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Immune Response to Rabies Vaccine in Water-in-Oil Emulsion.

JULES FREUND, MURRAY M. LIPTON, AND T. M. PISANI.

From the Division of Applied Immunology, the Public Health Research Institute of the City of New York, Inc.

The formation of neutralizing antibodies was compared in guinea pigs after the subcutaneous injection of rabies vaccine (rabbit brain containing inactivated rabies virus) given either in salt solution or in a water-in-oil emulsion with or without *Mycobacterium butyricum* (Table I). The technic of preparing the emulsions and other details of the method have been described.¹

Groups of 5 or 6 animals were given a single subcutaneous injection and bled 34 and 71 days later. The pooled sera were kept at -15° C until the neutralization tests were made. Equal volumes of serum-dilutions and infected rabies mouse brain suspensions were mixed, held at 37° C for 1 hour and at 4° C for another hour and injected into the brain of mice. The inoculum was 0.03 ml to which were added 20 Oxford Units of penicillin. The results are shown in Table II.

It is seen from this table that in the groups with vaccine in oil emulsions the neutralizing antibody titers were higher than with vaccine in saline solution. In another experiment using Vaccine B by the intraperitoneal route

the titers in the group with water-in-oil emulsion were at least 8 times higher than in the group with Vaccine B in salt solution. The killed mycobacteria did not seem to potentiate antibody production. In this connection it is interesting that *M. butyricum* and other mycobacteria have a potentiating effect on immunity responses to the influenza virus² and *S. typhosa*.³ Allergic encephalitis occurred only in the groups II, V, and VIII with *M. butyricum*. It may be emphasized here that single or few injections of brain in water-in-oil emulsion do not cause encephalitis unless mycobacteria or actinomycetes (*Nocardia asteroides*)⁴ are added to the emulsion.

Unpublished experiments on the formation of complement fixing antibodies against rabies virus are in harmony with the above results. They also show the potentiating effect of water-in-oil emulsion.

Titers of neutralizing antibodies in the blood are of significance with respect to protection against viral infection of the central nervous system. They are in a well-defined

TABLE I.
Material Injected.

Group	Vaccine A, ml	Vaccine B, ml	Vaccine C, ml	NaCl, 0.85%	Falva, ml	Paraffin oil, ml	Killed, <i>M. butyricum</i> , mg
I	0.8	—	—	1.2	—	—	—
II*	0.8	—	—	—	0.4	0.8	0.8
III	—	0.8	—	1.2	—	—	—
IV	—	0.8	—	—	0.4	0.8	—
V†	—	0.8	—	—	0.4	0.8	0.8
VI	—	—	0.8	1.2	—	—	—
VII	—	—	0.8	—	0.4	0.8	—
VIII†	—	—	0.8	—	0.4	0.8	0.8

* 3 of 5 animals died of allergic encephalitis before testing for antibodies.

† 2 of 5 animals died of allergic encephalitis before testing for antibodies.

¹ Freund, J., Stern, E. R., and Pisani, T. M., *J. Immunol.*, 1947, **57**, 179.

² Friedewald, W. F., *J. Exp. Med.*, 1944, **80**, 477.

³ Freund, J., Thomson, K. J., Hough, H. B., and Pisani, T. M., *J. Immunol.*, in press.

⁴ Freund, J., and Lipton, M. M., *Proc. Soc. Exp. Biol. and Med.*, 1948, **68**, 373.

TABLE II.
Neutralizing Antibody Titers of Sera of Guinea Pigs After a Single Injection of Rabies Vaccine.

Group	Serum dil.	1:5*	1:10	1:20	1:40	1:80	1:100	1:1000	1:10,000
34 Days—									
I	Vaccine A		0/6†				6/6	6/6	6/6
II	" A in oil + <i>M. butyricum</i>		0/5				1/5	5/5	6/6
III	" B		1/6				4/4	6/6	6/6
IV	" B in oil		0/5				0/6	0/6	6/6
V	" B " " + <i>M. butyricum</i>		0/6				0/6	3/6	6/6
VI	" C		0/6				3/5	6/6	4/5
VII	" C in oil		0/5				0/6	2/6	4/5
VIII	" C " " + <i>M. butyricum</i>		0/6				0/6	6/6	5/6
71 Days—									
III	Vaccine B		2/7				8/8		
IV	" B in oil		0/8				1/8	8/8	
VI	" C	0/7	4/7	6/7	7/7	7/7	8/8		
VII	" C in oil	1/7	0/8	0/7	1/7	6/7	7/8		

* 0.015 ml of a 1:2.5 dilution of serum + 0.015 ml of a fixed virus-brain suspension diluted 1:300,000.
Titer of virus suspension: 10⁻⁶: 8/8; 10⁻⁷: 5/8; 10⁻⁸: 3/8.

† 0 of 6 mice died of rabies.

numerical relationship to the antibody content of the CNS.⁵ Deviation from the ratio occurs when antibodies are produced in the CNS itself as a result of infection.⁶ Immunization with the aid of water-in-oil emulsion may be applicable to prophylactic immunization of dogs and to the production of "hyperimmune" sera for the treatment of man and lower animals after exposure to rabies infection. Habel⁷ found that greater protection

against rabies can be obtained in experimental animals with the combination of passive and active immunization than with the latter alone.

Summary. The incorporation of inactivated rabies vaccine into water-in-oil emulsion enhanced the antigenic property of the vaccine as judged by the formation of neutralizing antibodies. Allergic encephalitis did not occur unless mycobacteria were added to the emulsion.

⁵ Freund, J., *J. Exp. Med.*, 1930, **51**, 889.

⁶ Morgan, I. M., *Am. J. Hygiene*, 1947, **45**, 390.

⁷ Habel, K., *Pub. Health Rep.*, 1945, **60**, 545.

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Demonstration of Acid Phosphatase in *Endamoeba histolytica*.*

G. M. CARRERA AND G. W. CHANGUS.† (Introduced by E. C. Faust.)

From the Department of Tropical Medicine and Public Health and the Department of Pathology and Bacteriology, Tulane University School of Medicine, New Orleans.

Acid phosphatase activity can be demonstrated in *Endamoeba histolytica* using Gomori's histochemical method.¹ The amebae, originally isolated from human cases and grown in Balamuth's liquid media² or ob-

tained by rectal aspiration from an experimentally infected dog, consistently give strongly positive histochemical acid phosphatase reaction.

Although the method used is fundamentally the one described by Gomori, slight technical modifications are necessary to make it adap-

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† Fellow, American Cancer Society.

¹ Gomori, G., *Arch. Path.*, 1941, **32**, 189.

² Balamuth, W., *Am. J. Clin. Path.*, 1946, **16**, 380.