and spacing of doses of the drug to ensure the optimum effect might be more easily settled in this way.

CONCLUSIONS.

1. Rabbits injected intravenously with heat-killed bovine tubercle bacilli were protected by repeated injections of sanocrysin from the lethal action of an intravenous injection of old tuberculin about a month later, while control animals that had received no sanocrysin succumbed. In conformity with this the controls showed extensive histological tuberculous lesions in the liver, but those rabbits treated with sanocrysin had slight, or at any rate, less extensive ones corresponding with the considerably smaller degree of allergy developed.

2. The experiments prove that *sanocrysin exerts an inhibitory effect on tuberculous lesions, apart from any bactericidal action it may have on the living tubercle bacillus.*

3. It is pointed out that such experiments form a ready means for testing the possible efficacy of new therapeutic drugs in tuberculosis and the best methods of administering them.

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IMMUNIZATION WITH KILLED HERPES VIRUS.

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UNTIL comparatively recently it was generally accepted that filterable viruses gave rise to little or no immune response unless they were employed in the living state; rabies virus provides an exception to this statement. The demonstration, however, that the viruses of foot and mouth disease (Vallée, Carré, Rinjard, 1925; Bedson, Maitland and Burbury, 1927), distemper (Laidlaw and Dunkin, 1928) and yellow fever (Hindle, 1928) can be inactivated by low concentrations of formalin, yet retain their antigenic power to a considerable degree has materially altered the outlook, and at the present time it is admitted that virus rendered inactive by such means is still capable of producing an appreciable degree of immunity. But the question as to whether dead virus can immunize is still in dispute, many workers holding that these inactive vaccines contain a residuum of living virus, and that their efficacy is

attributable to this living residue. The point is one of considerable interest and of practical importance, for if inactive vaccines of filterable viruses are to be used in prophylaxis—and there is reason to think they will be of service, particularly if the problem of cultivation of filterable viruses on artificial media is solved—their usefulness would be curtailed considerably if their efficacy depended on a variable quantity of living virus. It was in order to find out whether herpes virus inactivated by formalin possessed any immunizing power, and if so whether this was due to dead virus, that the following experiments with herpes virus were carried out.

EXPERIMENTAL.

Herpes virus adapted to the guinea-pig's skin was used in this work. Primary inoculation from human herpes vesicles was made in the skin of the guinea-pig's foot, and the strain maintained by weekly passage in the same tissue. Two strains were used, both of which were well adapted to the guinea-pig, and which maintained an almost constant titre. A skin strain of herpes virus was chosen in preference to one adapted to the brain or testicle, because the availability of skin titration made possible the quantitative estimation of immunity with a smaller expenditure of animals.

Choice of Experimental Animal.

The guinea-pig was selected in preference to the rabbit because it is more readily immunized ; to this extent the experiments were biased in favour of the vaccines. There is a tendency to regard the guinea-pig as relatively insusceptible to herpes virus, but nothing could be more incorrect. During the last few years the author has isolated a considerable number of herpes strains by direct inoculation from man to guinea-pigs, and although a few of those strains lapsed by the fourth of fifth passage, the majority were readily maintained in the guinea-pig in an active state. The guinea-pig, however, is less susceptible to herpes virus than the rabbit.

Criterion of Death of Virus.

It is quite certain that simple inoculation is insufficient to determine whether all the virus in a given suspension is dead ; a negative result would not rule out a sub-infecting dose of living virus. It has been found possible to reactivate sub-infective doses of herpes virus by serial passage in the plantar skin of the guinea-pig, so this procedure was made use of to determine whether any living virus remained in the formalized vaccine. If serial passage through three guinea-pigs failed to reveal living virus, the vaccine was considered to be composed entirely of dead virus.

Immunization with Formalized Virus.

Preparation of Vaccine (*B.*893/6).

Herpetic guinea-pig pads weighing 2·4 gm. were ground with sand and 4·8 c.c. 9 per cent. salt solution added. After standing overnight in the refrigerator, sterile distilled water was added to bring the volume to 48·0 c.c., the

whole thoroughly mixed, centrifuged lightly, and the supernatant pipetted off. The suspension was then titrated for virus, the 10^{-3} dilution proving infective, while 10^{-4} was negative. Formalin (40 per cent. formaldehyde) was added in a concentration of 0·2 per cent., and the suspension placed in the refrigerator. After ten days in the refrigerator the vaccine was tested for the presence of active virus by serial passage through three guinea-pigs. This occupied twelve days and proved negative.

Efficacy of Vaccine.

Immunization of guinea-pigs was commenced when the vaccine B.893/6 was 22 days old. The details of immunization and the resulting immunity are to be found in Table I, and require no further description. The neutralizing power of the serum of the immunized guinea-pigs was tested seven days after the last dose of vaccine ; the details of these estimations are given in Table II. The experiment shows that this vaccine, which had been formalized for twenty-two days prior to use, and contained less active virus than could be reactivated by serial passage through three guinea-pigs, was capable of exciting a high degree of immunity in guinea-pigs.

TABLE I.—*Immunization with Formalized Herpes Virus.*

Guinea-pig.	Immunization.	Weight in gm.		Test of immunity. Intradermal inoculation of falling concentrations of virus in 0·2 c.c. volume. Test made 10 days after last dose of vaccine.			
		Before.	After.	10^{-1}.	10^{-2}.	10^{-3}.	10^{-4}.
B.914	Three doses of 1·0 c.c. of vaccine B.893/6 given subcutaneously at intervals of 7 days	495	535	A	—	—	—
B.915		580	720	A	—	—	—
B.916		538	580	A	—	—	—
B.917		500	560	A	—	—	—
B.936	None	..	..	++	+	±	—

A. = Transitory allergic reaction. ++ to ± = degrees of reaction to virus.

TABLE II.—*Neutralizing Power of Serum of Guinea-pigs Immunized with Formalized Herpes Virus.*

Serum.	Final concentration of serum.			
	1 in 2.	1 in 10.	1 in 25.	1 in 50.
B.914	—	∓	+	+
B.915	—	—	—	--
B.916	—	—	—	+
B.917	—	—	—	+
Normal control	++	++	++	..

Equal parts of herpes virus diluted 1 in 20 in M/50 phosphate pH 7·6 and serum dilutions in phosphate. Allowed to stand for 1 hour at room temperature. Intradermal inoculation of 0·2 c.c. quantities of the various mixtures. Titre of virus used in test, 1 in 1000.

Immunization with Heat-killed Virus.

This experiment was carried out in order to see whether herpes virus killed by heat was capable of producing an immunity comparable with that given by formalized virus.

Preparation of Vaccine (*B.*982).

Herpetic guinea-pig pads weighing 0·8 gm. were ground with sterile sand, and 1·6 c.c. 9 per cent. salt solution added. After standing overnight in the refrigerator sterile distilled water was added to bring the volume up to 16·0 c.c., the whole thoroughly mixed, lightly centrifuged, and the supernatant pipetted off. The virus titre of this suspension was 10^{-3}. It was inactivated by heating for 45 minutes at 60° C. The presence of active virus was tested for in the way described with a negative result.

*Efficacy of Vaccine B.*982.

The findings in this experiment are given in detail in Table III, and leave no doubt that herpes virus inactivated by heat has little or no immunizing power.

TABLE III.—*Immunization with Herpes Virus Killed by Heat* (60° *C. for* 45 *Minutes*).

Guinea-pig.	Immunization.	Weight in gm. Before.	Weight in gm. After.	Test of immunity. Intradermal inoculation of falling concentrations of virus in 0·2 c.c. volume. Test made 12 days after last dose of vaccine. 10^{-1}.	10^{-2}.	10^{-3}.	10^{-4}.
B.987	Three doses of 1·0 c.c. of vaccine B.982 given subcutaneously at intervals of 5 days	440	410	++	+	±	∓
B.988		440	440	+	±	∓	−
B.989		560	490	+	±	∓	−
B.990	None	..	..	++	+	±	−

Immunizing Power of Formalized Normal Guinea-pig-Pad Tissue.

The possibility existed that the immunity produced by the formalized vaccine was due to the formalized guinea-pig tissue, and not to the virus which it contained. The preparation of this control vaccine was similar to that of vaccine B.893/6, with the exception that normal guinea-pig pads replaced herpetic ones. The negative results obtained with this control vaccine given in Table IV show that the efficacy of vaccine B.893/6 must have depended on the virus present in it.

TABLE IV.—*Immunization with a Formalized Suspension of Normal Guinea-pig-" Pad " Tissue.*

Guinea-pig.	Immunization.	Weight in gm. Before.	Weight in gm. After.	Test of immunity. Intradermal inoculation of falling concentrations of virus in 0·2 c.c. volume. Test made 11 days after last dose of vaccine. 10^{-1}.	10^{-2}.	10^{-3}.	10^{-4}.
B.979	Three doses of 0·1 c.c. of vaccine given subcutaneously at intervals of 5 days	420	400	++	+	±	−
B.980		420	430	++	+	±	−
B.1003	None	..	..	++	+	±	−

Comparison of Immunity Produced by Equal Quantities of Living and Formalized Herpes Virus.

This was compared by giving three guinea-pigs one inoculation each of the formalized vaccine B.893/6, and three other guinea-pigs an equivalent amount of living virus. The neutralizing power of the serum of these animals was tested before and 8 days after immunization. The results of this experiment are given in Tables V and VI. As one would expect, the living virus gave the better immunity, but the one dose of formalized vaccine gave quite a surprising degree of immunity—in two out of the three animals practically as much as that evoked by the living vaccine. This experiment of itself rather negatives the viewt hat the activity of the formalized vaccine was due to a residue of living virus. This point, however, is dealt with specifically in the next experiment.

TABLE V.—*Comparison of Immunity Produced by Equal Quantities of Living and Formalized Herpes Virus.*

Guinea-pig.	Immunization.	Weight in gm. Before.	Weight in gm. After.	Test of immunity. Intradermal inoculation of falling concentrations of virus in 0·2 c.c. volume. Test made 7 days after last dose of vaccine. 10^{-1}	10^{-2}.	10^{-3}.	10^{-4}.
B.952	One dose of 1·0 c.c. living herpes virus subcutaneously. (Contained the same amount of virus as the vaccine before formalization)	440	385	±	∓	−	−
B.953		445	420	+	±	−	−
B.954		425	410	+	±	?	−
B.955	One dose of 1·0 c.c. of formalized vaccine B.893/6 given subcutaneously.	440	380	++	+	+	±
B.956		420	380	±	∓	−	−
B.957		380	360	+	∓	−	−
B.972	None	..	..	++	+	±	∓

TABLE VI.—*Neutralizing Power of Serum of Guinea-pigs Immunized with Single Doses of Living and Formalized Herpes Virus.*

Serum.	Immunization.	Final concentration of serum. Before Immunization. 1 in 2.	After immunization. 1 in 2.	1 in 10.	1 in 20.	1 in 50.
B.952	One dose of living virus	+	−	−	−	±
B.953		+	−	−	∓	±
B.954		+	−	−	±	+
B.955	One dose of formalized virus	+	−	?	±	+
B.956		+	−	±	+	+
B.957		+	−	−	+	+

Equal parts of herpes virus diluted 1 in 100 (titre 1 in 1000), and serum dilutions in M/50 phosphate pH 7·6. Inoculated intradermally in 0·2 c.c. quantities after 1 hour at room temperature.

Immunizing Power of Minimal Doses of Living Herpes Virus.

Preparation of Vaccine (*R.*25.)

A virus suspension (guinea-pig pad) whose titre was 10^{-3} was diluted a thousandfold with a suspension of normal guinea-pig-pad tissue. Since a

dilution of 10^{-4} of this virus suspension produced no lesion on primary inoculation, but could be shown to contain active virus by serial passage, this vaccine contained ten times more virus per unit volume than could be demonstrated by this procedure, and must therefore have contained considerably more than the hypothetical quantity of living virus remaining in the formalized vaccine.

Efficacy of Vaccine.

Four guinea-pigs were immunized, the dosage and route of inoculation being the same as in the case of the formalized vaccine. The results are given in Table VII, and leave no doubt that such small doses of living virus are devoid of immunizing power.

TABLE VII.—*Immunization with Minimal Doses of Living Herpes Virus.*

Guinea-pig.	Immunization.	Weight in gm. Before.	Weight in gm. After.	Test of immunity. Falling dilutions of herpes virus inoculated by scarification. Test made 9 days after last dose. 10^{-1}.	10^{-2}.	10^{-3}.	10^{-4}.
B.1136	Three inoculations of 1·0 c.c. subcutaneously of vaccine R.25. Each dose of vaccine contained living virus diluted 1 in 1000 (titre of virus, 1 in 1000)	700	745	++	+	?	—
B.1137		520	590	++	+	?	—
B.1138		600	615	++	+	—	—
B.1139		490	500	+	±	—	—
B.1141	None	..	..	++	+	—	—

Effect of Heating at 100° C. on Formalized Virus.

It might be argued that treatment of herpes virus with low concentrations of formalin attenuated the virus but did not kill it. Such an attenuated virus might be capable of multiplication in the animal's tissues and yet not produce an herpetic lesion. A virus suspension B.1088/9 was prepared in the way described ; its titre was 1 in 1000. This was divided into two portions, one of which was steamed for twenty minutes, whilst to the other formalin was added to a concentration of 0·1 per cent. Both were stored in the refrigerator. After 7 days' formalization this second portion was divided in two, one of which was steamed for twenty minutes. These three vaccines, steamed, formalized and steamed and formalized, were then used to immunize 6 guinea-pigs, 2 with each vaccine. The resultant immunity is shown in Table VIII, and the neutralizing power of the serum of these animals 7 days after the last inoculation is given in Table IX. A duplicate experiment gave a very similar result, and the fact that herpes virus which has been formalized and then steamed retains some antigenic power, though not as much as after formalization alone, whereas the steamed virus is devoid of immunizing power, suggests that formalin does not act by attenuating the virus, but that it kills it without destroying the majority of its antigenic power.

TABLE VIII.—*Immunization with Herpes Virus Inactivated by:* (A) *Formalin,* (B) *Formalin followed by Steaming,* (C) *Steaming.*

Guinea-pig.	Immunization.	Weight in gm. Before.	Weight in gm. After.	Test of immunity. Intradermal inoculation of falling concentrations of virus in 0·2 c.c. volume 10 days after last dose. 10^{-1}.	10^{-2}.	10^{-3}.	10^{-4}
B.1094	Received 3 doses of 1·0 c.c. steamed virus subcutaneously at 5 days' interval	400	450	+	+	±	∓
B.1095		390	420	+	±	∓	−
B.1096	Received 3 doses of 1·0 c.c. formalized and steamed virus subcutaneously at 5 days' interval.	435	370	+	±	−	−
B.1097		350	370	±	∓	−	−
B.1098	Received 3 doses of 1·0 c.c. formalized virus subcutaneously at 5 days' interval	380	400	−	−	−	−
B.1099		380	370	−	−	−	−
B.1107	None	..	..	++	+	±	−

TABLE IX.—*Neutralizing Power of Serum of Guinea-pigs B.*1093/1099.

Serum.	Immunization.	Final concentrations of serum. 1 in 2.	1 in 10.	1 in 20.
B.1094	Steamed virus	±	+	+
B.1095		±	+	++
B.1096	Formalized and steamed virus	−	∓	±
B.1097		−	∓	±
B.1098	Formalized virus	−	−	−
B.1099		−	−	−

CONCLUSIONS.

1. Herpes virus treated with low concentrations of formalin is capable of producing a high degree of immunity in guinea-pigs.
2. This immunity is produced by dead virus.
3. Herpes virus killed by heating at 60° C. or 100° C. is devoid of immunizing power.
4. If the virus is treated with formalin before heating it at 100° C. it is still capable of producing some immunity.

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