who reported that low copper synthetic rations were more effective than whole milk diets in curing anemia. Others have disputed this finding however(11).

Conclusions. Black rats subsisting on a purified diet containing 14.6 μg of copper per g grew well and maintained normal hemoglobin levels but developed marked achromotrichia. The achromotrichia could be cured by adding copper sulfate to the diet and could be prevented by diets containing approximately 20 μg of copper per g. The activity of liver, yeast and alfalfa in preventing and curing the achromotrichia was due to their copper content. Evidence suggesting that the basal purified diet used in these experiments may have been deficient in an unidentified growth factor has been discussed.

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Formation of Neutralizing Antibody in Monkeys Injected with Poliomyelitis Virus and Adjuvants.* (17964)

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It has been long known that rhesus monkeys receiving repeated injections of poliomyelitis virus produce neutralizing antibodies. Recently Morgan, Howe, and Bodian(1) and Morgan(2) studied the effect of dosage, route of administration of the antigen and other factors on the hyperimmunization against this virus. No evidence was found that antibody formation was augmented by the incorporation of the Lansing type of virus into a water-in-oil emulsion with or without tubercle bacilli (1,3).

Our hope for the effectiveness of these adjuvants in relation to poliomyelitis virus was raised by results obtained recently with another neurotropic virus, namely, that of rabies(4). It was found that rabbits or guinea pigs, given a single injection of brain containing inactivated rabies virus emulsified in paraffin oil and one month later a few more injections of the same antigen in salt solution, produced neutralizing and complement fixing antibodies in great abundance.

Material and methods. As an immunizing agent, monkey spinal cord, containing active poliomyelitis virus of the Lansing type, was used. It was suspended in salt solution with the aid of a Waring blendor. The antigen was injected either in this form or emulsified in paraffin oil with or without the addition of a small amount of killed *Myco. butyricum.* The technic of emulsification and the ingredients used have been described previously (5). Immature rhesus monkeys (*Macacca mulatta)* as a rule, were given 5 simultaneous injections. The sites were the pectoralis, and the flexor muscles of the femur and the subcutaneous tissue of the nuchal region. One monkey (Group IIIa) received the primary injection in the footpads. The "booster" injections were the same for all groups, namely, the antigen in salt solution. The data are given in Table I.

The neutralizing antibodies were titrated using 4 or 5 fold serial dilutions of serum

* Aided in part by a grant from The National Foundation for Infantile Paralysis, Inc.

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TABLE I.
Material Injected per Monkey. (First injection).

Group	No. of monkeys	Lansing virus monkey cord, g	Salt sol., ml	Paraffin oil, ml	Arlacel A, ml	*Myco. butyricum*, mg
I	6	1	9	0	0	0
II	6	1	4	4.5	.5	.1
III	6	1	4	4.5	.5	0
IIIa	1	0.4	1.6	1.8	.2	0
		Booster injections 5 and 11 wk after primary injection				
All monkeys		1	10	0	0	0

TABLE II.
Neutralizing Antibody Titers in Monkeys Injected with Poliomyelitis Virus With and Without Adjuvants. Dilution of sera before adding equal volumes of virus dilution.

Group	Immunizing antigen: Lansing virus with	Rhesus No.	Neutralizing antibody (serum endpoint)* 1 mo. after 1st inj.	Mean	2 wk after 2nd inj.†	Mean	1 wk after 3rd inj.†	Mean
I	Saline	1	2.4, 1.9	1.9	3.4, 3.6	3.1	3.6	3.4
		2	2.4, 2.5		3.3, 3.3		2.9	
		3	2.1		3.7, 3.4, 3.4		3.7	
		4	1.7		3.3		3.7	
		5	1.7		2.6		3.1	
		6	1.3, 1.8		2.6		3.3	
II§	Paraffin oil	7	3.8	3.3	4.4, 4.3	4.2	4.6, 4.3	4.3
	Arlacel	8	3.7		4.4, 4.3, 4.0‡		—	
	Myco. butyricum	9	3.3		4.3, 4.5		4.2	
		10	2.7, 2.5		3.8‡		—	
III	Paraffin oil	11	3.3	3.1	3.9	3.9	3.6, 3.5	3.7
	Arlacel	12	3.3		3.9, 3.5		3.6	
		13	2.9		4.1, 4.1		3.8	
		14	2.9		3.8		3.7	
		15	2.8		3.9		3.9, 3.6	
		16‖	3.3, 3.3		4.0		3.6	

* = Reciprocal log of the highest dilution of serum neutralizing the virus, 50% of mice being protected. (Reed and Muench formula).
† = 2nd and 3rd inj.; virus in saline (lg. Rh. CNS).
‡ = Titers 1 wk after 2nd inj.
§ = Two animals died of allergic encephalitis before the 1st bleeding.
‖ = Group IIIa 1st inj.; 4 ml in footpads.

mixed with a constant amount of virus; the mixture was held at room temperature for 1 hour and injected into the brain of mice in groups of 8 or 9. Although the virus suspension used in all tests was from the same pool, the number of LD_{50} doses of virus ranged from 10 to 100 (calculated from the final dilution of virus *after* the latter was added to serum). The mice were observed for 4 weeks. All deaths occurring after the first day were considered as specific deaths. The serum 50% endpoints were calculated by the method of Reed and Muench(6). The titers are expressed as the serum dilution endpoints *before* the addition of an equal volume of virus dilution.

Results. The mean antibody titers for each group of monkeys are given in Table II. One month after the first injection they were about 10 times higher in the 2 groups receiving adjuvants than in the group given the saline suspension. Two weeks after the second injection, the titers rose in all groups, but a conspicuous difference was still sustained. One week after the third injection, the mean levels were only slightly different

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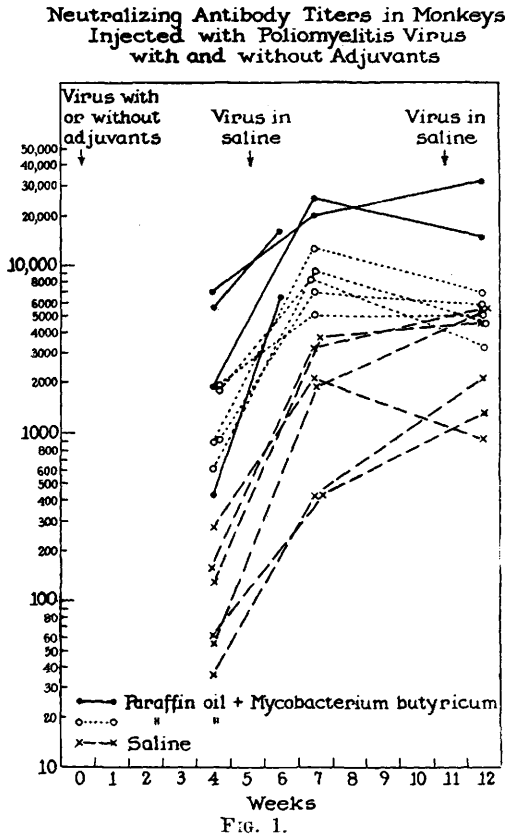

FIG. 1.

from those found in the preceding test. However, since the mean titers rose slightly in the group with saline suspension and fell slightly in the group with oil in the absence of *Myco. butyricum,* the difference between these 2 groups became less.†

In Table II, the multiple figures under the same heading represent titers found on repeating the tests. Some of the samples were titrated in the two different laboratories by different persons.

Fig. 1 presents graphically the data of Table II using the average values for samples titrated repeatedly.

As customary, with each neutralization test, the infectivity of the virus suspension was titrated. Groups of 8 or 9 mice were injected with 10-fold serial dilutions of the virus suspension beginning with 10^{-2} and including 10^{-5} dilutions. The arithmetic mean titer was $10^{-3.5}$ (range $10^{-3.1}$ to $10^{-4.0}$). Accordingly, as mentioned above, the LD_{50} values in the different tests varied from 10 to 100. Since some of the samples of the sera were titrated repeatedly, a tabulation of the results may reveal the effect of the variation in the concentration of virus on neutralizing titers obtained. Table III shows that within this range, this variation had little if any effect on the serum titers.

In another experiment, using 4 monkeys, the synergetic effect of paraffin oil was confirmed. Table IV includes a monkey (No. 20) which had an inapparent infection following intracerebral injection of Lansing virus, as shown by the presence of antibody, before immunization was started. The titer of the serum before immunization was log 2.5 and reached the unusual titer of log 4.6 4 weeks after the first injection.

In 4 of the 6 monkeys which were given *Myco. butyricum* in addition to infected spinal cord and paraffin oil, fatal allergic encephalitis occurred. Two of them died before the first bleeding. One monkey had some of the symptoms of this experimental malady, while in another one no symptoms were seen. This complication was not unexpected in view of the experience of Morgan(3) and Kabat, Wolf, and Bezer(7) with tubercle

TABLE III.
Antibody Titer and Concentration of Virus in Test

	Log. of LD_{50} doses of virus					
Serum	1.0	1.3	1.4	1.5	1.7	2.0
6–I*				1.8		1.3
1–I		2.4			1.9	
12–II	3.9		3.5			
8–II	4.4		4.0	4.3		
15–III			3.9		3.6	
7–III					4.6, 4.3	
1–II	3.6			3.4		
10–I		2.7			2.5	
7–II	4.4				4.3	
2–II	3.3			3.3		
13–II	4.1			4.1		
16–I				3.3		3.3
3–II	3.4			3.7	3.4	
2–I		2.4			2.5	
11–III			3.5			3.6
9–II	4.3			4.5		

* 6–I = Rhesus No. 6, 1st bleeding.

† One week after the third immunizing injection, all the monkeys were challenged by intracerebral injection of 3000 LD_{50} doses of Lansing virus. None developed signs of poliomyelitis.

TABLE IV.
Neutralizing Antibody Titers in Monkeys Injected with Poliomyelitis Virus Combined with Paraffin Oil and Arlacel A. Dilution of sera before adding equal volumes of virus dilution.

Rhesus No.	Before immunizing inj.	4 wk after 1st inj.	8 wk after 2nd inj.
17	0†	4.1	3.6
18	0	3.3	3.9
19	0	2.4	3.9
20*	2.5	4.6	3.9

* Inapparent infection following intracerebral inoculation.
† Undiluted serum did not neutralize the virus.

bacilli and our own in guinea pigs using either species of acid-fast bacilli(8). In the present experiment, the *Myco. butyricum* was used since it may be a slightly more effective potentiating agent(5,9) than tubercle bacillus. A relatively small amount was injected, 0.1 mg per monkey with the hope of diminishing its untoward effect.

In the present experiments, the "booster" injections were of antigen in saline. With rabies virus high antibody titers were obtained by one injection of antigen in water-in-oil emulsion followed by a "booster" injection of antigen in saline(4). Following an injection of a bacterial antigen in water-in-oil emulsion, the injection of antigen in saline was as effective a "booster" as antigen emulsified in paraffin oil(5).

Conclusions. 1. The formation of neutralizing antibodies in rhesus monkeys is enhanced when the antigen, spinal cord containing poliomyelitis virus, is incorporated into a water-in-oil emulsion. 2. The addition of a small amount of killed *Myco. butyricum* to the water-in-oil emulsion further augments antibody formation. Some of the monkeys, however, develop fatal allergic encephalitis. 3. By repeating the injections of antigen without these adjuvants, antibody titers can be produced which are equal to those found after the injection of antigen in water-in-oil emulsion, but not as high as in monkeys receiving the combination of antigen, paraffin oil and killed *Myco. butyricum.*

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Lack of Effect of Lactogenic Hormone on Mammary Adenocarcinoma in Mice.* (17965)

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Many clinical observations have indicated that the growth of mammary cancer in women is accelerated by pregnancy and lactation (1,2). The effect of lactation alone is not known, although Geschickter(3) believes that it does not influence the growth rate of infiltrating cancer of the breast. Pregnancy stimulation is usually ascribed to the high estrogen titre, a situation which does not apply during lactation(3).

In mice, estrogens exert a carcinogenic effect on the mammary gland(4), but the estrogen preponderance of pregnancy ap-

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