therefore, that the publication of this yearly record of American progress in the geophysical sciences may be adequately maintained not only in the national interest but also because of its value in the stimulation, orientation and coordination of international endeavor in these fields.

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SPECIAL ARTICLES

THE PROTECTIVE ACTION OF RABBIT SERUM FOR VACCINIA VIRUS AT HIGH TEMPERATURES[1]

VACCINIA virus rapidly loses its viability at temperatures higher than 5° C. The desirability of a medium by which its viability could be maintained at higher temperatures over a reasonable length of time has long been recognized. Otten[2] succeeded in preparing a dried calf pulp which, stored in vacuo, kept its potency for many months at tropical temperatures. This method has the distinct disadvantage of not eliminating the bacterial contamination in vaccine prepared from this source. Recently Rivers and Ward,[3] taking advantage of the fact that the vaccine virus cultivated by their method in tissue culture is free from bacterial contaminants, added one part of 30 per cent. gum acacia solution to eleven parts of tissue culture virus; and after rapid drying and sealing in vacuo, they obtained a preparation which withstood a temperature of 37° C. for at least one month. Lloyd and Mahaffy,[4] using Rivers's tissue culture virus, prepared a desiccated virus which had been diluted previously with equal parts of normal horse serum. This material stored at 28° C. showed some diminution of potency during a period of 69 days. Stored at 37° C. it could not be kept at undiminished titre longer than 14 days.

Green[5] has shown that glycerinated vaccine loses its viability completely when stored at 37° C. in about 12 days. It is generally agreed that even at a low temperature glycerine has a deteriorating effect on vaccinia virus. The great advantage in its use has been its bactericidal action on the contaminants necessarily present in fresh calf pulp. In a bacteria-free vaccine, such as can be prepared by cultivating the virus in tissue culture or in the chorio-allantoic membrane of the chick embryo, the use of glycerine is of no distinct advantage. Other suspensoids, preferably of a colloidal nature, which, when used with the calf virus, might possibly act as a culture medium for the accompanying contaminants, might prove to have distinctly more protective action for vaccine virus against high temperatures, provided no contaminants are present.

Vaccine virus cultured in the chorio-allantoic membrane of the chick embryo[6] provides an excellent bacteria-free material for testing the effect of various substances upon the viability of the virus at high temperatures. The following substances were used to prepare suspensions of the virus: 30 per cent. solution of gum acacia, 3 per cent. mucin in saline, sterile egg yolk and normal inactivated rabbit serum. Vaccine suspensions were made with these materials in the proportions of 1 part of the ground membranal lesion, which contained the virus from the 200th generation in this tissue, to 4 parts of the suspensoid. The samples were controlled by making the usual 1 to 4 suspension in 50 per cent. glycerine in .9 per cent. NaCl solution, as well as in .9 per cent. NaCl alone. The different suspensions were put up either in capillary tubes or in 1 cc amounts in sealed ampoules. These were stored at 0°C., 25° C. and 37° C. At weekly intervals the different batches were tested for potency by cutaneous inoculation on the rabbit. Dilutions of 1–10, 1–100, 1–1000 and 1–10,000 of the virus suspensions were inoculated in 0.4 cc amounts over a scarified area of skin 2.5 × 5 cm square. Readings were made on the fourth or fifth day. A freshly prepared vaccine from this source induces by this method a confluent lesion in a dilution of 1–1000 and scattered pustules at 1–10,000.

From these comparative experiments it is found that at room temperature for one month the virus suspended in gum acacia and in mucin produces only a very mild reaction in the rabbit in dilutions of 1–10. A moderate reaction is obtained from the virus suspended in glycerine, in saline and in sterile egg yolk in dilutions of 1–100. After 6 weeks at 25° C. in normal inactivated rabbit serum there was practically no diminution in the titratable potency of the virus. Fresh unheated normal rabbit serum seems to reduce the activity of the virus slightly in the first few hours. This observation is being investigated further.

Stored at 37° C. the virus suspended in mucin and gum acacia had lost its viability completely at the end of two weeks. After 3 weeks at 37° C. the glycerinated virus and that in egg yolk was completely inactive. In saline at this temperature a mild reaction was obtained at the end of three weeks, but the virus was completely inactive after a period of four weeks. These were all tested in dilutions of 1–4.

The vaccine suspended in normal inactivated rabbit

[1] Aided by grants from the Divisions of International Health and of Medical Sciences, Rockefeller Foundation.

[2] H. Otten, *Zeits. f. Hyg.*, 107: 677, 1927.

[3] T. M. Rivers and S. M. Ward, *Jour. Exp. Med.*, 62: 549, 1935.

[4] W. Lloyd and A. F. Mahaffy, *Proc. Soc. Exp. Biol. and Med.*, 33, 154, 1935.

[5] A. B. Green, *Jour. Hyg.*, 8: 536, 1908.

[6] E. W. Goodpasture and G. J. Buddingh, *Am. Jour. Hyg.*, 21: 319, 1935.

serum at the end of four weeks at 37° C. produced a strong reaction in dilutions of 1–100 on the scarified rabbit skin and at the end of five weeks it was still moderately active in the same dilution.

When suspended in serum and desiccated *in vacuo* in the frozen state and stored in sealed ampoules at 37° C., only a slight diminution in the titratable potency of the virus takes place over a period of four weeks.

These experiments indicate that normal inactivated rabbit serum, as a suspensoid, serves to maintain the viability of vaccinia virus at temperatures as high as 37° C. over much longer periods of time than do 50 per cent. glycerine or various other substances which we have used. Such a method of preserving the activity of vaccinia is of great practical importance, especially in view of the use of vaccine virus in tropical climates where transportation under unfavorable conditions is necessitated.

These investigations are being carried on further with the purpose of adapting this method to preservation of vaccine for human prophylaxis. The protective action of sera from sources other than the rabbit is also being investigated. The various details of the method will be published at a later date.

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FURTHER OBSERVATIONS ON FACTORS FROM NORMAL TISSUES INFLUENCING THE GROWTH OF TRANSPLANTED CANCER[1]

THE presence of an inhibiting factor associated with the transmitting agents of fowl tumor has been demonstrated in the extracts and filtrates of these neoplasms. When properly concentrated it has the property of neutralizing the tumor agent and furthermore will retard the growth of a transplantable mouse sarcoma (Murphy and Sturm). On the basis of these observations, Murphy suggests as a working hypothesis that growth and differentiation of cells is controlled by a balanced system comprising a stimulating force and a retarding force, a suggestion which is in line with other known physiological processes. On the basis of this hypothesis a break in the balanced mechanism leading to uncontrolled growth might take place either by reinforcement of the stimulating force or by suppression of the inhibiting force. Murphy's and Sturm's demonstration that a substance extracted from placenta and embryo skin definitely retards the growth of both transplanted and natural cancers of mice seemed to offer partial support to the general hypothesis. The present report gives further confirmation in showing two factors in active normal tissues.

The tumor utilized was Bashford Mouse Carcinoma No. 63, and the source of the factors tested was the prelactating mammary gland of 6 rabbits representing different stages of pregnancy and of one cow at about the fourth month of pregnancy. The fresh tissues were first desiccated *in vacuo* in a freezing box, and the finely ground powder was used in preparation of the extracts. The details of the experiments will be published later, but it should be stated here that they were so designed and controlled as to give each test solution equal representation in the variables associated with tumor growth energy and host susceptibility. The essential point of the procedure was the inoculation of pairs of grafts into each mouse in a series after one graft had been exposed to a test solution concurrently with the exposure of the other graft of the pair to a control solution (Tyrode's solution). For each experiment an additional group of animals was inoculated with two grafts each from the control solution. These data made it possible to analyze the results on the basis of the system developed by Karl Pearson, in particular the Chi square test for "goodness of fit" of frequency distribution.

Significant inhibiting action on the tumor growth was shown in 60 or more cases by the aqueous extracts at pH 7.0–7.3 of the desiccated mammary gland of the six different rabbits. There was no significant difference in the intensity of inhibition between the different rabbits representing a range from 12 to 28 days of pregnancy. The mammary tissue from pregnant mice and from a cow gave equally definite results. The fact that similar extracts of placentas from several of the above animals failed to show any inhibiting action at pH 7.0–7.3[2] indicates that the results with

TABLE I

TUMOR INHIBITION AND TUMOR STIMULATION AS SHOWN BY DIFFERENT FRACTIONS OF RABBIT MAMMARY GLAND

Fraction	Concentration Per cent.	Frequency of: Inhibition	No effect	Stimulation	N	χ^2	n′	P*
Unfractionated ..	2.6	16	22	4	42	4.06	3	.13
Ether-insoluble ..	1.3	25	16	6	47	6.03	3	.049
Ether-soluble ...	2.0	3	16	25	44	11.05	3	.0040
Double controls .	0.95	12	19	11	42	Standard of reference		
Theoretical normal distribution ...		11	22	11	44	0.21	3	.90

* P = "Probability that deviations as great or greater would occur by chance."

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

[2] Definite inhibition was noted when the placenta extracts were used at pH 5.8–6.3. The meaning of this observation is not clear, but it can not be ascribed to the pH, as this is within the range of non-injury to tumor cells.