

THE KEEPING QUALITIES OF VACCINE LYMPH

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(With 8 Charts in the Text)

THE object of this investigation is to gain more precise information concerning the retention of potency of vaccine lymph at those temperatures which are likely to be encountered by lymph after issue from the laboratory and before use by public vaccinators. These temperatures are—broadly speaking—likely to be those of the ice-box (say 5–7° C.) and average room temperature (say 15–16° C.).

Many indirect observations on this question have been recorded previously—Blaxall & Fremlin (1906–7), Fremlin (1902–3), (Green 1902–3)—but as the potency test used was the reaction of calves to the application of undiluted lymph they are not comparable with the results of the present inquiry where compliance with the requirements of the British Therapeutic Substances Regulations, 1931—the use of a thousandfold dilution of lymph for testing—has been considered necessary. In general, these former results show that when undiluted lymph was tested on calves a diminution of potency after 2–3 months at +10° C. was noted and that this became more noticeable at room temperature. Blaxall (1902–3) states that the duration of potency of lymph in emulsion with glycerol diminishes with increase of the temperature at which it is stored: he gives no details however. Green ((1908), using the same type of test, reported some observations on the potency of glycerinated calf lymph after various periods of storage at 10–15, 4 and –4° C., etc. He found glycerinated lymph was infective up to 203 days when stored at 10–15° C. and up to 1 year when stored at 4° C. Noguchi (1918), however, gives less optimistic figures.

The main cause of deterioration in potency of glycerinated lymph after issue is exposure to heat above 0° C. and because the potency at the time of issue varies equal exposure even to small rises in temperature may affect different samples of lymph very differently, rendering one comparatively feeble in a few days while another may be effective for a much longer period. This is a cause of irregular results in the vaccination of children and is unsatisfactory to the public vaccinator and those interested in lymph production.

The method employed in this investigation has been to divide a sample of vaccine lymph into three portions of approximately 5 c.c. each and to store these at –10, +5 (ice-box) and +15° C. At intervals sufficient material was removed from each sample to enable the necessary dilutions to be made and

their potency to be determined by a method complying with the British Therapeutic Substances Regulations, 1931. In the course of time it was feared that the frequent removal of the samples from storage at -10 and $+5^{\circ}$ C. with the necessary exposure to room temperature in order to carry out dilutions might be having some detrimental effect; subsequently therefore a repetition of the experiment was made using samples stored in capillary tubes so that only what was actually required for the testing needed to be removed from the place of storage.

There are several methods of performing a potency test so as to conform to the requirements of the Therapeutic Substances Regulations. The Calmette-Guerin or scarification test (*see* Blaxall, 1930) has been employed for the three lymphs in this investigation; it has been modified by reducing the area of inoculation and dose to one-tenth. Thus the test is actually the application of 0.1 c.c. of the unfiltered thousandfold dilution of lymph in saline to an area of lightly scarified skin measuring 14.4 sq. cm. on the shaved side of a rabbit.

In addition an intradermal method of potency testing, using falling dilutions, has been employed for two of the lymphs as also has the application of a higher dilution (1 in 10,000) of lymph when using the scarification method. The results from these additional tests have not been recorded here because of their irregularity. In the main they confirm the observations made from the scarification tests of 1 in 1000 dilution material and recorded below. Such irregularities are due to disabilities inherent in the technique of intradermal testing, especially of higher dilutions of unfiltered material where gross flocculi of tissue may pass over and to varying susceptibilities of test animals, particularly brought out, it is thought, by the employment of high dilutions.

The materials used in the experiments were samples of the ordinary glycerinated vaccine lymph produced at the Government Lymph Establishment; each consists of the vesicular material or "pulp" collected from calves vaccinated with vaccinia virus 5 days previously and then diluted and triturated with four times its weight of 50% glycerol (by weight) with 0.1% clove oil added as a mild antiseptic. This lymph when diluted 1 in 1000 with normal saline for the potency test thus actually represents a dilution of 1 in 5000 of pulp or vesicular material.

The result of the application of the 1 in 1000 dilution of lymph to a rabbit when seen after 5 days may vary from a few isolated vesicles, which can be easily counted, to a confluent patch of vesicles. The lymph is graded in terms of the vesiculation produced; when these reach 20-30 vesicles over the area they tend to run together and become semi-confluent or confluent. In practice, lymphs giving semi-confluent results in the 1 in 1000 dilution on a susceptible animal are regarded as just satisfying the Therapeutic Substances Regulations, the purpose of which is to ensure the activity of the lymph at the time of issue and retention of that activity for at least 7 days after issue.

Three lymphs were used in this investigation, C. 1010, C. 1297 and C. 1262. Lymph C. 1010 was a lymph of high potency and did in fact become a reference

lymph, that is, one which is used to compare and assess the value of other lymphs when carrying out potency tests. It has given a case and insertion success of 97% on 2368 children in the hands of public vaccinators. The results from this lymph are given in Chart I. Lymph C. 1297 was a lymph collected only for this investigation; its results are given in Chart II. Lymph C. 1262 was a portion of a lymph collected for general issue which proved quite recently of insufficient potency for that purpose, confirming thereby what this investigation had revealed previously. Other portions of the pulp from which this lymph was derived were also examined when made up with the same volume of 50% glycerol (*a*) without any antiseptic, (*b*) with $\frac{1}{2}$ % phenol and (*c*) with 1% phenol. This was done in order to discover if there was any harm or advantage from the presence or absence of any of these antiseptics: Noguchi (1918) has reported on the advantages of phenol. The tests with these four samples of lymph were the initial ones made: experience of certain precautions necessary was lacking and the results are therefore probably less reliable than those obtained in later tests. The results from this lymph are graphically recorded in Charts III *a*, *b* and *c*. Charts IV *a*, *b* and *c* give the combined results of the three lymphs at each test temperature in order to bring out the difference between the lymphs.

For convenience the results have been given in graphical form; the terms in which a lymph is graded appear as ordinates and 5-day intervals as abscissae. The graphs are compiled from those rabbits giving the better readings when more than one animal was used for the test. Many photographic records were made of the scarification test results at different intervals but they have not been reproduced here.

The results obtained may now be briefly summarized. It should be noted that the result of the potency test of a sample stored at -10° C. constitutes a control to the results obtained at other temperature levels each having been tested on the same rabbit on any one particular day.

Chart I gives the results of a lymph (C. 1010) which proved to be highly effective on children. It will be seen that at room temperature it rapidly loses potency and probably would be only just effective by the 26th day. When stored at ice-box temperature it would probably remain effective up to the 75th day.

Chart II gives the results of a lymph (C. 1297) whose qualities as regards vaccination of children are unknown. It probably fell below effective level after 35 days' storage at room temperature; it was probably at effective level at the 62nd day of storage in the ice-box but fell below that level between the 62nd and 109th day. The similarity of this lymph to lymph C. 1010 as regards retention of potency suggests that it would be highly effective on children.

Charts III *a*, *b* and *c* give the results of a lymph (C. 1262) which has not been issued for human use. It will be seen that this lymph fell below effective level of potency by exposure for 9–26 days in an ice-box (Chart III *b*) or 6–15 days when kept at average room temperature (15° C.), Chart III *a*, whatever

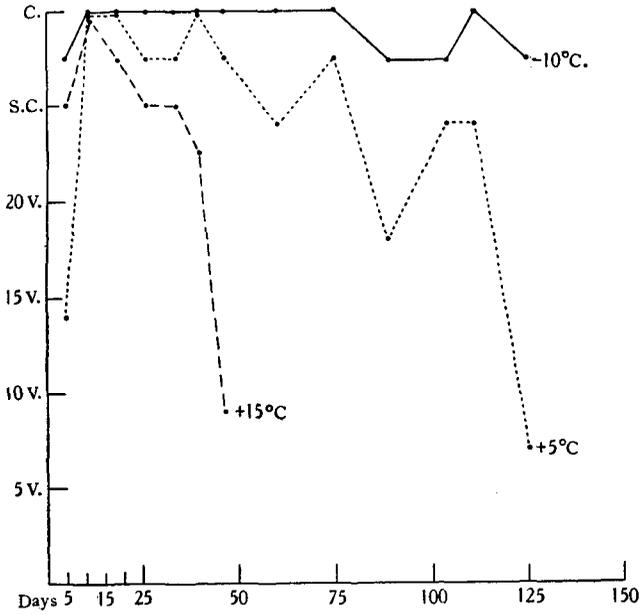


Chart I. Calf lymph C. 1010. Stored in capillaries. Scarification test with 1 in 1000 dilution. C = confluent; S.C. = semi-confluent; V. = vesicles.

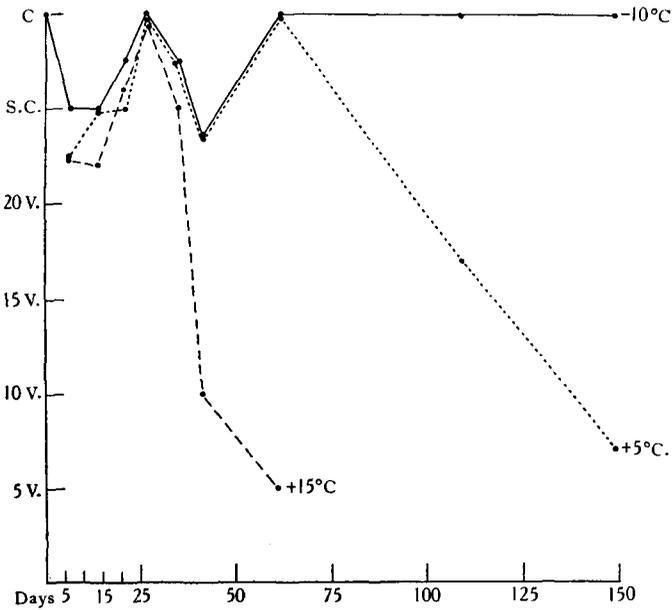


Chart II. Calf lymph C. 1297. Stored in bulk. Scarification test with 1 in 1000 dilution.

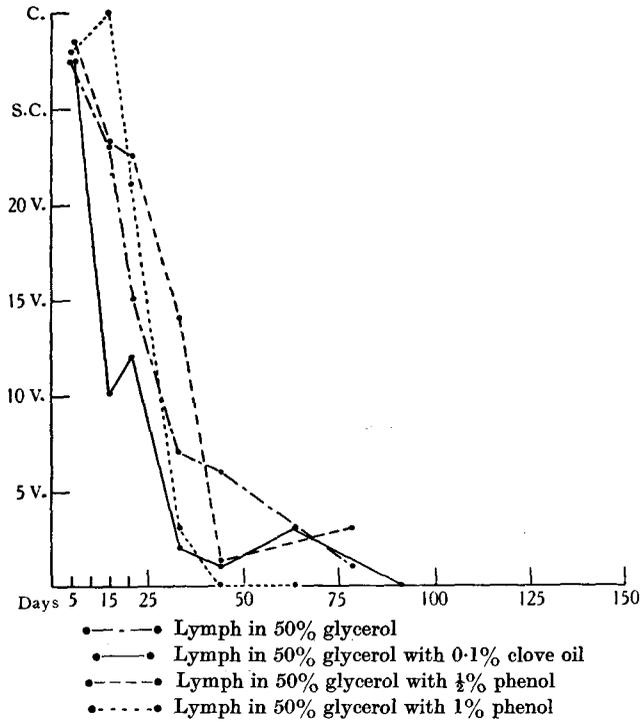


Chart IIIa. Calf lymph C. 1262. Stored at +15° C. Scarification test with 1 in 1000 dilution.

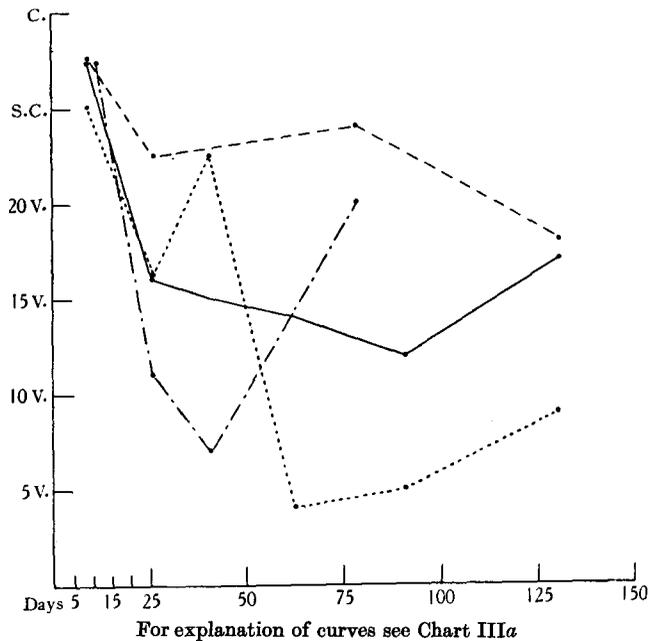
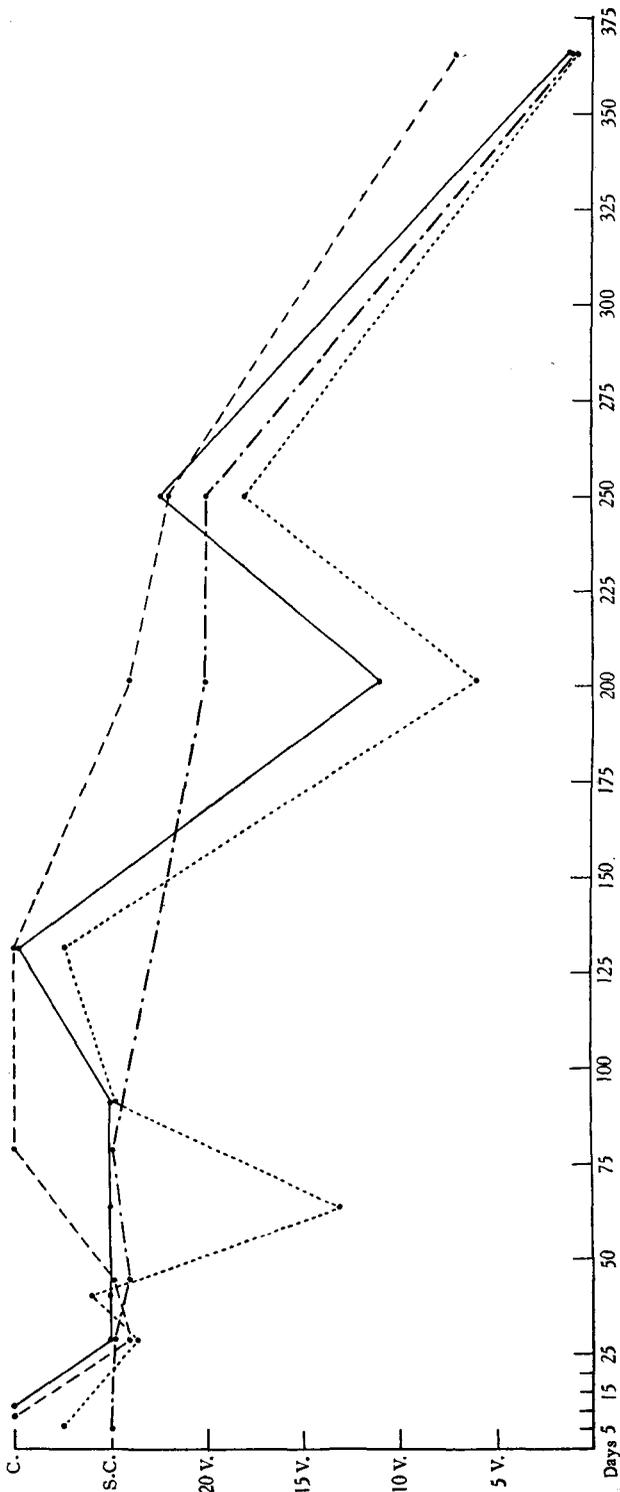
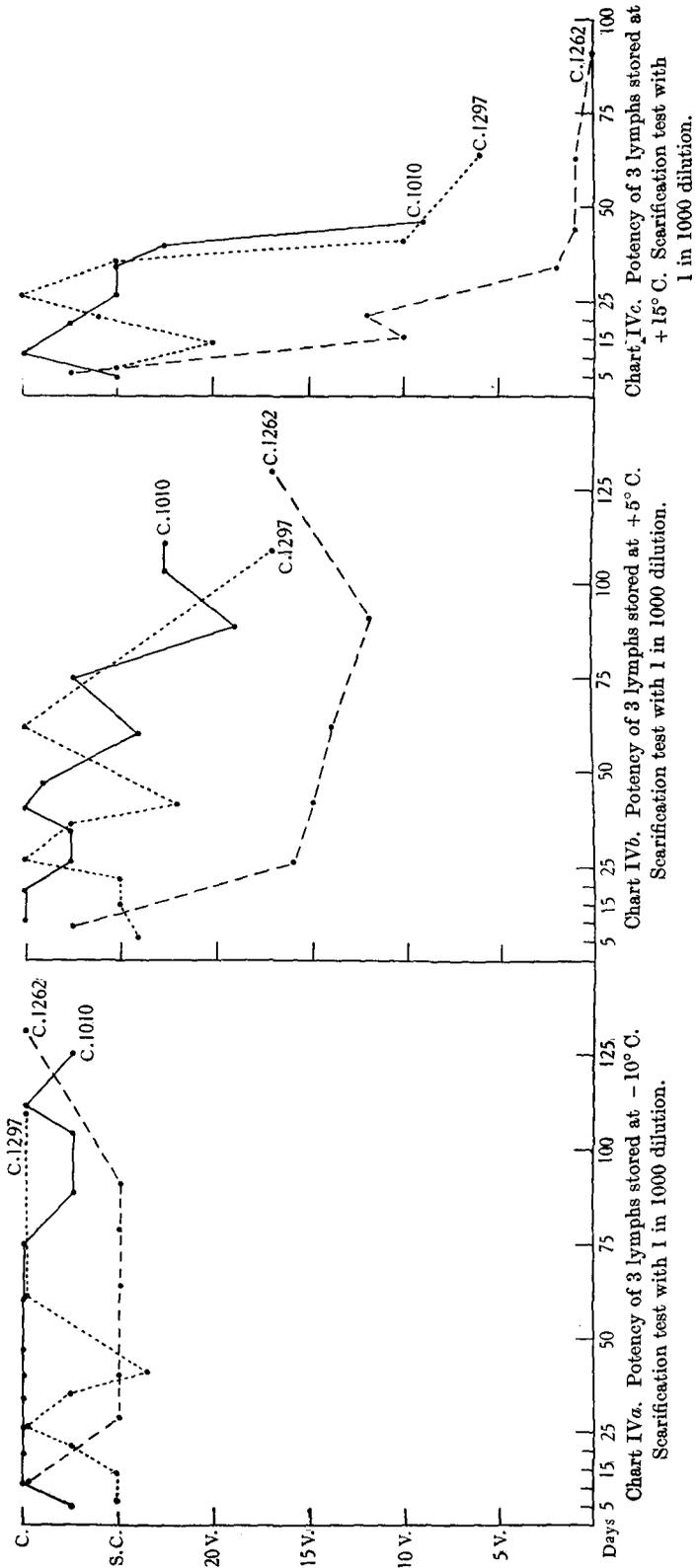


Chart IIIb. Calf lymph C. 1262. Stored at +5° C. Scarification test with 1 in 1000 dilution.



For explanation of curves see Chart IIIa

Chart IIIc. Calf lymph C. 1262. Stored at - 10° C. Scarification test with 1 in 1000 dilution.



medium the pulp had been mixed with. The general trend of the three graphs (Charts III*a*, *b* and *c*) suggest a better retention of potency in the sample containing $\frac{1}{2}$ % phenol.

There is a difference in the results from the lymphs recorded in Charts I and II and from that sample of lymph made up in the usual manner, with glycerol and clove oil, recorded in Charts III*a*, *b* and *c*. To bring this out Charts IV*a*, *b* and *c* have been constructed. It will be seen that during 125 days' storage at -10° C. (Chart IV*a*) there is no appreciable difference in the apparent potencies of the lymphs, but when these lymphs are stored at room temperature (Chart IV*c*) or ice-box temperature (Chart IV*b*) a marked weakness in retention of potency is revealed in one of the lymphs (C. 1262) indicating a degree of initial potency (at the stage of removal from cold storage at -10° C.) having insufficient reserve to withstand storage at higher temperatures. It was considered possible that re-examination of the potency of these three lymphs stored at -10° C. at a considerably later date than 125 days might bring out not only the initial comparative weakness of lymph C. 1262 but possibly also any difference between the other two. Recently, since these experiments have been completed, that portion of lymph C. 1262 which had been kept continuously in storage for 27 months at -10° C. with a view to issue for human vaccination has been found, in the routine potency tests carried out before issue, to be of insufficient potency. The examination, however, of the potency of samples of this lymph stored at room (15° C.) or ice-box temperature (5° C.) did reveal its poor potency at a much earlier date.

It should be stated that the duration of effective potency revealed in these experiments are the extreme limits of retention of such potency. A guide to the issue of a lymph of satisfactorily high efficiency would be the evidence of retention of high potency (using the standard test) in a sample that had been subjected to storage at $+15^{\circ}$ C. or other temperatures to which the lymph is likely to be subjected for the period of guarantee. This we term the reserve potency of our lymph.

Conclusions

1. Vaccine lymph (derived from the calf and glycerinated) varies in initial and therefore in reserve potency. The practice of carrying out the standard potency test on lymph while in storage at -10° C. may not reveal for a considerable time any weakness in a fresh lymph. This weakness may, however, be revealed by carrying out the standard test after the lymph has been exposed to temperatures above 0° C., e.g. ice-box temperature ($5-7^{\circ}$ C.) and room temperature ($15-16^{\circ}$ C.).

2. A lymph of proved high potency on children has in these experiments retained sufficient effective potency for 9-10 weeks of exposure to the temperature of an ice-box, or 4 weeks of exposure to the average temperature of a room. Another lymph which behaved similarly may be anticipated to prove of equal value in human vaccination.

3. A lymph of less initial potency—unless grossly weak—may appear for a considerable time to be fully potent by the standard test applied during storage at -10° C. Such a lymph has been rendered insufficiently potent by exposure for 9 days to the temperature of an ice-box and by exposure for 6 days to average room temperature.

4. A lymph made up in 50% glycerol containing $\frac{1}{2}$ % phenol has retained its potency rather better than lymph made up in the normal manner when exposed to temperatures of -10 , $5-7$ and 15° C.

5. A test of reserve potency in a lymph is suggested as a method of assuring the efficiency of a lymph at the time of issue and for the period of guarantee provided the conditions of storage are adhered to.

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In his room in the Old Laboratory in 1901