begins to twist and fold, place the material in water again.

The method is not recommended for tissue which is tough in a fresh condition, nor will it take the place of a sharp knife. The writer prefers a regular microtome knife, stropped at frequent intervals, to any of the razor blade holding devices. Investigations are to be continued along this line to find a water or alcohol soluble substance which has greater softening qualities than pure water that can be used successfully in connection with the 95 per cent. alcohol treatment to soften tissue which is definitely tough in a fresh condition.

Chamberlain recommends a method used by Dr. Land of storing the paraffin cakes in water. The writer is not in a position to make comparisons. Obviously, however, the 95 per cent. alcohol treatment upon the partially exposed material would facilitate the infiltration of water later. It is hoped that this modification of Dr. Land's method may meet with some favor among paraffin workers.

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SPECIAL ARTICLES

IMMUNIZATION WITH ALUMINUM HYDROXIDE MIXTURES OF POLIOMYELITIS VIRUS

THE recrudescence of poliomyelitis in the United States and Europe during the past two or three years has led to a restudy of the disease from many points of view. This brief report deals with the experimental evidence that the virus of poliomyelitis, inactivated by adsorption on particles of aluminum hydroxide, is still capable of producing immunity when inoculated into *Macacus rhesus* monkeys. Previously several investigators had shown that a variety of viruses could be adsorbed and rendered ineffective by a number of colloidal and particulate chemical substances. No one seems, however, to have tested the inactivated materials for the production of artificial, active immunity.

The aluminum hydroxide employed was the type C suspension of Willstätter containing 22.5 grams of aluminum per liter. The virus was either Berkefeld N filtrate of fresh monkey pooled virus,[1] or suspension of glycerolated material of the same strain. Mixtures of virus and suspension were allowed to stand 30 minutes at room temperature. The experiments carried out were of three types: simple observations on the inactivation of poliomyelitis virus by aluminum hydroxide; studies of the effect of the pH of the mixture on the inactivating power; and determinations of the value of the inactivated virus in the production of immunity. Intracerebral inoculations of the aluminum suspension alone were without pathological effect.

In respect to these three tests it was found first, that the filtrate and aluminum hydroxide mixed in equal volumes became inactive; second, that inactivation was promoted by acid (5.5) and prevented by alkaline (8.8) reactions; and third, that repeated subcutaneous injections of the inactivated virus led to active immunity.

[1] Rhoads, C. P., *Jour. Exper. Med.*, 49: 701, 1929.

The immunity thus induced was tested in three ways. First, glycerolated virus was repeatedly instilled into the nostrils. All the previously treated animals resisted infection, although the control developed typical poliomyelitis. The second test, carried out 28 days after the first, consisted of intracerebral inoculation of fresh virus. Of three treated animals so tested, one developed poliomyelitis, as did the control, and two resisted infection. The third test was made with the blood serum of the treated monkeys. Each of the three sera was tested separately and each neutralized the virus.

It may, therefore, be concluded that the virus, when adsorbed on aluminum hydroxide, is incapable of producing poliomyelitis, but still capable of inducing active immunity in *Macacus rhesus*. In a small series of animals thus immunized, no symptoms of experimental poliomyelitis arose, and in one only was the degree of immunity, although adequate to protect against nasal instillation, insufficient to protect against intracerebral injection of virus. That all three treated monkeys developed immunity is shown by the serum neutralization tests.

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THE EFFECT OF TESTICLE EXTRACT AND NORMAL SERUM ON THE GROWTH OF A TRANSPLANTABLE EPITHELIAL TUMOR OF THE RABBIT[1]

EARLIER investigations in this laboratory[2, 3] have shown that extracts of the testes considerably enhance

[1] From the laboratories of the Rockefeller Institute for Medical Research.

[2] F. Duran-Reynals, *Soc. Biol.*, 1928, 99, 6; *J. Exp. Med.*, 1929, 50, 327.

[3] F. Duran-Reynals and J. Suñer Pi, *Soc. Biol.*, 1928, 99, 1908.

the infectivity of vaccine virus and staphylococcus, while blood serum interferes with the action usually observed with these agents. These observations have been extended by Hoffman,[4] who has demonstrated the same phenomena with other filterable viruses and by Pijoan[5] with many other bacteria.

The present report deals with the effect of testicle extract and serum on the Brown-Pearce rabbit tumor,[6] a malignant, transplantable neoplasm of epithelial origin. In each experiment, three sets of test inoculations were made as follows: A suspension of the tumor cells was made with (a) an equal volume of testicle extract, (b) an equal volume of normal rabbit serum, (c) an equal volume of Ringer's solution as a control. These mixtures were incubated for 2 to 3 hours at a temperature of 37° C. and then injected intradermally in the shaved skin of the side of the body. Each rabbit was inoculated in one or more areas with each test mixture.

The results obtained in 10 rabbits inoculated in 84 different areas are shown in the accompanying table.

TABLE I

EFFECTS OF TESTICLE EXTRACT AND SERUM ON THE BROWN-PEARCE TUMOR

Tumor cell suspension plus	Number of inoculations	Larger growth than control	Same growth as control	Smaller growth than control	No growth
Rat testicle extract	16	0	0	2	14
Rabbit testicle extract	16	0	1	6	9
Rabbit serum	32	19	10	3	0
Ringer's solution (control)	20				0

In addition, an experiment carried out with the intratesticular inoculation of tumor tissue and rat testicle extract resulted in a less active primary growth and a greatly decreased distribution of metastases as compared with the results obtained by the intratesticular inoculation of suspensions prepared with Ringer's solution. This result is an apparent paradox, for the method used in carrying this tumor is by intratesticular injection, with which active growth is usually associated.

It may be concluded from these experiments that testicle extract exerts an inhibitory effect on the growth of a transplantable rabbit tumor, while normal rabbit serum, on the contrary, appears to stimulate its growth. These findings are in contrast to those obtained with viruses and bacteria, in which the testicle extract augments and normal serum inhibits the effects of these agents.

F. DURAN-REYNALS

[4] D. C. Hoffman, *J. Exp. Med.* (in press).

[5] M. Pijoan, *J. Exp. Med.* (in press).

[6] W. H. Brown and L. Pearce, *J. Exp. Med.*, from 1923 to 1929.

THE NECESSITY AND FUNCTION OF MANGANESE IN THE GROWTH OF CHLORELLA SP.[1]

THE importance of manganese in plant growth has been emphasized by the experiments of McHargue,[2] and more recently Samuel and Piper[3] have shown very clearly its essential nature for a fairly large number of species of seed plants. In the experiments of the latter workers practically no development of the plants beyond the seedling stage was obtained without manganese. Titus and Cave[4] have also shown the beneficial effect of manganese in hemoglobin building in the cases of animals made anemic on a whole milk diet. The necessity of manganese for a single-celled organism has not been shown and is of fundamental importance.

In connection with my own studies on iron in relation to Chlorella sp., a unicellular green alga, it has also been found that manganese is essential for growth. Increases of from 10 to 600 fold in the growth have been obtained by the addition of one part of manganese in five million parts of culture solution from which the manganese had been removed by adsorption on calcium phosphate. The accompanying tables present the data from two experiments which are typical of many performed. The experiments were carried out in pure culture.

TABLE I

THE NECESSITY OF MANGANESE FOR THE GROWTH OF CHLORELLA SP.

pH 7.0			pH 8.0		
Cult no.	Manganese	Dry weight	Cult no.	Manganese	Dry weight
1	none	2.6 mgs	9	none	0.6 mgs
2	"	5.1	10	"	0.4
3	"	2.2	11	"	0.2
4	"	0.9	12	"	0.1
5	1: 5,000,000	58.7	13	1: 5,000,000	77.2
6	"	57.1	14	"	45.7
7	"	64.7	15	"	52.8
8	"	60.3	16	"	53.2

The results shown in Table I demonstrate the necessity of manganese for Chlorella sp., since there is practically no growth without it. At pH 7.0 the increase due to manganese is about 17 fold and at pH 8.0 about 170 fold. The fact that there is more

[1] The investigation upon which this article is based was supported by a grant from the Heckscher Foundation for the Advancement of Research established by August Heckscher at Cornell University.

[2] *Ind. and Eng. Chem.*, 18: 172–175, 1926.

[3] *Ann. Appl. Biol.*, 16: 493–524, 1929.

[4] SCIENCE, 68: 410, 1928.