FOLIA PARASITOLOGICA (PRAHA) 25: 109–113, 1978.

LATEX REACTION WITH TOXOPLASMA ANTIGEN

V. BOZDĚCH

Institute of Medical Microbiology und Immunology, Faculty of General Medicine, Charles University, Prague

Abstract. The technique of latex reaction with toxoplasma antigen is briefly described. The results of latex reaction are compared with those obtained by complement-fixation test, indirect fluorescence reaction and microprecipitation in gel.

Sixteen years ago the latex particles were first used as carriers of soluble toxoplasma antigen (Bozděch and Jíra 1961). The original technique was then gradually modified by other authors. The number of individual components of the test proper was lowered, the time-consuming centrifugation was replaced by shaking before incubation in the refrigerator overnight and another shaking after the incubation (Lunde and Jacobs 1967). Some authors assumed that the latex reaction with toxoplasma antigen (further LR) too often gave negative results (Kwantes et al. 1972) or 23% of erroneously negative results (Neto et al. 1975).

We have studied in detail the LR technique and we assume that we have succeeded in improving it by some technical modifications which will be described elsewhere. Practical results of these modifications appeared while comparing the LR positivity with that of other seroreactions to toxoplasmosis, which is the subject of the present paper.

MATERIAL AND METHODS

Latex and glycine buffer (GLR) were supplied by Imuna, Šarišské Michalany, toxoplasma antigen for the complement-fixation and microprecipitation reaction was obtained from the Institute of Sera and Vaccines, Prague. The microprecipitation reaction (MPA) was carried out after Hübner and Uhlíková (1973), complement-fixation reaction (KFR) after the technique described in detail in other papers (Bozděch 1965, Pokorný et al. 1972), indirect fluorescence reaction (IFAT) after Kramář et al. (1964).

0.1 ml of latex particles were mixed with 3.9 ml of GLR, and 0.8 ml of toxoplasma antigen diluted after the titre for KFR given by the producer were mixed with 3.2 ml of GLR. Both reaction mixtures were mixed in a tube and sensibilized for two hours in water bath at 37 °C. The sensibilized latex was further diluted in the ratio of 1 : 2.5 GLR and added by 1.0 ml to graduated double dilution of inactivated serum in the titres 10, 20, 40, 80, 160, 320 and 640. After incubation in the refrigerator overnight the reaction mixture was centrifuged for 10 min at 4500 g.

The agglutination was read in a dark room with an oblique light and dark background. The positive reaction was manifested by the formation of fine flakes floating after shaking in an uncleared supernatant (+) or by formation of rough flakes either in an uncleared or completely cleared supernatant (+++).

RESULTS

If the sera are divided into 7 groups according to the results of LR and into 9 groups according to the results of KFR (Table 1), in negative LR the positive KFR in the titre of 512 occur only exceptionally and do not occur at all in higher titres.

A more pronounced relationship between these two reactions appears while comparing the percentage of occurrence if the results are divided into negative, weakly positive (KFR 8—32, LR 10—20), positive (KFR 64—512, LR 40) and strongly positive (KFR above 512, LR above 40).

The occurrence of negative LR decreases with increasing positivity of KFR (Table 2), whereas the occurrence of strongly positive LR increases with the posivity of KFR (66.3 % — 15.2 % — 8.1 % and 2.7 % — 10.4 % — 40.4 % — 71.4 %). The occurrence of weakly positive KFR is highest in weakly positive KFR (51.7 %) and the occurrence of positive LR is highest in weakly positive and positive titres of KFR (22.7 % and 25.0 %). Negative LR occurs rarely with positive IFAT. No negative LR was found in strongly positive IFAT (Table 3).

Table 1. Relationships between positivity of latex reaction with toxoplasma antigen and positivity of complement fixation reaction (in total numbers of sera examined)

			Latex reaction with toxoplasmine						
			Negative	Positive					
				10	20	40	80	160	320
Complement fixation reaction	positive	Negative	316	74	53	21	9	4	
		1 : 8	41	63	43	17	10	1	
		1 : 16	16	23	46	35	13	1	
		1 : 32	10	24	30	47	17	4	
		1 : 64	5	11	8	19	19	4	
		1 : 128	2	2	7	7	9	7	1
		1 : 256	3	3	1	3	6	3	1
		1 : 512	1	3	1	5	2	3	
		1 : 1024 and higher	0	2	0	2	5	5	

Table 2. Relationships between positivity of latex reaction with toxoplasma antigen and complement fixation reaction (in %)

			Latex reaction with toxoplasma antigen positive in titre				Total examined
			negative	10—20	40	80 and higher	
Complement fixation reaction	positive in titre	negative	66.3	26.6	4.4	2.7	477 (= 100 %)
		8—32	15.2	51.7	22.7	10.4	441 (= 100 %)
		64—512	8.1	26.5	25.0	40.4	136 (= 100 %)
		1024 and higher	0.0	14.3	14.3	71.4	14 (= 100 %)

Table 3. Relationship between positivity of latex reaction with toxoplasma antigen and fluorescenec reaction (IFAT) (in whole numbers)

			Latex reaction with toxoplasma antigen Positive in titre						
			negative	10	20	40	80	160	320
IFAT	positive in titre	negative	232	58	47	21	4		
		8	51	47	37	10	5		
		16	24	31	31	29	8		
		32	12	31	36	25	14	4	
		64	3	9	17	28	13	5	
		128	1	5	9	13	17	3	
		256	2	2	3	5	6	4	1
		512		2	6	4	3		
		1024		1		2	4	3	1

Table 4. Relationship between positivity of latex reaction with toxoplasma antigen and positivity of fluorescence reaction (IFAT) (in %)

			Latex reaction with toxoplasma antigen Positive in titre				Total examined
			negative	10—20	40	80 and higher	
IFAT	positive in titre	negative	64.1	29.0	5.8	1.1	362 (= 100%)
		8—16	27.5	53.5	14.3	4.7	273 (= 100%)
		32—64	7.6	47.2	36.9	18.3	197 (= 100%)
		128 and higher	3.1	22.7	26.8	47.4	97 (= 100%)

Table 5. Relationship of positivity of microprecipitation reaction according to Hübner and Uhlíková (MPA) and positivity of latex reaction with toxoplasmin (LR)

			MPA positive %	Total examined
LR	positive in titre	Negative	0	183
		10—20	3.0	174
		40—80	12.9	101
		160—320	41.6	12

In negative LR (Table 4) the occurrence decreased with the increasing positivity of IFAT (64.1 % — 27.7 % — 7.6 % — 3.1 %). The occurrence of strongly positive LR increased with increasing positivity of IFAT (1.1 % — 4.8 % — 18.3 % and 47.4 %). Weakly positive LR occurred most frequently in sera with weakly positive IFAT (titre 8—16, 53, 5 %). The positive LR occurred most frequently in sera with positive (titres 32—64) IFAT (36.9 %).

No positive MPA occurred in sera with negative LR. The occurrence of MPA positivity was in direct correlation to the positivity of LR (0 % — 3.0 % — 12.9 % — 41.6 %) (Table 5).

DISCUSSION

If the results of LR and KFR positivity in our original paper (Bozděch and Jíra 1961) are compared with those obtained in the present communication, the correlation between both tests is markedly improved. It cannot be judged to what extent this success is due to the quality of latex or antigen (Pokorný et al. 1971) prepared by Tween aether method.

We have not yet succeeded in omitting centrifugation in the LR test (Lunde and Jacobs 1967) without decreasing the reproducibility of results. Further studies will be devoted to the LR technique in terms of miniaturization and automation.

We have found that the sensibilized particles of latex keep their ability to agglutinate with positive sera for many months (more than 11). By a central production and distribution of sensibilized latex to the laboratories the main obstacles in the standardization would be overcome and this easy serological reaction would be introduced into the laboratory diagnostics of toxoplasmosis.

Acknowledgement. My thanks are due to Mr. I. Květoň for his skilful technical assistance.

РЕАКЦИЯ ЛАТЕКСА С ТОКСОПЛАЗМЕННЫМ АНТИГЕНОМ

В. Боздех

Резюме. Автор кратко описывает технику реакции латекса с токсоплазменным антигеном. Результаты реакции латекса сравнены с результатами реакций связывания комплемента, непрямой флуоресценции и микропреципитации в геле.

REFERENCES

BOZDĚCH V., Bemerkungen zur Methodik der Komplementbindungsreaktion. Z. Immun. Allergie Forsch. 128: 483—498, 1965.

—, JÍRA J., Latex-Agglutinationstest mit dem Toxoplasmaantigen. Dtsch. Gesundheitswes. 15: 2398—2400, 1961.

HÜBNER J., UHLÍKOVÁ M., Use of the microprecipitation method in agar gel (MPA) in the diagnosis of toxoplasmosis. J. Hyg. Epidem. Microbiol. Immunol. 17: 70—84, 1973.

KRAMÁŘ J., ČERNÁ Ž., CHALUPSKÝ J., Immunofluoreszenzreaktionen in der serologischen Diagnostik der Toxoplasmose. Zbl. Bakt. I. Abt. Orig. 193: 523—534, 1964.

KWANTES W., PAYNE R. A., LUDLAM G. B., BRIDGES J. B., FLECK D. G., An assessment of a latex agglutination slide test for toxoplasma antibody. J. clin. Pathol. 25: 359—360, 1972.

LUNDE M. N., JACOBS L., Evaluation of a latex agglutination test for toxoplasmosis. J. Parasitol. 53: 933—936, 1967.

NETO V. A., CAMARGO M. E., SILVA da L. Y., ROCCA A., Análise da sensibilidade do "Agglutinotest" para o diagnostico da Toxoplasmose. Rev. Inst. Med. Trop. Sao Paulo 17: 277—282, 1975.

POKORNÝ J., FRÜHBAUER Z., ČUŘÍK B., ZÁSTĚRA M., Complement fixation antigen

of *Toxoplasma gondii* prepared by tween-ether preparation. Čs. epidem. 20: 73—74, 1971 (In Czech).
—, ZÁSTĚRA M., ČUŘÍK B., Proposal of standardization of microtechnique of complement binding for serodiagnosis of toxoplasmosis. Čs. epidem. 21: 225—234, 1972. (In Czech).

Received 13 June 1977.

V. B., Ústav lékařské mikrobiologie a imunologie, Studničkova č. 7, 120 00 Praha 2, ČSSR

FOLIA PARASITOLOGICA (PRAHA) 25: 113—114, 1978.

RELATIONSHIPS OF LEDNICE (YABA 1) ARBOVIRUS TO SOME SMALL MAMMALS

In the effort to determine the range of the hosts of Lednice virus, susceptibility of various species of mammals living in the surroundings of the ponds near Lednice (Southern Moravia), where the virus was isolated (Málková D. et al., Acta virol. 16: 93, 1972; Folia parasit. (Praha) 21: 363—372, 1974), was investigated. All species of the littoral vegetation and also the reeds above water-level, are often inhabited by the harvest mouse — *Micromys minutus*, which builts its nest suspended on the reeds and surrounding bushes and which has the greatest possibility to get into contact with the vector of virus. *Apodemus sylvaticus* and *A. flavicollis* were the other mice, which occurred more frequently. From other most numerous animals the shrews and the voles (*Clethrionomys glareolus* and *Microtus arvalis*) were present (Pelikán, J. Acta Sci. Nat. Acad. Sci. Bohemoslov., Brno, nova series 9, no 12, 45 pp., 1975). Besides susceptibility of young animals assayed experimentally, contact of virus with small mammals in nature was investigated by serological survey.

MATERIALS AND METHODS

Animals. Experimental susceptibility was assayed in *M. minutus*, *A. sylvaticus*, *M. arvalis* and *C. glareolus*. The animals were captured in live traps in the Břeclav district of Southern Moravia in November 1976 and young approx. 2—4 months old animals were included in the experiment. The animals used for serological survey were captured into live traps situated in the reed belts of Lednice ponds and of the pond Nesyt at the beginning of autumn 1975 and 1976. (For the capturing of animals in the pond Nesyt partially devoid of water in 1976 see Pelikán J., Hodková Z., Folia zool. 26: 99 to 113, 1977). Species and numbers of serologically investigated animals are presented in Results.
Virus and experimental infection. The lyophilized mouse brain suspension of the 6118 SM2 strain of Lednice virus was used. After the rehydration in redistilled water, the virus was titrated in medium containing 10% calf serum in PBS and antibiotics. Animals were inoculated scut into the occipital part of the head per 0.1 ml of suspension of virus diluted 10^{-2} (equal to 5 000—10 000 mouse icer LD_{50}).
Collection of material for isolation experiments was done 24, 72, and 120 hours p.i. Blood was collected from sinus retraorbitalis into heparin. Moreover, the regional lymph nodes (cervical bilateral) and the spleen were dissected from 1—2 animals in each interval (Málková D., Acta virol. 4: 290—295, 1960). Collections of plasma for serological investigation were done the 3rd and 6th week p.i. Pools of plasma from 2—4 animals were assayed. For serological survey of animals captured from nature blood was collected by bleeding from the axillary vein.
Detection of virus. Blood and suspensions of organs were titrated (log_{10}) and each dilution was inoculated icer in the amount of 0.01 ml into 1—2 litters of suckling mice.
Serological assay. After experimental infection neutralization antibodies were established. Plasma was diluted 1 : 2 and added to the tenfold diluted antigen. After incubation at 37° C for 60 minutes, the pools of plasma with respective diluted antigen were inoculated icer in the amount of 0.01 ml into 1—2 days old suckling mice. The virus titres were calculated according to Reed and Muench. For serological survey the microhemagglutination-inhibition test according to Clarke and Casals (Am. J. trop. Med. 7: 561—573, 1958) was used. The saccharose-acetone antigen was prepared by ultrasonic disintegration (Kolman J., Meergansová J., J. Hyg. Epid. Microbiol. Immunol. 17: 503—504, 1973). For antigen preparation the strain 6118 was used in both tests. In HI tests moreover, the antigens of Ťahyňa, Čalovo, Sindbis, Uukuniemi-Poteplí and of tick-borne encephalitis were used.

RESULTS

Experimental infection. Viremia was not ascertained in any of the experimentally infected animals of the above mentioned 4 species. The virus was not even detected in the regional lymph nodes; only after inoculation of suspension of the spleen—dissected 72 hours p.i.—single deaths of mice after incubation time of 8—10