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.

TABLE II

THE EFFECT OF CONCENTRATION OF MANGANESE ON THE GROWTH OF CHLORELLA SP.
pH 8.0

Concentration manganese	0	1 to 5,000,000	1 to 1,000,000	1 to 500,000	1 to 100,000	1 to 50,000
Dry weight mgs. av.	1.4(4)*	52.9(4)	53.7(4)	48.1(4)	36.2(3)	11.9(3)

* The figures in parenthesis refer to the number of cultures included in the average.

growth without added manganese at pH 7.0 may be due either to the difficulty of completely removing the manganese impurity or to the greater ionization of the manganese at this reaction.

In Table II the toxicity of manganese is also shown as the amount added is increased. It should be stated that to each culture of the above experiments was added 0.1 mg of iron and 0.04 gms of sodium citrate, and therefore soluble iron which is essential for this organism[5] was not a limiting factor. An important point in connection with these experiments is that the alkaline limit for the growth of this species as reported by Wann and Hopkins[6] must now be extended to higher pH values since pH 8.0 is very close to the limiting reaction reported by them. Other experiments show that, when manganese is added to manganese-free cultures which have shown no development of the organism in two weeks, growth then begins. The cells with which the cultures were inoculated were not dead but were unable to develop without manganese. I have also found that manganese will not replace iron—both are essential.

In most of the literature on manganese an explanation of its action has not been attempted. The present writer wishes to suggest that manganese functions physiologically in an indirect manner by its action on the state of oxidation of iron. In other words, manganese tends to control the ratio $[Fe^{++}] : [Fe^{+++}]$ in the culture or in the cell. Experiments *in vitro* have shown that the reduction of ferric iron to ferrous which is brought about slowly by sodium citrate tends to be prevented by the presence of manganese. For example, a solution of ferric chloride and sodium citrate on being allowed to stand in a stoppered flask lost its original greenish-yellow color after some time. A similar solution which contained manganese did not change color. On testing them the first one showed only a slight test for ferric iron and a strong test for ferrous iron, and the second solution showed just the reverse.

Culture experiments with yeast also indicate that the reduction of the iron by the yeast organism tends to be prevented by the presence of manganese. Further, oxidation-reduction potential measurements on culture solutions containing ferric iron and sodium citrate show that when manganese is added a higher potential is developed.

On this basis it is believed that not only the necessity of manganese but its toxicity can be explained. In the first case, sufficient manganese must be present to insure the reoxidation of the iron after its reduction by the organism. In the second case, a large amount of the element either results in too high a concentration of ferric ions or prevents its reduction by the organism. Different species may be expected to vary in their relation to manganese depending on the reducing power of their cells.

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[5] E. F. Hopkins and F. B. Wann, "Iron Requirement for Chlorella," *Bot. Gaz.*, 84: 407–427, 1927.

[6] *Bot. Gaz.*, 83: 194–201, 1927.

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