the increasing number of cases of bovine cysticercosis in European countries as well as growing interest of developing countries in bovine production in Africa caused that some methods of ante-mortem diagnosis have been tried. Among others the intradermal test was employed. In a number of papers the intradermal test was described to diagnose experimental or spontaneous cysticercosis. The achieved results were contradictory; the test was determined as useless because of the lack of specificity and cross-reactivity with other parasites (Froyd 1963, Graber and Thome 1964), otherwise the skin test was described as a useful, reliable diagnostic tool (Skvorcov et al. 1941, Bugyaki 1961). The main feature of the above-mentioned papers which made the results uncomparable was the ununiformity of antigens. The authors used different antigens without the determination of protein contents. Several examinations did not include objective measurements of skin reaction, other papers did not show the dynamics of the test.

Our examinations were made on twenty bulls experimentally infected with *C. bovis*, and on twenty-three naturally infected animals. Antigenic fractions of *T. saginata* proglottids and antigen prepared from *T. saginata* oncospheres were used as allergens.

The aim of our studies was to examine these antigens in intradermal test, to establish relationship between titre of passive haemagglutination test and skin test in animals naturally infected with *C. bovis*, and to detect dependence of skin reaction intensity on the number of cysticerci in the infected animal.

MATERIAL AND METHODS

The examinations were made on Lowland cattle kept indoors. The intradermal test was performed on ten healthy animals selected at random. In four of them the examination was repeated after three months.

Twenty-one naturally infected bulls were selected from the flock on the basis of the positive reaction of passive haemagglutination test. This test was performed as described by Machnicka (1974) with *C. bovis* fluid as antigen. The post-mortem examinations of these animals showed very similar numbers of cysticerci as in the artificially, weakly infected bulls. Two calves were tested as controls, showing unexpected positive reactions of the intradermal test. The post-mortem examination revealed in both animals the presence of cysticerci: in the first calf two cysticerci in tongue, in the second one two cysticerci in heart. These animals were also infected naturally, so that a total of 23 naturally infected animals were examined.

Six bulls about 7—8 months old, weighing cca 200 kg each were used for experimental infection. All animals were infected with 160 000 *Taenia saginata* oncospheres each. Thorough post-mortem examination revealed a very weak infection when slicing predilection muscles as: masseters, heart, intercostal muscles, muscles of neck and oesophagus, and inspecting of viscera. About 30 cysticerci were found in each animal. The animals of this group were slaughtered successively: 2, 3, 6 and 8 weeks, 6 and 10 months after infection. Intradermal test was performed before each slaughter.

The second experimentally infected group was composed of fourteen 2—4-month old bulls weighing 75—190 kg. Each animal was infected with 280 000 *T. saginata* oncospheres. Eleven of these animals were examined by skin test and slaughtered four months after infection. One bull was checked by intradermal test nine months after infection. In three animals the intradermal examination was performed twice. The number of cysticerci in animals from this group was estimated at one thousand in each carcass.

The animals were infected with *T. saginata* oncospheres by stomach tube. Before infection the oncospheres were tested by Silverman's method (1954) demonstrating the per cent of viability expressed by the embryonic hook movements.

Antigenic fractions of *T. saginata:* C, C_{alk}, B_1 and $N_{0.1}$ prepared as described by Machnicka—Roguska (1965) were used as allergens. Lyophilized homogenate of *T. saginata* was delipidized successively with organic solvents: ether and chloroform (1 : 1), ethanol and 1% HCl (1 : 1). The lipids were discarded and the sediment extracted with triple volume of 1% CH_3COOH, after extraction fraction C was precipitated with three volumes of 96% ethanol from the supernatant. The remaining sediment was extracted with 0.1 N NaOH; fraction B_1 was separated from the supernatant by precipitation with 15% CH_3COOH, fraction B_1 by action with 40 % CCl_3COOH, and fraction C_{alk} was precipitated from the remaining supernatant by means of excess 96% ethanol. All steps of antigen preparations were carried out at 4 °C. Each fraction was dissolved three times and precipitated after each run. When being solubilized it was centrifuged and only the supernatant was precipitated. In lyophilized fractions the protein contents were determined by the method of Lowry et al. (1951).

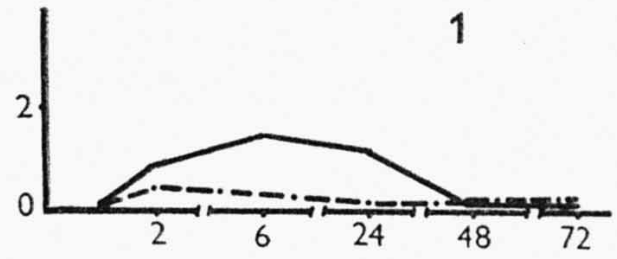
Fractions C and C_{alk} were soluble in water. Before use fractions B_1 and $N_{0.1}$ were solved in a small amount of 0.1 NaOH then adjusted to pH 7.2 with 0.1 N HCl and completed to a stable amount of protein per one ml with saline as follows: fraction C — 0.07 mg, C_{alk} — 0.05 mg, $N_{0.1}$ — 1.28 mg, B_1 — 1.4 mg. The preparations of antigens for the skin test were made in sterile conditions with sterile fluids. The antigens were sealed in glass ampoulles.

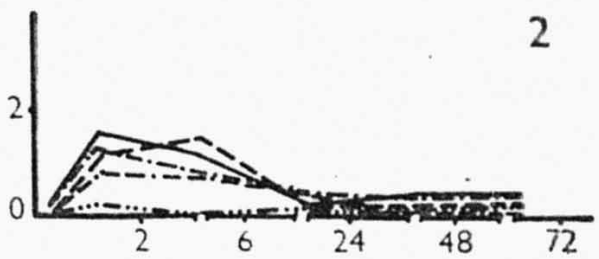
We also employed two allergens prepared from activated oncospheres prepared as follows: *T. saginata* oncospheres taken from mature proglottids were digested in 1% peptic solution with 1% HCl at 37 °C for 3 h, solution being changed 5—7 times. After that the oncospheres were washed 3 times in saline and hatched according to Silverman (1954). Then they were washed 4 times in saline centrifuging for 5 min at 7000 r.p.m. after each change. The oncospheres prepared in this way were frozen at — 30 °C in deionized water (1 : 1 with sediment), thawed at room temperature and centrifuged at 4 °C for a minute at 12 000 r.p.m. The supernatant was discarded to store and the same procedure was repeated three times. The protein determination by the Lowry method 1951 showed 1.06 mg/ml in the first supernatant, 2.5 mg/ml in the second and 1.18 in the third one. The first and the second supernatant marked O_I and O_{II} were used as allergens.

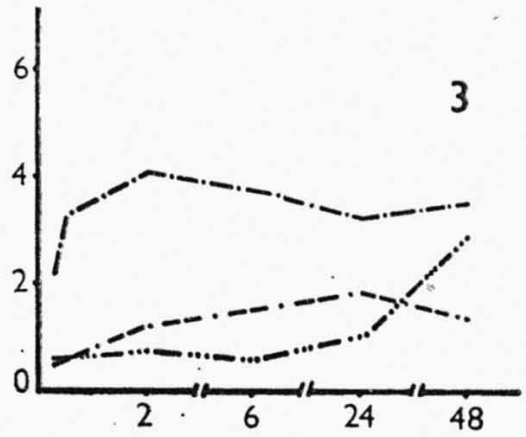
The allergens were given intradermally in the neck in this order: B_1, C_{alk}, C, $N_{0.1}$, O_{II}, O_I. Skin thickness was measured with calipers before infection of the antigen, and then 0.5, 1, 2, 6, 24, 48, 72 and in some animals 94 hours post injection (p.i.). Each allergen was administered at a dose of 0.1 ml.

RESULTS

Skin test reactions given by the uninfected animals are shown in Figs. 1, 8. Figs. 5, 8 present the results in four animals tested twice. In two haifers (Figs. 5, 6) the examination was performed after a month's brake. The remaining two animals were tested for the second time after a five months' interval (Figs. 7, 8).







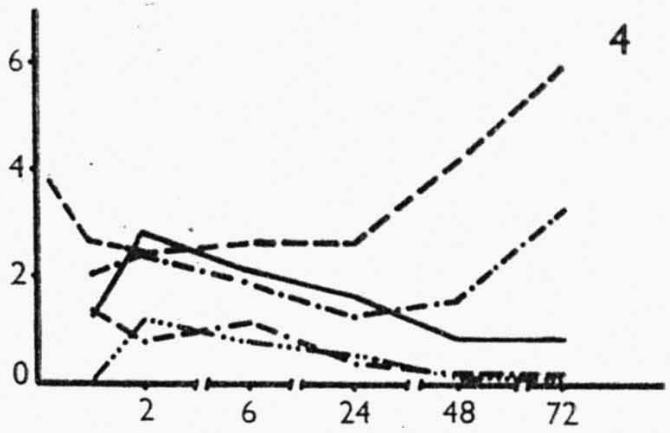


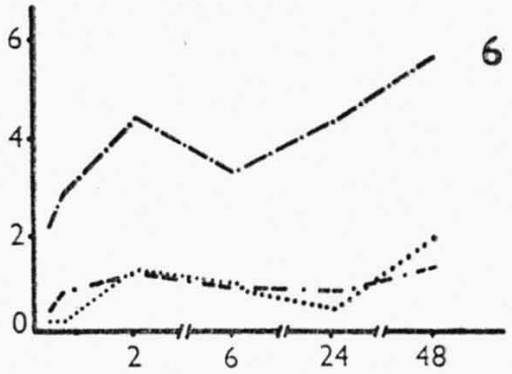
Fig-. 1—4. Skin reaction in uninfected animals.

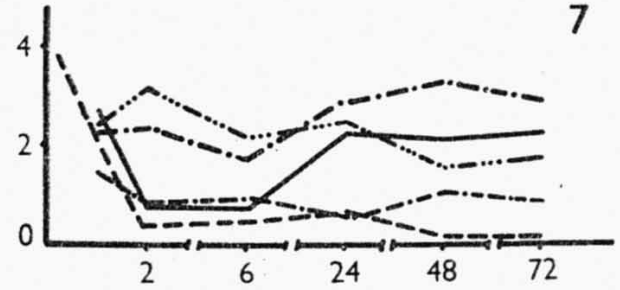
Explanation Figs 1—20:

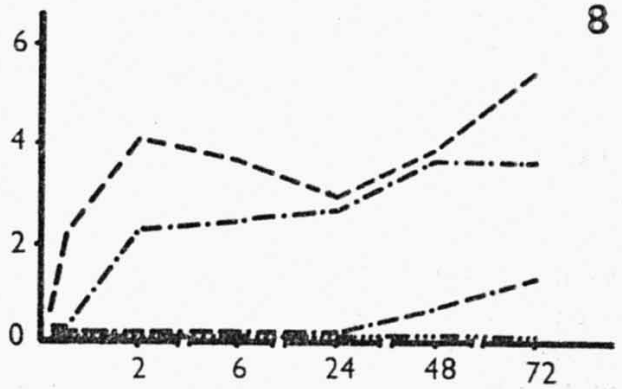
———— B_I ---------- C ·-·-·-·-· $N_{0.1}$

·····-····· C_{alk} ---·---·--- O_I ····------···· O_{II}

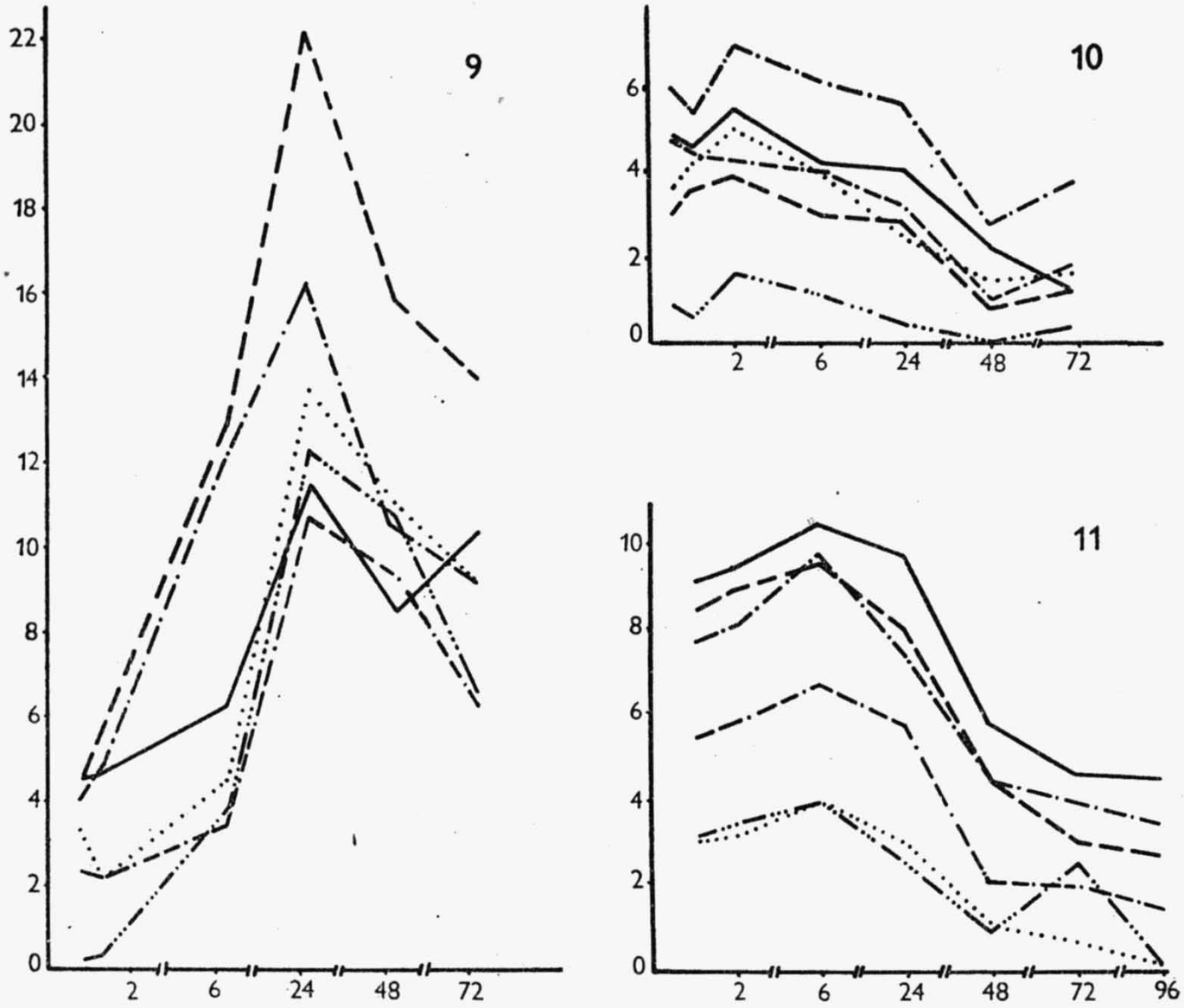
ordinate — skin thickness (mm), abscissa — time (hours). The graphs are in most cases selected as representative for each group of animals.







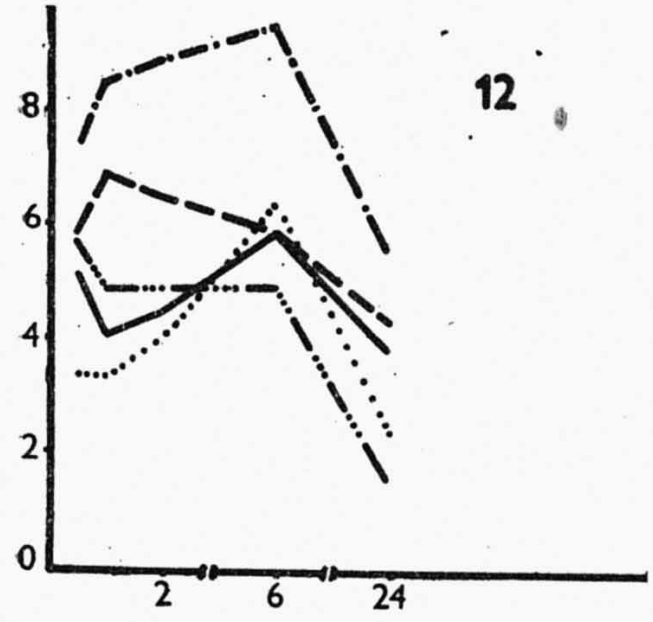
Figs. 5, 7. Skin reaction in uninfection animals. **Fig. 6.** Skin reaction in uninfected animals performed for the second time after a month's brake. **Fig. 8.** Skin reaction in uninfected animals performed for the second time after five months' brake.

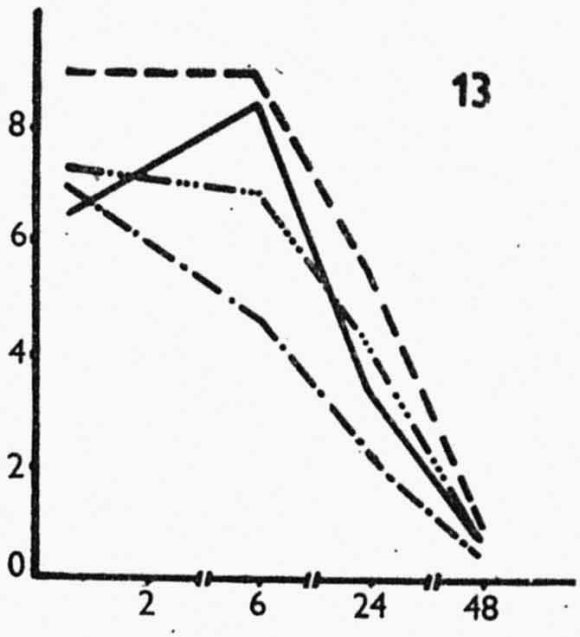


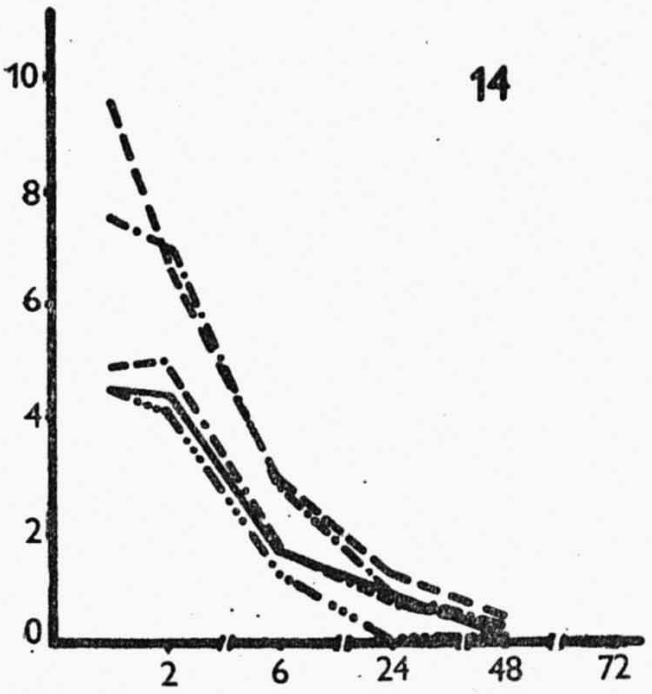
Inoculation of antigen into the skin of uninfected animals showed 2—4 mm thick edema in places which appeared soon post infection, in some cases it was negligible 24 or 48 h p.i. (Figs. 1, 2). In other animals the reaction was more marked, 48 or 72 h p.i., than earlier (Fig. 4). In animals examined for the second time the reactions were more intensive: 1, 2 or 6 h p.i. the thickness of edema was above 4 mm, and at least with some antigens it did not disappear 24 or 48 h p.i. (Figs. 6, 8). The most distinct reactions gave antigens $N_{0.1}$, B_1 and C.

In the young bulls weakly infected with *C. bovis* the skin test was performed progressively. The first animal was examined two weaks after infection (Fig. 9) demonstrating the maximum reaction 24 h p.i. In the next two bulls examined three weeks after infection (Fig. 10) and 6 weeks after infection (Fig. 11) skin edema being well expressed 2 h p.i. did not fade clearly until 24 h p.i. In the second of these bulls as well as in two additional ones tested 8 weeks after infection (Fig. 12) and 6 months after infection (Fig. 13) the strongest reaction was marked 6 h p.i. The last animal from this group tested 10 months after infection (Fig. 14) showed the biggest skin edema 1 h p.i. lasting until 2 h p.i. and quickly disappearing.

Fig. 18 presents the skin test in bulls (14 head) which showed considerable amount of *C. bovis* during meat inspection. All animals were tested 4 months after infection and all of them except one (Fig. 15) were treated with Praziquantel (Bayer) three months after infection. In three animals the skin test was performed twice: for the second







Figs. 9—11. Skin reactions in bulls weakly infected with *C. bovis*. **Figs. 12—14.** Skin reactions in bulls weakly infected with *C. bovis*. **Fig. 9.** 2 weeks after infection. **Fig. 10.** 3 weeks after infection. **Fig. 11.** 6 weeks after infection. **Fig. 12.** 8 weeks after infection. **Fig. 13.** 6 months after infection. **Fig. 14.** 10 months after infection.

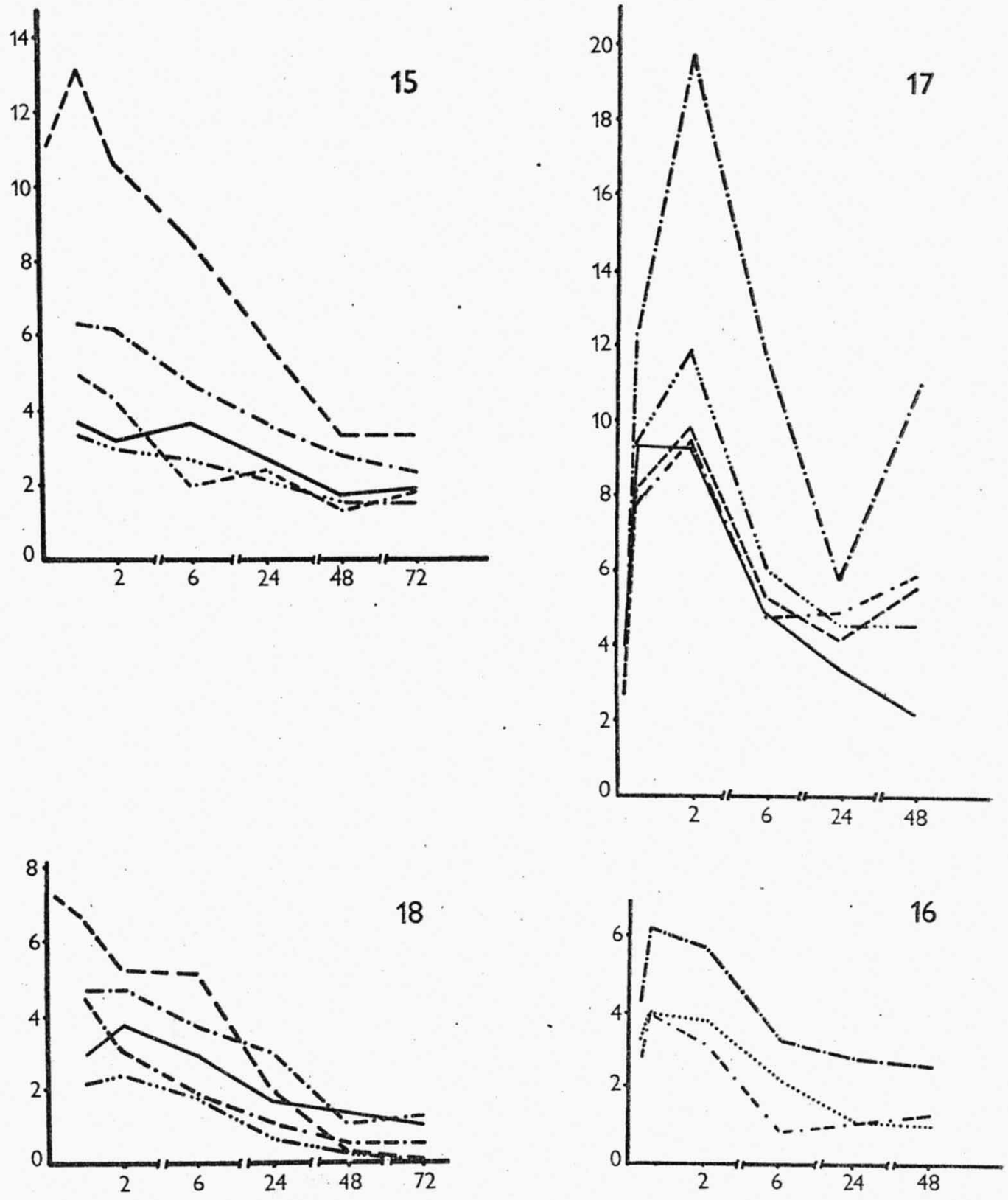
time, five months after infection in two animals (Fig. 16) and 9 months after infection in one animal (Fig. 17).

In most animals the skin edema due to inoculated antigens are most conspicuous between 15 min and 6 h p.i., however, peak values are noted mainly 1 h p.i. (Fig. 26). The most distinct reactions are manifested by fractions B_1, $N_{0\cdot1}$ and C, but in particular cases other antigens give higher reactions, e.g., antigen O_{II} (Fig. 17). The skin test, performed in the same animal for the second time (Figs. 16, 17) was characterized by a more conspicuous edema than during the first examination.

In naturally infected animals tested as controls by five antigens each the maximum skin edema was observed from 15 min to 2 h p.i. (Figs. 19, 20).

The sera taken from animals bred in a farm where the focus of cysticercosis had been detected were examined by passive haemagglutination test, and as the results 21 animals responding positively were selected (Table 1). In these 21 animals infected with *C. bovis* as was detected in meat inspection, the skin test was performed with one antigen only —

O_I or O_{II} (Figs. 21—25). In the majority of animals the test gave a distinct edema 2 h post injection of antigen, its intensity declined 6 h p.i. and grew again 24 h p.i. Usually the greatest skin edema was noted 2 h p.i. except for Nos. 4 (Fig. 21), 14, 16 (Fig.



Figs. 15, 18. Skin reactions in bulls infected with *C. bovis*, performed 4 months after infection. **Fig. 16.** Skin reactions in heifers infected with *C. bovis*, performed for the second time five months after infection. **Fig. 17.** Skin reaction in a bull infected with *C. bovis*, performed for the second time 9 months after infection.

Table 1. Results of passive haemagglutination test with the sera of naturally infected cattle selected for skin test

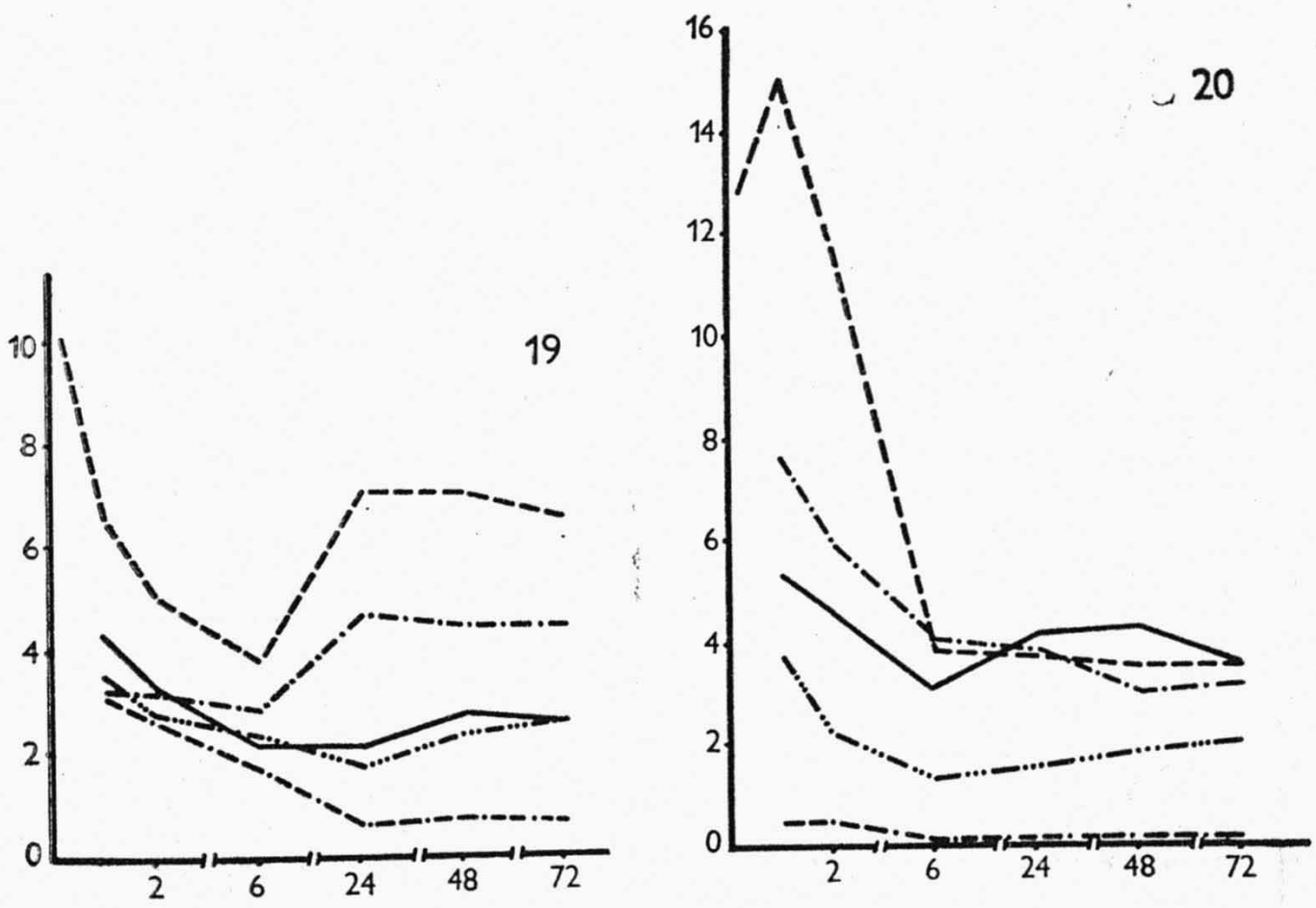
No. of animal	titre of HA test	No. of animal	titre of HA test	No. of animal	titre of HA test
1	160	8	640	15	160
2	160	9	80	16*	640
3	1280	10	40	17	160
4*	640	11	1280	18	80
5	20	12	160	19	20
6	80	13	160	20	20
7	320	14*	80	21*	160

* animals in which Arthus' skin reactivity was registered

24) and 21 (Fig. 25); the graphs of skin thickness demonstrated the most intensive reactions 6 h p.i. In these animals exudation at the site of antigen inoculation was manifested 24 h p. i. and later (Fig. 27).

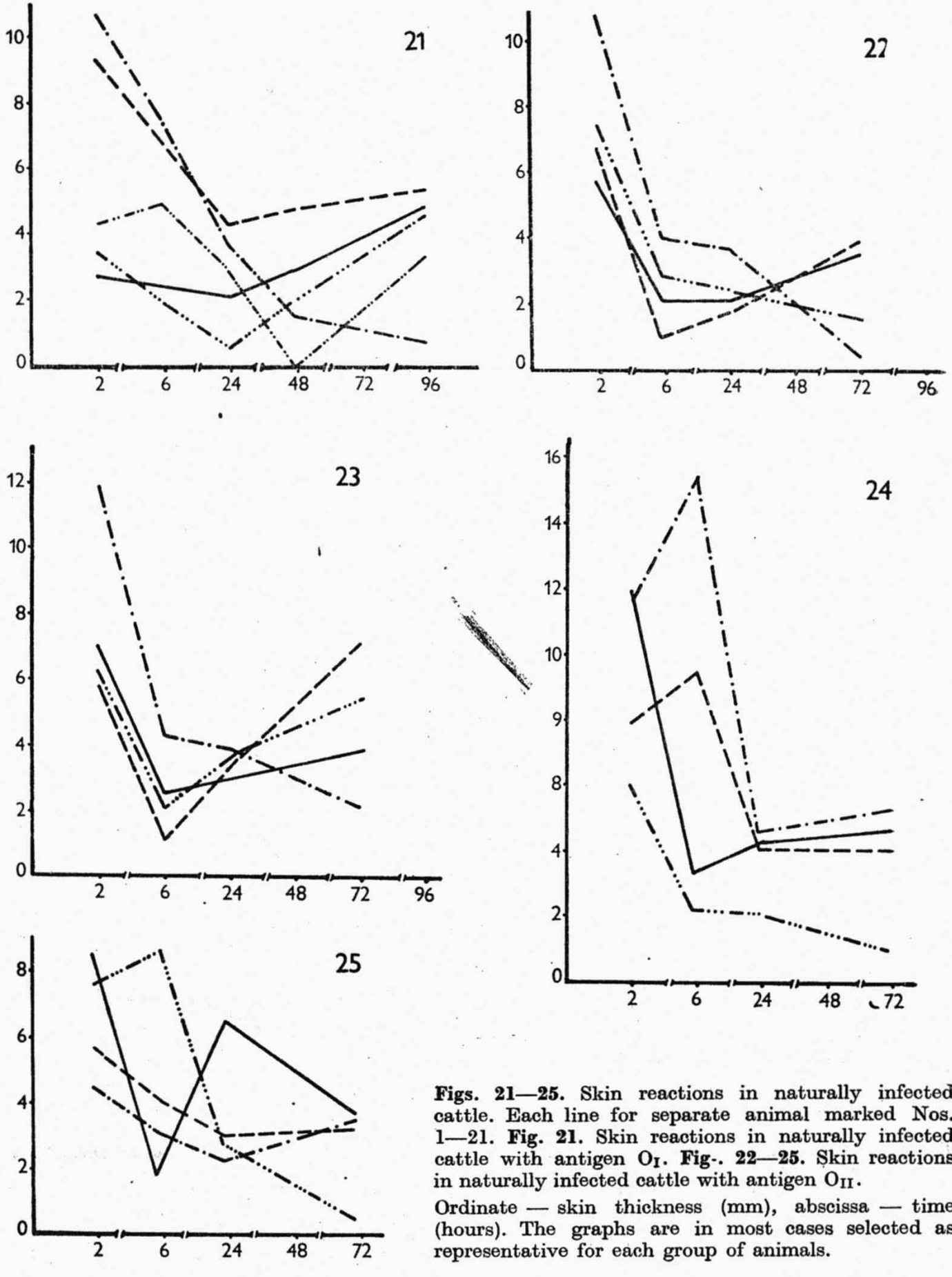
DISCUSSION

Of antigens used in the skin test to diagnose *C. bovis* infection in cattle the most active, i.e. giving the biggest edema, were those prepared from *T. saginata:* B_1, $N_{0.1}$, C.



Figs. 19, 20. Skin reactions in naturally infected heifers, test performed in each animal with 5 antigens.

It was not due to the sequence of inoculation of particular antigens which could desensitize the animal after two or three injections. It may be shown on the bulls infected heavily with *C. bovis* (Figs. 15—18) and inoculated successively with antigens B_1,



Figs. 21—25. Skin reactions in naturally infected cattle. Each line for separate animal marked Nos. 1—21. **Fig. 21.** Skin reactions in naturally infected cattle with antigen O_I. **Fig. 22—25.** Skin reactions in naturally infected cattle with antigen O_{II}.

Ordinate — skin thickness (mm), abscissa — time (hours). The graphs are in most cases selected as representative for each group of animals.

C_{alk}, C, $N_{0.1}$, O_{II} that antigens containing large quantities of protein appeared most active. Fraction O_{II} is an exception containing the highest per cent of protein and showing the smallest activity when inoculated as the last one. The same fraction when used as a single antigen in naturally infected animals gave positive reactions in the form of a more developed edema then when injected as the fifth antigen. The same may be observed from Figs. 19 and 20 illustrating reactions in naturally infected heifers examined with five antigens each.

It seems that all antigens used in these studies are active in intradermal test probably due to protein component which is, at least partly, common for them (Machnicka 1974).

As demonstrated by individual figures for each tested animal, generally, the reactions in healthy animals can be differentiated from those in infected animals. The most striking features are rather small edema of short duration or reactions developing after 6 h p.i.

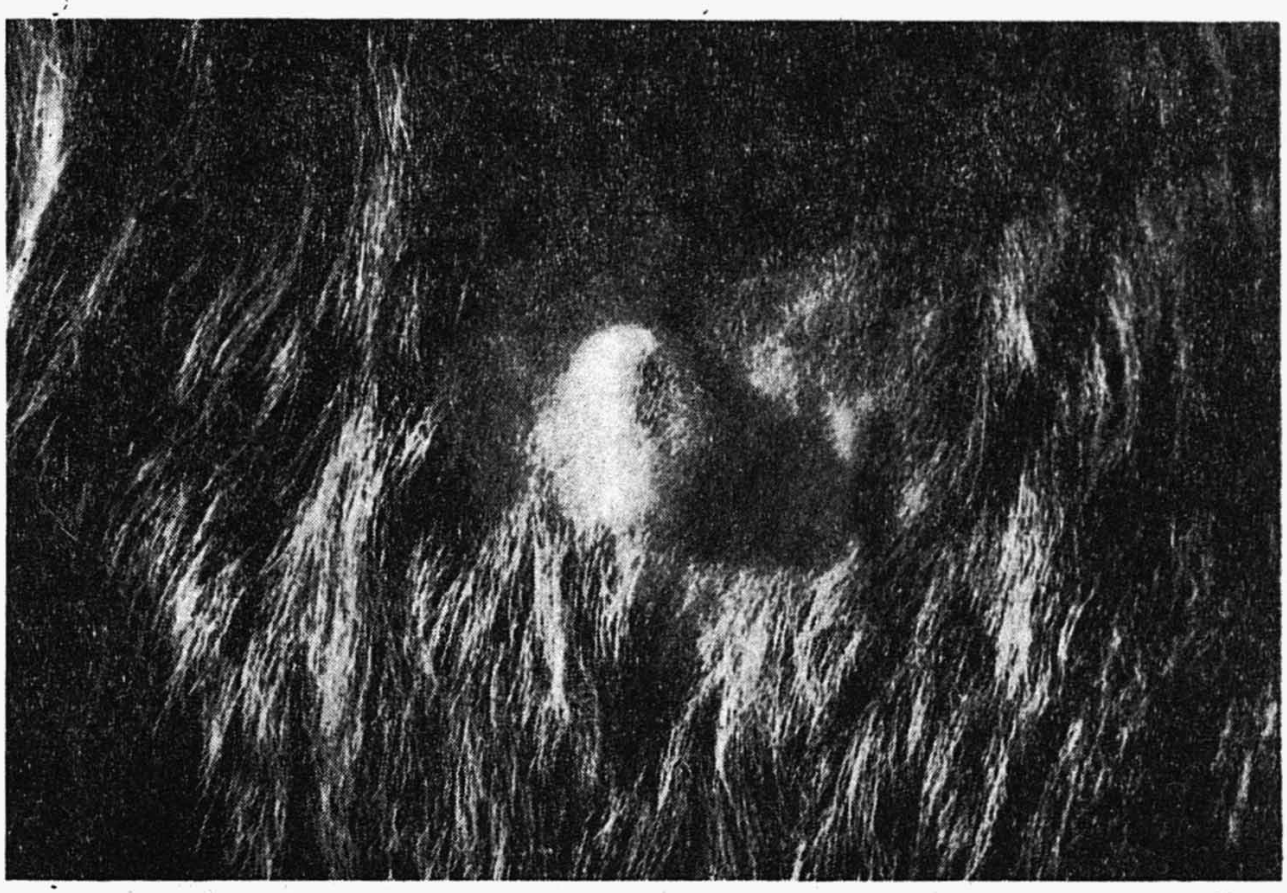


Fig. 26. Intradermal test in bovine cysticercosis. Intensive skin reaction 1 hr post antigen injection.

The observations on the character of skin test at a given period after infection demonstrated delayed skin hypersensitivity two weeks after infection with the peak reaction 24 h p.i. (Fig. 9). This reaction may be considered uspecific according to observations of Bugyaki (1961), or specific characterizing early infection, however, there is a need for verification in larger number of animals. From three weeks till 6 months after infection the immediate type of skin reaction occurred, but Figs. 11, 12 and 13 illustrating animals tested 6 weeks, 8 weeks and 6 months after infection respectively show the Arthus' reactions. Immediate type of skin reaction was demonstrable in the last animal of this experiment (Fig. 14) tested 10 months after infection. The type of skin reaction was possibly connected with the intensity of infection with *C. bovis* or with the action of Praziquantel. In animals with heavy infections treated with Praziquantel 3 months

after infection and tested four months after infection we failed to demonstrate Arthus' reactions. The maximum edema developed 2 h post injection or earlier.

In naturally infected animals antigens O_I or O_{II} produced immediate-type reactions, only in four of 23 animals examined Arthus' reactions have been observed.

Comparing the results of skin tests with passive haemagglutination it may be seen that these two types of reaction do not give parallel results. High haemagglutination titre does not condition the intensity of skin edema and is not connected with Arthus' reaction. Observations on the intensity of intradermal reaction in animals with mild and heavy infection did not allow us to find any conclusive differences as did Leikina et al. (1962). The authors found that the intensity of the skin test in experimental calves was infective dose dependent; however, the test was performed with antigens prepared from *C. bovis*. In our examinations positive reaction was mostly manifested by 4—10 mm skin thickness.

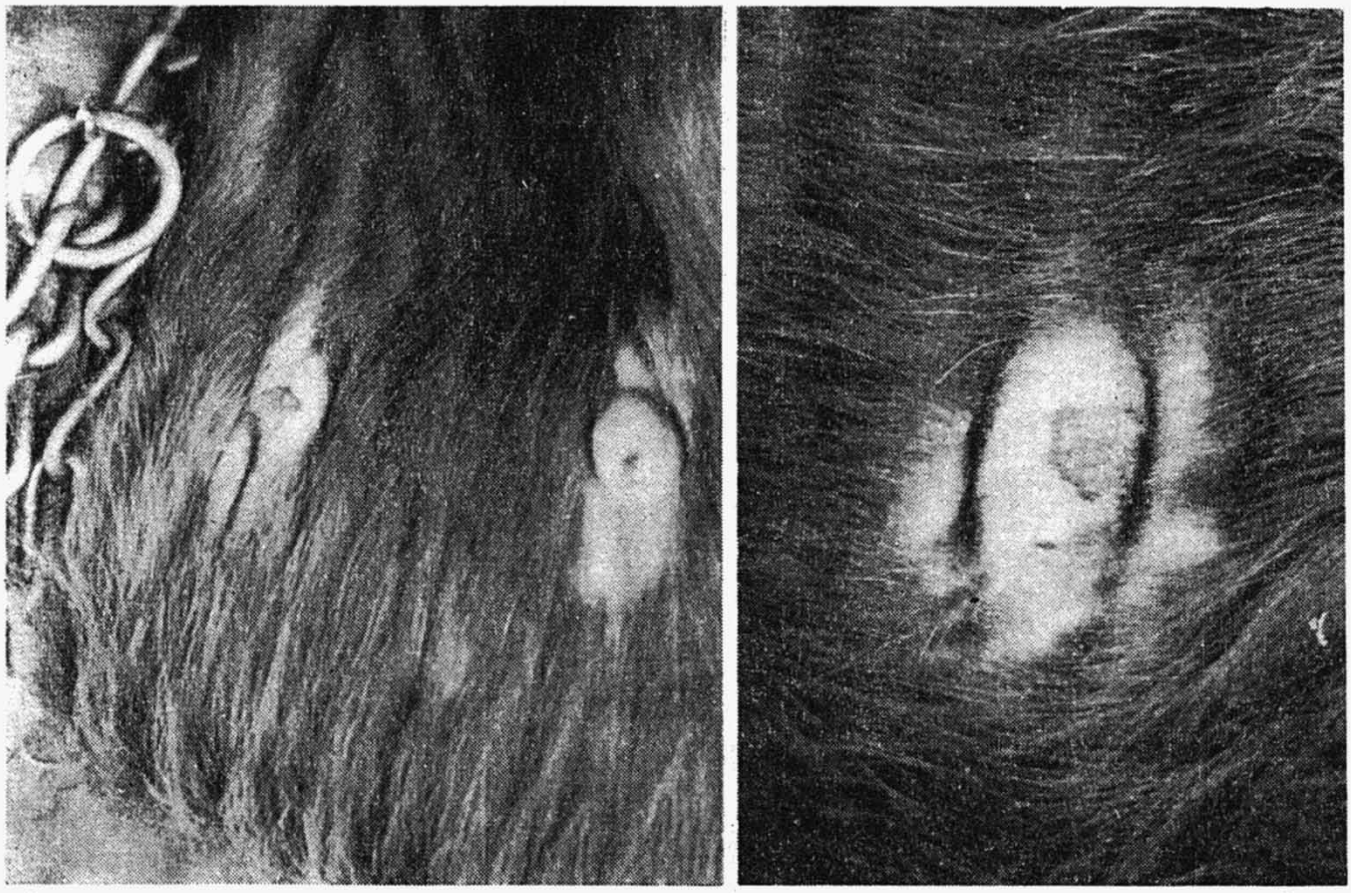


Fig. 27. Intradermal test in bovine cysticercosis. Intensive skin reaction 24 hrs post antigen injection. On the right — detail of skin reaction with exudation.

The majority of hitherto published literature on skin tests in the diagnosis of bovine cysticercosis described the results achieved in naturally infected animals employing *C. bovis* antigens. Dewhirst et al. (1960) and Bugyaki (1961) stressed that positive reactions of immediate type were observed even in animals harbouring calcified cysticerci. Bugyaki (1961), Graber and Thome (1964) and Froyd (1963), the latter working with *T. saginata* antigen as well, noted positive unspecific reactions in infections of cattle with various digeneans. In our studies we have no opportunity to verify the reliability of the skin test in cattle heavy infected with other parasites than *C. bovis*.

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ВНУТРИКОЖНАЯ РЕАКЦИЯ ПРИ ДИАГНОЗЕ ЦИСТИЦЕРКОЗА КРУПНОГО РОГАТОГО СКОТА (*CYSTICERCUS BOVIS*)

Б. Махницка, Й. Штерба и В. Шандл

Резюме. Изучена внутрикожная реакция у крупного рогатого скота, зараженного *C. bovis*. Обследовано 23 естественно зараженных и 20 искусственно зараженных животных в разных промежутках времени после заражения. Антигены, приготовленные из *T. saginata* (B^1, $N_{0,1}$, C, C_{alk}) и антигены из активированных онкосфер *T. saginata* (O_I и O_{II}) применяли для внутрикожной прививки. Активнее всех оказались антигены B_1, $N_{0,1}$ и C, приготовленные из *T. saginata*. В большинстве случаев антигены вызывали у животных реакцию немедленного типа, с максимальной интенсивностью 2 часа после заражения. Очень часто встречалась реакция Артуса. У незараженных животных эти антигены вызывали слабую реакцию, не выше чем 4 мм тольщины кожи. После одного или пяти месяцев те-же животные давали более интенсивную реакцию, немного походящую на реакцию зараженных животных. Не наблюдались соотношения между титрами пассивной реакции гемагглютинации и внутрикожной реакции у естественно зараженных животных. Количество цистицерков, применяемых для заражения животных, не оказывало влияния на отеки кожи.

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