served in whole serum by ourselves and others.

1. Samachson, J., Lederer, H., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 867.
2. Likins, R. C., Posner, A. S., Kunde, M. L., *Arch. Biochem. Biophys.*, 1959, v83, 472.
3. Carr, C. W., Woods, K. R., *ibid.*, 1955, v55, 1.
4. Clegg, R. E., *Chem. Analyst*, 1949, v38, 87.
5. Carr, C. W., Anderson, D., Miller, I., *Science*, 1957, v125, 1245.
6. Svensson, H., *Arkiv. Kemi, Mineral Geol.*, 1946, No. 10, v22A.
7. Deutsch, H. F., Kcenig, V. L., *Handbook of Biological Data*, Spector, W. W., ed., p55, Saunders, Philadelphia, Pa., 1956.

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A Standardized Antigen and Skin Test for Toxoplasmosis.* (27431)

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The poor correlation between the presently used skin test(1) for toxoplasmosis and the more reliable methylene blue dye test for serum antibodies has been reported(2,3). This discrepancy may arise from use of an essentially unstandardized skin test antigen(4). One of the technical difficulties in securing a standardized antigen has been the efficient separation of the toxoplasma from the cells of the peritoneal exudate of the infected mouse.

The procedure described here provides a method to secure a skin test antigen essentially free of exudate cells and standardized by count of the toxoplasma. The use of this standard antigen made possible an investigation of standard procedures for reading and evaluating the skin test.

Preparation of skin test antigen. Aseptic technic, sterile solutions and sterile equipment were employed at all times. All centrifugations were in No. 1 International Centrifuge, rotating radius 20 cm.

The peritoneal exudate from 25 adult Webster mice, which had been infected 3 to 4 days previously with RH strain *T. gondii*, was collected into a 50-ml centrifuge tube and diluted 1:5 with saline. After centrifugation of this supension at 2500 rpm for 30 minutes, the supernate was removed. The solids were resuspended in one ml saline by expelling the total contents several times through a 27-gauge needle attached to a one-ml syringe. A 30-gauge needle was then substituted on the syringe and the total contents expelled 10-15 times while exerting as great pressure as possible on the plunger of the syringe and holding the needle at right angles to, and at a distance of one to two cm from the bottom of a 20-30-ml vaccine bottle. Air bubbles were allowed to escape between expulsions as foaming appeared to interfere with the splitting process. Rubber gloves were worn and work proceeded under a protective hood at this stage.

Microscopic examination determined the complete disruption of the exudate cells. The total contents were diluted with 10 cc saline, transferred to a 15-ml siliconized centrifuge tube with a water tight seal, and placed in a 56°C water bath for 60 minutes. Subsequently the solids were evenly dispersed by withdrawing and expelling the total contents several times through a 27-gauge needle on a 20-ml syringe. The cellular debris was separated from the toxoplasma by centrifugation at 600 rpm for 60 minutes and the supernate discarded. This procedure was repeated, if indicated, for better separation.

The toxoplasma were next suspended in a sufficient volume of saline to permit reliable counting with a haemacytometer, and total number of organisms was determined. The suspension was placed in a 56°C water bath for 60 minutes, then centrifuged in a 50-ml

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TABLE I. Efficiency of Exudate Cell Removal.

	Exudate (million/ml)		
	Original	Expelled	% change
R.H. strain			
Exudate cells	164	6	96% decrease
Toxoplasma	312	385	23% increase
Gracewood strain			
Exudate cells	116	7	95% decrease
Toxoplasma	159	285	79% increase

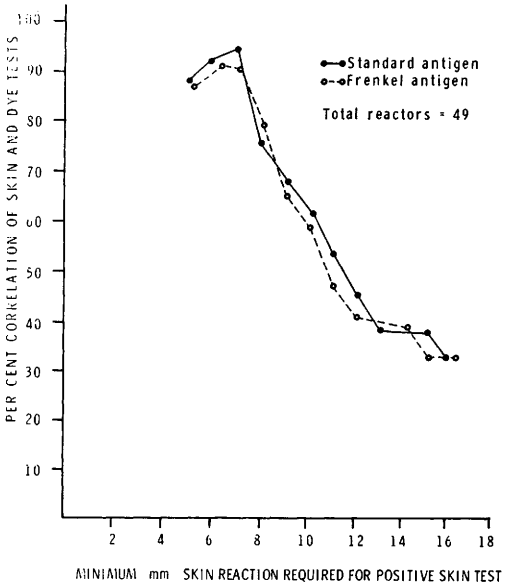
centrifuge tube at 2500 rpm for 45 minutes. After the supernate was discarded, the toxoplasma were resuspended in 0.3% phenolized saline to the desired concentration and recounted. The final preparation was checked for sterility by mouse inoculation and thioglycollate culture.

The efficiency of this technic is shown in Table I. The Gracewood strain was isolated from a swine herd with subclinical toxoplasmosis(5). The percentage increase in toxoplasma varied with the stage of infection and with the 2 strains used.

Standardization of skin test antigen. The following standard procedures for skin testing were maintained. A skin test dose of 0.1 ml was injected intracutaneously on the inner forearm, and the erythema and induration produced at 48-72 hours (preferrably observed in daylight) were recorded in millimeters. Simultaneous skin tests on the right and left arms of the same individual provided comparative reactions. Preliminary skin tests were made on 13 known positive white individuals and provided evidence that degree of skin reaction was related to number of organisms injected. All produced at least 6 mm of erythema or induration to an antigen of 2 million toxoplasma/ml, but only 8 had this much reaction to an antigen of 200,000 toxoplasma/ml.

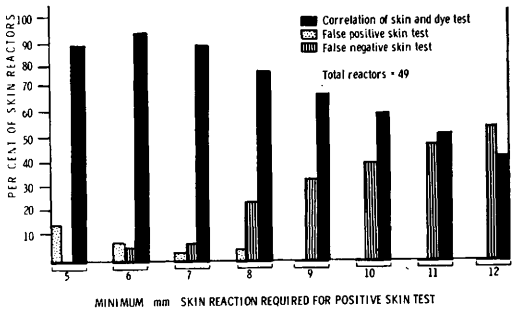
Further skin testing, with concentrations up to 5 million organisms per ml, indicated that an antigen containing 1-1½ million toxoplasma per ml would produce easily read but few extensive reactions on known positive individuals. This concentration of toxoplasma was then established as the standard antigen. A comparative study of the standard antigen and the Frenkel‡ antigen was made on 126 selected individuals and showed 96% agreement when a minimum of 5 mm of either erythema or induration was required for a positive reaction. The 2 antigens produced approximately equal areas of erythema. However, the standard antigen produced more induration. The data showed 43 reactors with at least 5 mm of induration in response to the standard antigen, but only 33 similar reactors to the Frenkel antigen.

‡ Prepared by Eli Lilly and Co.



GRAPH I. Comparison of standard and Frenkel antigen.

Standardization in reading the skin test. Blood samples for the methylene blue dye test were secured from those individuals in the previous study who produced at least 5 mm of erythema or induration to the standard antigen. A titer of 1:4 was considered positive. Graph II shows the correlation of



GRAPH II. Correlation of area of skin reaction with dye test.

the dye test and skin test when related to degree of skin reaction.

The false positive skin test is defined as one in which the skin test is recorded as positive, but the dye test is negative. The false negative skin test is one in which the skin test is recorded as negative, but the dye test is positive. Graph II shows a definite curve with a peak of 95% correlation when a minimum of 7 mm of skin reaction is required for a positive skin test. Increasing numbers of false positive skin tests occur as smaller reactions are accepted as positive. Increasing numbers of false negative skin tests occur as larger reactions are required for positivity, *e.g.,* there is 95% correlation with the dye test if 7 mm of reaction is required for a positive skin test, but there is only 67% correlation if 10 mm is required.

Methylene blue dye tests were made on blood samples from 47 skin test negative individuals of the comparative study. Only one individual had a positive dye test, *i.e.,* presented a false negative skin test. It has been established that the incidence of toxoplasmosis in most populations increases in older age groups(6). Also it is known that the dye test becomes positive before the skin test during the course of infection(2). Therefore, dye tests were made on 19 skin test negative individuals over 20 years of age who resided in an area of known high incidence of toxoplasmosis(7). Only 2 false negative skin tests were revealed, although this group presented a sample of population possibly expected to show many false negative skin reactions.

Discussion. All skin tests cannot be measured with exactness, but it is evident from Graph II that there is a range of skin reaction to a standard toxoplasma antigen which encompasses a high degree of correlation with the dye test. The identification of this range of skin reaction for a standard antigen would increase the reliability of the skin test for epidemiological and clinical use.

The present data suggest that false negative skin reactions are caused not only by the absence of skin allergy to *T. gondii,* but also by the lack of a standard antigen and standard procedures for reading the skin test.

Further study of the whole body antigen showed that the toxoplasma did not remain intact over a long period. Four months after preparation, the toxoplasma count had decreased by 50%. It may be that the more stable antigen will be one that is standardized by count of the organisms, but that the final preparation consists of the soluble products of the toxoplasma. This point is being investigated. The present study was conducted with a standard antigen one to two months after preparation.

Summary. A skin test antigen for toxoplasmosis was prepared by liberation of the intracellular organisms and separation of the toxoplasma from the cellular debris. Standardization by count showed that an antigen of 1-1½ million organisms per ml produced skin reactions of a desirable clinical range. This standard antigen produced skin tests resulting in 95% correlation with the dye test when a minimum of 7 mm of either erythema or induration was required for a positive reaction.

1. Frenkel, J. K., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 634.

2. Jacobs, L., Naquin, H., Hoover, R., Woods, A. C., *Bull. Johns Hopkins Hosp.,* 1956, v99, No. 1, 1.

3. Feldman, H. A., *J. Chr. Dis.,* 1959, v10, no. 6, 488.

4. Jirovec, O., Jira, J., *Zbl. f. Bakt.,* 1961, v181, 110.

5. Wende, N. M., Dienst, R. B., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 400.

6. Beattie, C. P., *The Epidemiology of Toxoplasmosis,* 175, *Human Toxoplasmosis, Süm, J. Chr.,* Williams and Wilkins, Co., Baltimore, Md., 1960.

7. Wende, N. M., Dienst, R. B., *J. Ga. Med. Assn.,* 1962, Feb., in press.

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