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# An improved smallpox vaccine

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## INTRODUCTION

It is generally agreed that smallpox vaccines should, if possible, be distributed in the form of a lyophilized preparation. Only in this way can a standardized vaccine of proper potency at the time of use be made available throughout the world. Collier's (1955) method of preparing a partly purified dried vaccine has met with considerable success in the field (Frederiksen, Torres Muñoz & Jaurigui Molina, 1959) and there seems little doubt that a vaccine of this kind, or one derived from mammalian tissue cultures, will eventually be adopted for general use. There are, however, serious obstacles to be overcome before this can be achieved. It is difficult to prepare calf or sheep skin pulp of high viral potency and low bacterial content under the conditions usually present in tropical and subtropical countries where the need for universal vaccination is most urgent. In temperate climates skin pulp of high quality can be more easily prepared but production costs are considerably higher, particularly in the case of a lyophilized vaccine which demands expensive equipment and skilled operators. For these reasons, it is likely that there will continue to be a need for a highly potent but inexpensive liquid vaccine which is more stable than the presently available glycerinated lymph. The preparation of such a vaccine is here described. It is suitable for immediate conversion to a lyophilized product if so desired.

The use of glycerol as a preservative in smallpox vaccine dates back at least 100 years (Copeman, 1898; Collier, 1954). Glycerol is a good dispersing agent, it possesses valuable bacteriostatic properties and it has the necessary viscosity for this purpose. Until now these favourable properties have outweighed its disadvantages. In the presence of 50% glycerol and at a holding temperature of 37°C. bacterial growth is completely inhibited whereas the virus will retain an acceptable potency for about 5 days. In the absence of glycerol the rate of inactivation is much slower (Green, 1908). Some experiments on the stability of partly purified vaccinia elementary bodies were reported by the present author many years ago (Amies, 1934). The results showed that the virus particles suspended in peptone broth retain their activity for considerable periods when held at room temperature and for several weeks at 37°C. It was suggested that purified elementary bodies suspended in a simple peptone medium might be of value for jennerian vaccination. Now, 28 years later, the same suggestion is made and some alternatives are advanced; this time, perhaps, with more success. A new suspending

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medium to take the place of glycerol-saline is now recommended. This contains 12·5 % of polyvinylpyrrolidone, a biologically inert synthetic polymer, and 1·0 % of peptone. Experiments demonstrating the favourable influence of these substances on the survival of vaccinia virus are presented. This same solution serves as an excellent menstruum for the production of a lyophilized vaccine.

## MATERIALS AND METHODS

### *The preparation of vaccinia elementary body suspensions*

The first essential in preparing a stable smallpox vaccine is the separation of the virus from the cell debris and fatty material which constitutes the bulk of the crude skin pulp. Bacteria must also be eliminated. These requirements can be met by a combination of methods designed to bring about mechanical disintegration, maintenance of dispersion, fractional centrifugation and bacterial disinfection. The process is that described by Collier (1955) with improvements developed from our own researches.

Two strains of vaccinia virus were used. One was a dermal lapine which has been maintained for many years at the Lister Institute, London, England: the other was the Connaught Laboratories vaccinia strain which is maintained exclusively by serial passage in calf skin. The procedure to be described gave equally good results with each strain. Rabbit skin pulp was harvested on the 3rd or 4th day; but with calves the highest yield was usually obtained by sacrificing the animal on the 6th day following inoculation. If brilliant green solution has been sprayed on the calf skin during the incubation period this must be very thoroughly removed by prolonged gentle washing of the skin prior to removal of the pulp. Purified virus elementary bodies are flocculated and inactivated by brilliant green in extremely high dilutions. There is no advantage to be gained by working with fresh pulp: it can be stored frozen at – 20° C. until it is convenient to commence the purification process. Disintegration and homogenization are effected by means of a Virtis '45' Homogenizer or similar apparatus. A Waring blender can also be used for this purpose provided that steps are taken to avoid aerosol formation. The model specially designed for bacteriological work is recommended. Of the two machines mentioned, the former gives a more finely dispersed homogenate and is much preferred. The suspending medum is 4 mM McIlvaine buffer of pH 7·0 containing 1·0 % Tween 80 and 0·5 % phenol. The subsequent steps will be more readily followed by giving a concrete example.

(1) Approximately 25 g. of calf skin pulp was placed in the 250 ml. Virtis homogenizing flask together with 75 ml. of suspending medium. The machine was then run at three-quarters maximum speed for 3 min.

(2) The homogenate was clarified by centrifugation at 1000*g* for 10 min. A swinging bucket type of centrifuge was employed.

(3) The supernate $S_1$ was removed and the deposit together with the fatty layer which had formed at the surface of the supernate was re-suspended in 75 ml. of dispersing medium. Homogenization and clarification were carried out as before. The supernate $S_2$ was added to $S_1$.

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(4) This process was repeated a third time.

(5) Pooled supernates, $S_1$, $S_2$, $S_3$, were again submitted to clarification at 1000*g*. All deposits were discarded.

(6) The clarified pooled supernate, volume approximately 225 ml., was centrifuged in an angle centrifuge (Servall SS 1) for 30 min. at 6780*g* (7500 r.p.m.)

(7) The deposit consisting mainly of virus was suspended in 10 ml. of McIlvaine buffer pH 7·0 containing 0·5 % phenol but no Tween 80. Very thorough dispersion was necessary: this was secured by repeatedly drawing the suspension into a syringe through a 24 gauge needle, and then forcibly expelling it. Frothing was avoided. The suspension was then clarified by centrifugation for 5 min. at 1000*g*.

(8) The supernate was removed and saved. The deposit was suspended in 10 ml. of buffer-phenol and processed as before. A third extraction followed.

(9) The 3 supernates were pooled and then submitted to a light centrifugation. The resulting 30 ml. of milk-like fluid was the final purified elementary body suspension. This had an infective potency of about $10^8$ pfu/ml. as determined by monkey kidney tissue culture assay.

(10) The concentrated virus suspension was held at 20–22° C. for 48 hr. During this period, bacterial sterilization was effected by the phenol.

(11) The concentrated virus suspension was finally diluted 1/10 with 4 mM McIlvaine buffer containing 13·9 % polyvinylpyrrolidone, 1·1 % Bacto peptone (Difco) and 0·39 % phenol. The final concentrations of these substances thus became PVP, 12·6 %; peptone 1·0 %; phenol 0·4 %. The phenol is omitted from this diluent if it is intended to lyophilize the product.

A considerable amount of virus is lost at each stage of this process of purification. Treatment of the residues by further homogenization and centrifugation will recover much of this but the extra work entailed is not justifiable on economic grounds. In spite of the mechanical losses, purification results in an *increase* in the amount of smallpox vaccine obtainable from a given quantity of skin pulp. In one experiment, for example, the total yield of pulp from one calf was divided into two equal parts. One half was converted into glycerinated vaccine by the conventional method while the other half was submitted to purification by the procedure outlined above. The results obtained were as follows:

(*a*) Conventional method. 175 g. of skin pulp yielded 1500 ml. of glycerinated vaccine at a potency of $1{\cdot}44 \pm 10^7$ pfu/ml.

(*b*) Purification method. 175 g. of skin pulp yielded 2000 ml. of purified vaccine at a potency of $2{\cdot}0 \pm 10^7$ pfu/ml.

The apparent gain in virus resulting from purification is attributed to the elimination of interfering substances present in the crude suspension. It is not possible at present to decide whether this phenomenon is due to viral auto-interference or to the influence of material, soluble or particulate, derived from the host cells.

To those who are only familiar with official potency tests which usually employ a rabbit skin scarification technique of titration it may be surprising to note the high potency figures shown above. There is clearly a need for an internationally acceptable standard method of testing the potency of smallpox vaccines by the sensitive and reproducible techniques now available.



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The procedure described above has been in use on an experimental scale for more than 2 years. Many alternative methods were tried during this period but none was wholly satisfactory. The object was to separate the virus from finely particulate cell constituents by bringing the latter into solution either by enzyme action or by means of solubilizing substances such as sodium benzoate, sodium salicylate, KCNS and deoxycholate. Treatment with fluorocarbons (Gessler, Bender & Parkinson, 1956) was found to be an excellent method of purification but the low yields obtained precluded its use for large-scale production.

## THE INFLUENCE OF CERTAIN PRESERVATIVES ON THE SURVIVAL OF VACCINIA VIRUS

Concentrated vaccinia virus suspensions, prepared as described above, were added to solutions of the substances to be tested. The resulting mixtures were dispensed in approximately 1·0 ml. quantities into sterile glass ampoules which were at once sealed with the blowpipe. The ampoules were then immersed in a water bath set up in an incubator room maintained at 37° C. At intervals of a few days sample ampoules were removed from the bath and the infective potency of the contents was determined by titration on monkey kidney cell monolayers. In experienced hands the results obtained by this method have an experimental error (standard deviation) of ± 17 %.

The following is a list of the substances tested:

| | Final concentration used (%) |
|---|---|
| Glycerol | 50 |
| Sorbitol | 35 and 50 |
| Gelatin | 1·0 |
| Methyl cellulose (400 centipoise) | 0·6 |
| Carboxymethylcellulose | 0·6 |
| Peptone (Bacto) | 1·0 and 5·0 |
| PVP | 5·0 and 12·5 |

## RESULTS

The protocols of a few representative experiments will be enough to show that the stability of the virus is much improved by purification. Of the preservatives tested a mixture of peptone and PVP yielded the best results.

*Experiment* 28 *February* 1961. A purified elementary body preparation derived from the lapine strain was held at 30° C. (85° F.) in 3 different suspending media: 1·0 % peptone in 4 mM buffer of pH 7·0; in buffer alone; and in 50 % glycerol-saline. The results of the sample assays are presented in Table 1 and Fig. 1. The beneficial effect of peptone in maintaining viability is clearly demonstrated.

*Experiment* 9 *June* 1961. In this experiment the holding temperature was 37° C. (98·4° F.) and the protective effects of 5 different suspending media were compared. The full results obtained are given in Table 2. Three of the survival curves are shown in Fig. 2. The mixture of 1·0 % peptone and 12·5 % PVP consistently showed the best protection.

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Table 1. *Survival of vaccinia virus (purified elementary bodies) in 3 different suspending media maintained at* 30° C.

Potency of sample is expressed in plaque-forming units (pfu) per ml. Titrations performed on monkey kidney cell monolayers. (*a*) 1·0 % peptone in 4mM phosphate buffer; (*b*) 4 mM phosphate buffer alone; (*c*) 50 % glycerol-saline.

| Period of storage (days) | Suspending medium (*a*) Peptone-buffer (pfu/ml.) | (*b*) Buffer alone (pfu/ml.) | (*c*) Glycerol-saline (pfu/ml.) |
|---|---|---|---|
| 0 | — | $5{\cdot}8 \times 10^6$ | — |
| 3 | $6 \times 10^6$ | $6{\cdot}7 \times 10^6$ | $1{\cdot}2 \times 10^6$ |
| 6 | $6 \times 10^6$ | $5{\cdot}2 \times 10^6$ | $6{\cdot}4 \times 10^5$ |
| 9 | $2{\cdot}9 \times 10^6$ | $1{\cdot}3 \times 10^6$ | $3{\cdot}2 \times 10^5$ |
| 13 | $1{\cdot}5 \times 10^6$ | $2{\cdot}3 \times 10^5$ | $6{\cdot}2 \times 10^3$ |
| 17 | $1{\cdot}3 \times 10^6$ | $3{\cdot}1 \times 10^4$ | $1{\cdot}3 \times 10^3$ |
| 21 | $5{\cdot}9 \times 10^5$ | $4{\cdot}2 \times 10^4$ | $1{\cdot}2 \times 10^2$ |
| 23 | — | — | $1 \times 10$ |
| 24 | — | $1{\cdot}5 \times 10^4$ | 8 |
| 28 | — | — | 1 |
| 30 | $2{\cdot}2 \times 10^5$ | $4{\cdot}8 \times 10^3$ | 0 |
| 38 | $9{\cdot}3 \times 10^4$ | $3{\cdot}5 \times 10^2$ | — |
| 42 | $6{\cdot}0 \times 10^4$ | $7 \times 10$ | — |
| 49 | $2{\cdot}1 \times 10^4$ | $1 \times 10$ | — |
| 56 | $7{\cdot}0 \times 10^3$ | $2 \times 10$ | — |
| 63 | $4{\cdot}7 \times 10^3$ | $1 \times 10$ | — |

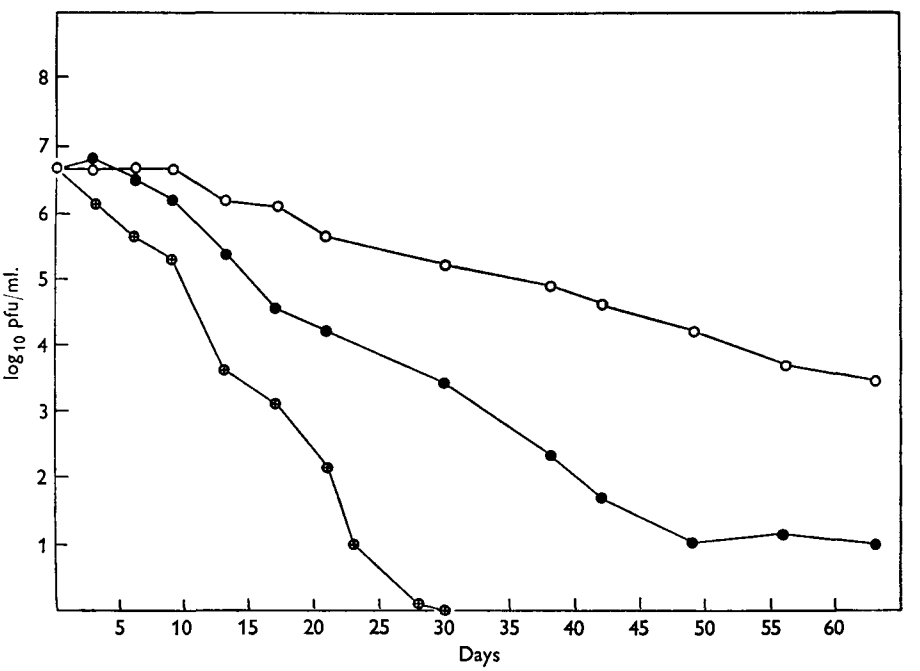


Fig. 1. Decline of infectivity at 30° C. in 3 suspending media: ○—○—○, 1·0 % peptone; ●—●—●, 4 mM buffer; ⊕—⊕—⊕, 50·0 % glycerol-saline.



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Table 2. *Survival of vaccinia virus (purified elementary bodies) in 5 different suspending media maintained at* 37° C.

Potency of sample is expressed in plaque-forming units (pfu) per ml. Titrations performed on monkey kidney cell monolayers. (*a*) 12·5% polyvinylpyrrolidone in water; (*b*) 12·5% polyvinylpyrrolidone in physiological saline; (*c*) 12·5% polyvinylpyrrolidone in 4 mM phosphate buffer; (*d*) 12·5% polyvinylpyrrolidone in 1·0% peptone; (*e*) 50% glycerol saline.

| Period of storage (days) | (*a*) pfu/ml. | (*b*) pfu/ml. | (*c*) pfu/ml. | (*d*) pfu/ml. | (*e*) pfu/ml. |
|---|---|---|---|---|---|
| 0 | $7{\cdot}7 \times 10^6$ | $2{\cdot}3 \times 10^6$ | $5{\cdot}9 \times 10^6$ | $8{\cdot}2 \times 10^6$ | $6{\cdot}6 \times 10^6$ |
| 3 | $2{\cdot}0 \times 10^6$ | $1{\cdot}1 \times 10^6$ | $1{\cdot}8 \times 10^6$ | $2{\cdot}9 \times 10^6$ | $7{\cdot}0 \times 10^5$ |
| 5 | $1{\cdot}9 \times 10^6$ | $2{\cdot}9 \times 10^6$ | $1{\cdot}5 \times 10^6$ | $7{\cdot}0 \times 10^6$ | $4{\cdot}6 \times 10^5$ |
| 7 | $7{\cdot}7 \times 10^5$ | $2{\cdot}0 \times 10^6$ | $2{\cdot}4 \times 10^6$ | $5{\cdot}8 \times 10^6$ | $3{\cdot}1 \times 10^5$ |
| 10 | $2{\cdot}4 \times 10^5$ | $6{\cdot}5 \times 10^5$ | $1{\cdot}0 \times 10^6$ | $2{\cdot}7 \times 10^6$ | $6{\cdot}6 \times 10^3$ |
| 12 | $1{\cdot}8 \times 10^5$ | $5{\cdot}0 \times 10^5$ | $1{\cdot}0 \times 10^6$ | $4{\cdot}5 \times 10^6$ | $2{\cdot}4 \times 10^2$ |
| 14 | $1{\cdot}2 \times 10^5$ | $4{\cdot}4 \times 10^5$ | $1{\cdot}8 \times 10^6$ | $3{\cdot}2 \times 10^6$ | $9 \times 10$ |
| 17 | $6{\cdot}7 \times 10^4$ | $2{\cdot}7 \times 10^5$ | $7{\cdot}5 \times 10^5$ | $1{\cdot}7 \times 10^6$ | $2 \times 10$ |
| 19 | $4{\cdot}5 \times 10^4$ | $2{\cdot}3 \times 10^5$ | $9{\cdot}1 \times 10^5$ | $3{\cdot}0 \times 10^6$ | $2{\cdot}8 \times 10$ |
| 21 | $1{\cdot}3 \times 10^4$ | $1{\cdot}4 \times 10^5$ | $7{\cdot}5 \times 10^5$ | $2{\cdot}4 \times 10^6$ | 12 |
| 25 | $3{\cdot}5 \times 10^4$ | $6{\cdot}2 \times 10^4$ | $2{\cdot}1 \times 10^5$ | $5{\cdot}7 \times 10^5$ | — |
| 26 | — | $9{\cdot}8 \times 10^4$ | $1{\cdot}9 \times 10^5$ | $8{\cdot}4 \times 10^5$ | — |
| 28 | $1{\cdot}9 \times 10^4$ | $5{\cdot}7 \times 10^4$ | $7{\cdot}8 \times 10^4$ | $4{\cdot}2 \times 10^5$ | — |
| 31 | $3{\cdot}3 \times 10^3$ | $3{\cdot}3 \times 10^4$ | $3{\cdot}2 \times 10^4$ | $9{\cdot}7 \times 10^4$ | — |
| 33 | $2{\cdot}9 \times 10^2$ | $3{\cdot}0 \times 10^4$ | $2{\cdot}1 \times 10^4$ | $9{\cdot}0 \times 10^4$ | — |
| 35 | $2{\cdot}2 \times 10^2$ | $4{\cdot}5 \times 10^3$ | $5{\cdot}2 \times 10^3$ | $1{\cdot}6 \times 10^4$ | — |
| 38 | $2{\cdot}0 \times 10^2$ | $2{\cdot}0 \times 10^3$ | $2{\cdot}1 \times 10^3$ | $7{\cdot}5 \times 10^3$ | — |
| 40 | $6{\cdot}3 \times 10$ | $4{\cdot}0 \times 10^3$ | $4{\cdot}2 \times 10^3$ | $4{\cdot}5 \times 10^3$ | — |
| 66 | 0 | 0 | 0 | $1{\cdot}3 \times 10^3$ | — |

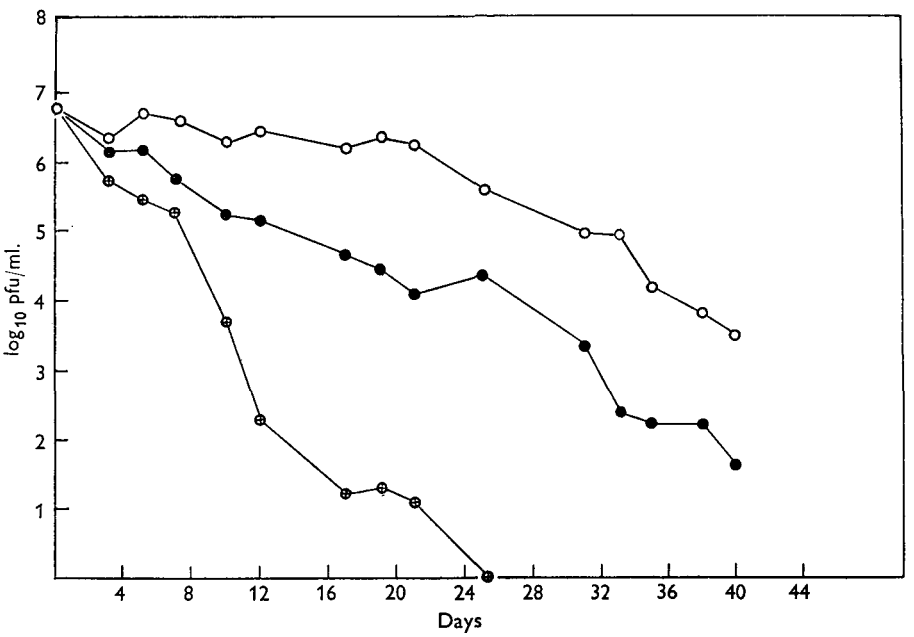


Fig. 2. Decline of infectivity at 37° C. in 3 suspending media: ●—●--●, 12·5% PVP alone; ○—○—○, 12·5% PVP + 1·0% peptone; ⊕—⊕—⊕, glycerol-saline.

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*Experiment* 1 *December* 1961. This experiment was one of several performed in order to obtain information about the effect of phenol on the survival of purified vaccinia virus suspended in various protective media. The results are given in Table 3. Although the phenol exerts some deleterious action of the virus this can be prevented to a large extent by peptone and PVP. This is an important finding because the official requirements or the established procedures of many countries include the addition of a small amount of phenol to smallpox vaccine in order to inhibit the growth of bacteria.

Table 3. *Survival of vaccina virus* (*purified elementary bodies*) *in* 6 *different suspending media maintained at* 37° C.

Potency of sample is expressed in plaque-forming units (pfu) per ml. Titrations performed on monkey kidney cell monolayers. (*a*) 12·5% PVP alone; (*b*) 12·5% PVP + 0·4% phenol; (*c*) 1·0% peptone alone; (*d*) 1·0% peptone + 0·4% phenol; (*e*) 12·5% PVP + 1·0% peptone; (*f*) 12·5% PVP + 1·0% peptone + 0·4% phenol.

| Period of storage (days) | (*a*) pfu/ml. | (*b*) pfu/ml. | (*c*) pfu/ml. | (*d*) pfu/ml. | (*e*) pfu/ml. | (*f*) pfu/ml. |
|---|---|---|---|---|---|---|
| 0 | $1{\cdot}0 \times 10^7$ | $5{\cdot}3 \times 10^6$ | $1{\cdot}1 \times 10^7$ | $1{\cdot}1 \times 10^7$ | $9{\cdot}6 \times 10^6$ | $6{\cdot}5 \times 10^6$ |
| 4 | $4{\cdot}0 \times 10^6$ | $1{\cdot}0 \times 10^6$ | $1{\cdot}6 \times 10^7$ | $1{\cdot}7 \times 10^7$ | $1{\cdot}1 \times 10^7$ | $8{\cdot}4 \times 10^6$ |
| 7 | $3{\cdot}7 \times 10^6$ | $2{\cdot}4 \times 10^5$ | $8{\cdot}4 \times 10^6$ | $4{\cdot}4 \times 10^6$ | $7{\cdot}6 \times 10^6$ | $6{\cdot}5 \times 10^6$ |
| 10 | $3{\cdot}3 \times 10^6$ | $1{\cdot}6 \times 10^5$ | $4{\cdot}9 \times 10^6$ | $3{\cdot}7 \times 10^6$ | $6{\cdot}0 \times 10^6$ | $5{\cdot}2 \times 10^6$ |
| 14 | $3{\cdot}4 \times 10^6$ | $8{\cdot}7 \times 10^5$ | $4{\cdot}0 \times 10^6$ | $6{\cdot}0 \times 10^6$ | $3{\cdot}6 \times 10^6$ | $3{\cdot}3 \times 10^6$ |
| 17 | $2{\cdot}2 \times 10^5$ | $3{\cdot}2 \times 10^4$ | $3{\cdot}8 \times 10^6$ | $5{\cdot}6 \times 10^6$ | $1{\cdot}6 \times 10^6$ | $1{\cdot}4 \times 10^6$ |
| 21 | $3{\cdot}4 \times 10^6$ | $2{\cdot}0 \times 10^4$ | $2{\cdot}5 \times 10^6$ | $1{\cdot}9 \times 10^6$ | $3{\cdot}0 \times 10^6$ | $2{\cdot}2 \times 10^6$ |
| 24 | $2{\cdot}0 \times 10^6$ | $4{\cdot}0 \times 10^3$ | $3{\cdot}8 \times 10^5$ | $2{\cdot}0 \times 10^6$ | $2{\cdot}5 \times 10^6$ | $1{\cdot}9 \times 10^6$ |
| 26 | $8{\cdot}0 \times 10^5$ | $7{\cdot}0 \times 10^3$ | $1{\cdot}4 \times 10^6$ | $1{\cdot}4 \times 10^6$ | $8{\cdot}4 \times 10^5$ | $2{\cdot}7 \times 10^5$ |
| 32 | $1{\cdot}1 \times 10^5$ | $1{\cdot}9 \times 10^2$ | $9{\cdot}8 \times 10^4$ | $5{\cdot}5 \times 10^3$ | $5{\cdot}0 \times 10^4$ | $1{\cdot}2 \times 10^4$ |
| 38 | $4{\cdot}0 \times 10^5$ | $2{\cdot}1 \times 10^2$ | $3{\cdot}6 \times 10^5$ | $8{\cdot}8 \times 10^2$ | $1{\cdot}2 \times 10^5$ | $1{\cdot}0 \times 10^5$ |
| 40 | $2{\cdot}9 \times 10^5$ | $4{\cdot}5 \times 10$ | $3{\cdot}1 \times 10^4$ | $6{\cdot}1 \times 10^2$ | $3{\cdot}1 \times 10^4$ | $3{\cdot}6 \times 10^4$ |

These experiments provide ample evidence in favour of the main contentions of this paper: that purified vaccinia virus is much more stable to heat than is usually supposed, and that glycerol has a deleterious effect upon the virus. The purified and stabilized vaccine retains its potency almost unimpaired for 3 weeks at 37° C.

## COMMENT

Improvements in the production and use of smallpox vaccine have long been overdue. It is hoped that the work described in this and the following paper (Amies, 1962) may serve as the basis for further research directed to these objectives. Clinical trials of the purified vaccine are in progress and it may already be stated that the results of a pilot study carried out on several hundred school children were successful.

Objections to the new vaccine will certainly be raised but it will be difficult to find any valid reasons for condemning it. The purification process succeeds in removing impurities without in any way modifying the virus itself. Cost of production cannot be used as an argument against the vaccine because the experimental data show conclusively that purification results in an increased number of doses from

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a given weight of skin pulp. Objections may be raised against the constituents of the suspending medium. These are readily refuted. It has long been the practice, particularly on the European continent, to administer polyvinylpyrrolidone intravenously in large doses to patients suffering from surgical shock. No serious consequences have occurred, either immediately or later (see PVP Handbook, 1951; Gall *et al.* 1953). Finally, there may be objections to the use of peptone in the suspending medium on the ground that some people are sensitive to this substance. To counter these objections it is only necessary to point out that diphtheria and tetanus toxoids which contain peptone have been administered to millions of adults and children without serious mishap. Examples of hypersensitivity to bacteriological peptone are occasionally reported but they are extremely rare. Some other antigen present in the inoculum is usually responsible for the reaction observed. A further safeguard against accidents of this type may be provided by the PVP. In some other experiments to be reported later it was found that this substance protects rabbits against shock induced by intravenous injection of an antigen to which the animals have developed hypersensitivity. This effect is probably due to delayed absorption of the antigen.

On the evidence collected in this paper it is concluded that purified stabilized vaccinia elementary body suspensions represent a distinct improvement over the crude glycerinated vaccines hitherto employed.

## SUMMARY

Detailed instructions are given for the preparation of a purified and stabilized suspension of vaccinia elementary bodies. This is intended to take the place of crude glycerinated calf or sheep lymph vaccine. The technique consists of a series of procedures involving mechanical disintegration of the skin pulp, maintenance of dispersion by means of a non-ionic detergent, fractional centrifugation and bacterial disinfection. Stability of the purified virus is secured by suspending it in a dilute buffer containing peptone and polyvinylpyrrolidone. Experiments are described which illustrate the remarkable heat resistance of the virus in this suspending medium. The PVP-peptone also serves as an excellent menstruum for lyophilization. Production of this vaccine should be well within the competence of any properly equipped virus laboratory. Clinical trials are in progress.

All the work involved in the performance of the tissue culture assays was carried out by my former assistant Mr Edmund Bischoff. It is a pleasure to acknowledge both his technical skill and his enthusiasm. Mr T. C. Campbell provided the crude calf pulp used in the preparation of some of the purified suspensions.

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