a much more important problem, but here the danger seems now infinitesimal when we consider that tuberculosis is rare in calves, and that lymph from a calf should not be used until the animal has been killed and proved to be free from tuberculosis. It will therefore be hardly necessary here to enter into a discussion on the possibilities of tubercle invagination under the former order of things. There is little advantage also in referring to cases of lupus vulgaris involving vaccination scars where the histories are too remote or vague to be of value. I have, however, observed three infants in whom one or more of the vaccination scars became the seat of a tuberculous patch on the healing, or very soon after the healing, of the wound. One child was inoculated with calf lymph by a very experienced vaccinator and other children vaccinated in the same batch experienced no ill-effects. Graham Little has recently published these cases in his paper on Vaccinal Lupus.⁷ Though they certainly raise unpleasant suspicions, I do not think any conclusion of scientific value can be drawn from them. The vaccination sores may have been inoculated secondarily, or a previous tuberculous centre may have existed and an embolus found its way to the scar.

With regard to leprosy, it is not proved, however probable it is, that the disease can be communicated directly by inoculation, and further by vaccination. The cases recorded in which the invagination of leprosy has been suspected will not stand investigation.

The recital of this list of possible complications, which may arise under every conceivable condition, is apt to excite a very false impression of the dangers attending vaccination, and may be taken undue advantage of to prejudice the public. Allowing for some variation in the local and systemic effects owing to the varying activity of the virus and the susceptibility of the subject, there are very few complications that it is not in our power to avert by using a pure lymph, conducting the operation in a proper manner, and preventing any secondary contamination. In terminating this sketch I will quote the following conclusion of the *Lancet* Commission, which is of great practical importance: "We are strongly of opinion that many of the bad results obtained in vaccination are due to the presence of one or other of the following conditions: Imperfect sterilization of the skin and want of protection against the invasion of the weakened and abraded tissues by extraneous organisms. We have found that, given a good lymph, the application of a plentiful supply of soap and water, a razor to take off hairs and surface epithelium, ether to remove fatty and sebaceous matter, alcohol to wash away the ether, superficial vaccination (by sterilized instruments), and protection of the vaccinated surface throughout the whole course of the local manifestation of vaccinia, most excellent results may be obtained. The best means of protecting the local lesion is the application of several folds of sterile lint containing no chemical disinfectant; that is held in position by strips of adhesive plaster; a layer of boric lint may then be placed outside this dressing, and the whole may be changed as soon as the slightest evidence of moistening by serum appears in the boric lint." By the observance of these conditions the vagaries and mishaps of vaccination will be reduced to a minimum, and the perfect result characterized by "typical vesicles, slow of development, with little inflammatory areolae, and late in coming to maturity," will be attained.

REFERENCES.

- ¹ *Lancet*, April 28th, 1900. ² *Ibid.*, June 7th, 1902. ³ *Journ. Cut. and Ven. Dis.*, vol. 1, 1883. ⁴ *Medical News*, August 11th, 1900. ⁵ *Journ. Cut. and Gen. Urin. Dis.*, February, 1902. ⁶ *Loc. cit.* ⁷ *Brit. Journ. Derm.*, March, 1901.

GLYCERINATED CALF LYMPH.

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[From the Government Lymph Laboratories, Chelsea Bridge, S.W.]

THE methods employed in the preparation of glycerinated calf lymph at the Vaccine Establishment of the Local Government Board are those which our experience has shown are most conducive to the production of well developed active vaccine vesicles, and to the subsequent manipulation of these

in such a way that the vaccine is never exposed and never comes in contact with other than sterile instruments. Further, repeated bacteriological test is employed to ascertain that such lymph before issue is entirely free from harmful micro-organisms.

The Selection and Preparation of the Calf.

The calf is the most important factor in the production of good vaccine, and calves vary so enormously amongst themselves in their response to vaccination that no care and no knowledge can be too great to assist in the selection of those most suitable. After a journey calves need a rest before vaccination. They should be housed in clean, airy, well-ventilated stables, kept as far as possible at an equable temperature; they need to be fed in the manner and with the materials most appropriate to their age and condition; indeed, in this respect it is hardly too much to say that each calf requires individual treatment. The most suitable calves are those from three to six months old, with smooth supple skins, clear eyes, and, generally, in the pink of condition. They should be kept under observation for a week, the weight ascertained, and the daily temperature. Everything being satisfactory the calf is ready to be prepared for vaccination.

For this purpose it is placed on a tilting table, constructed in such a way that every part of it can be kept thoroughly clean, that no moisture (urine, washings, etc.) can accumulate on the table, and with fastenings so arranged that no cramping of limbs can occur. The lower part of the abdomen of the calf is then shaved as far forward as the umbilicus and backwards as far as the thighs. This is an operation requiring care and skill, it being important that the skin should not be cut or abraded. The size and position of the area to be shaved for vaccinating purposes must, however, be actually determined by the condition of the particular calf, by the season of the year, and so forth. Sometimes calves respond much better to vaccination at one part of their skins than at another. The shaved area is well washed, rinsed with tap water, and finally cleansed with sterilized water. The moisture from such washings is removed from the shaved area and from the adjacent hair by means of sterilized gauze sponges.

The Vaccination of the Calf.

The calf is then vaccinated with glycerinated calf lymph, introduced into the skin in parallel linear incisions by a sharp scalpel. The incisions are of such a depth that they only pass through the epidermis and lay open the rete Malpighii. Care should be taken not to draw blood, as this is very apt to prevent the formation of perfect vesicles.

The vaccination of the calf requires practice and constant care, the incisions should all be of uniform depth, and there should be no gaps. As the incisions are made, more lymph is run in along the lines by the aid of a sterilized ivory spatula. The lymph must be distributed evenly along the line. The inoculation of the incisions must be effected as soon as they are made, otherwise the lips of the wound are apt to swell and close the opening.

After vaccination the calf is removed to a clean stall and kept in such a way as to prevent any injury to the vaccinated surface. The temperature of the stable should not fall below 60° F.

Collection of the Vaccine Material.

When the vesicles on the calf have reached maturity—generally in this country 120 hours after inoculation, but sometimes before, even as early as 96 hours—the animal is again placed on the operating table and the vaccinated surface is thoroughly washed with soap and warm water gently rubbed over it by the clean hands of the operator. It is well washed with tap water, and finally cleansed with sterilized water and the moisture removed with sterilized gauze sponges.

At this stage the site of each incision should present a line of even and continuous vesiculation; but this is by no means always the case. Sometimes the vesicles have been retarded in their development and show a tendency to dry up and form crusts, or they may be over-developed and advanced or may present an irregular and ragged appearance. It is from the careful consideration and from the knowledge of the value of all these points that the quality of the lymph has to be determined.

The skin of the calf is put firmly on the stretch and the vesicles and their contents collected with a sterilized Volkman's spoon, each line being treated in turn and scraped. The pulp thus obtained is received into a sterilized stoppered bottle. The abraded surface of the calf is then gently washed with warm water and the animal is removed from the table and weighed. The calves nearly always show a considerable gain in weight during their stay at the station and during the vaccination process. Each calf is transferred to the slaughter-house and is there slaughtered. A complete examination of the carcass and the viscera with a view to the recognition of tuberculosis is made on behalf of the Board by a veterinary surgeon appointed for that purpose. A report of the examination is received at the laboratories next morning. No lymph is used unless the animal in question is certified to have been healthy. The number of tuberculous calves at this age is very small.

Glycerination of the Vaccine Material.

The bottle containing the lymph pulp from each calf is taken to the laboratories, where the exact weight is ascertained. The pulp is next passed through a triturating machine consisting of a continuous screw, with diminishing worm revolving in a closely-fitting cylinder. The machines used are a modified form of the one invented by Dr. Chalybäus of Dresden. All the parts of the machine which come in contact with the lymph pulp are previously sterilized by prolonged steaming. The machines are driven by electric motors. Passage through the machine reduces the pulp to very fine particles, and this ground-up pulp is then gradually mixed with the proportionate quantity of glycerine and water. Usually in the laboratories the pulp is mixed with four times its weight of sterilized mixture of 50 per cent. pure glycerine in distilled water, but the amount of dilution should depend, whether more or less, upon the previously-adjudged quality of the lymph. The pulp mixed with some of the glycerine water is again passed through the machine, the remainder of the fluid being added during the passage. The resulting mixture is then once more passed through the machine, thus producing a fine and intimate emulsion.

At this stage a bacteriological examination is made of the glycerinated lymph; a loopful of the emulsion is withdrawn by a sterilized platinum needle, and agar-agar plates are established in order to estimate both the number and the quality of the organisms present in the lymph.

Storage of Emulsion.

The emulsion is next carefully poured into small sterilized test tubes capable of holding 4 to 10 c.cm. Each tube is filled as completely as possible, so that very little air is in contact with the emulsion. The tube is plugged with a sterilized cork, is sealed with melted paraffin rendered aseptic with carbolic acid, and is then placed in a cool cupboard or ice chest.

Bacteriological Examination of the Glycerinated Lymph.

As already stated at glycerination a bacteriological examination is made by means of agar-agar plates. After suitable incubation for seven days—twenty-four hours at blood heat and six days at room temperature—the colonies that have developed on these plates are counted and examined. Week by week this process is repeated, and invariably the number of colonies diminishes with the age of the emulsion. The elimination of the extraneous organisms is due to glycerine acting in conjunction with heat. The higher the temperature at which the glycerinated lymph is kept the more rapid the elimination; the lower, the reverse. The precise temperature at which glycerinated lymph should be kept for the elimination of the extraneous organisms and for the preservation of its specific activity is a matter requiring most careful consideration, presupposing as it does a very accurate knowledge of the quality of the lymph and the exact stage of maturation of the vesicles. In this way each lymph must be considered on its own merits.

The number of extraneous organisms contained in different lymphs varies enormously. Speaking generally, the better the quality of the lymph the fewer the number of organisms, and vice versa. During the process of glycerination the greatest destruction of the extraneous organisms takes place within the first week. Thus, if at glycerination a lymph

shows 25,000 organisms per loopful, at the end of the first week this number will probably be reduced to 2,000 or 3,000, at the end of the second week to 500 or 600, third week to 40 or 50, fourth week only 2 or 3 will be left or none. On the other hand, lymphs which show but few organisms at glycerination may be quite germ-free by the end of the second week.

The number of varieties of organisms found in our laboratories in glycerinated calf lymph is very small, and is practically limited to two—*staphylococcus aureus* and *staphylococcus albus*, *aureus* as a rule predominating. Numerous experiments with these *staphylococci* freshly isolated from calf lymph and injected into rabbits and guinea-pigs have shown that they have no pathogenic action for these rodents.

Also in glycerinated lymph are found *bacillus mesentericus* and its allies. These are more resistant, and if present frequently escape destruction. *Proteus vulgaris* sometimes occurs in lymphs, and is best discovered by means of fluid cultures, as shown by my colleague Mr. H. S. Fremlin. It is easily eliminated. Mr. Fremlin has also shown that no strictly anaërobic organisms occur in glycerinated calf lymph.

In this connexion it is interesting to note that the *Lancet* Commissioners, in their reports on glycerinated calf lymph, classify the extraneous organisms found in the various samples of lymph examined by them as aerobic and anaërobic. It appears that the latter term is assigned by the Commissioners to any organism capable of growth in the depth of glucose agar or glucose formate agar. Growth of the organisms in these media, however, does not necessarily entitle them to be called anaërobic unless it is shown that they will not grow equally well aerobically. Rather they should have been called facultative. In these laboratories exhaustive experiment has shown that, with the exception of *bacillus subtilis*, all the extraneous organisms found in lymph are facultative—that is, are capable of growing equally well under aerobic and under strict anaërobic conditions. In these circumstances, therefore, the Commissioners' classification cannot be accepted, and they have, in enumerating the organisms growing on surface agar and in glucose or glucose formate agar from the samples of lymph supplied to them, for the most part, undoubtedly counted the same kinds of organisms twice, and in any case have failed to show that they were not the same.

The streptococcus has never been found in the lymphs in these laboratories, nor have tubercle bacilli been discovered in lymphs which have been derived from tuberculous calves, and such lymphs when injected into guinea-pigs have invariably failed to produce any tuberculous lesions.

Use of the Lymph at the Animal Vaccine Establishment Prior to Distribution.

When the stage is reached at which the lymph is known to be free of any harmful organisms, and is practically germ free, samples of the lymph are drawn up into capillary tubes, and dispatched to the Animal Vaccine Establishment for the vaccination of children. The results of these vaccinations are recorded a week later, and from the number and size of the vesicles obtained an estimate of the potency of the lymph at this time is made.

Transference of the Glycerinated Lymph to Capillary Tubes for Distribution.

When the lymph of a given calf has been shown to be satisfactory, it is transferred to sterilized capillary tubes by means of special tube-filling machines worked by water power. The tubes contain sufficient lymph for the vaccination of one case, and are sealed in a small gas flame, every care being taken in the process to prevent overheating of the lymph. These tubes are then stored in boxes in an ice chest in such numbers, that any quantity can be at once be dispatched to the National Vaccine Establishment at Whitehall for issue to public vaccinators. During seasons, however, when small-pox is epidemic and the demand for lymph very great, all the lymph is not issued in capillary tubes in this way, but it has been found convenient and useful to send lymph out in small sterilized tubes, each containing sufficient lymph for ten or twenty-five vaccinations. These have a special advantage where several cases can be vaccinated or revaccinated in the same place.

Instructions with the Tubes.

Instructions are sent to each public vaccinator with the tubes, first, to ask only for such quantity of lymph as is immediately required; secondly, not to use lymph more than a week after it has been received, but to return it; thirdly, to keep the lymph in a cool and dark place. These instructions are designed to promote a proper care in the employment of lymph and to prevent waste.

Duration of the Activity of Glycerinated Lymph.

This depends primarily upon the quality of the lymph when collected. A first-class lymph, glycerinated and kept under suitable conditions, may retain its full activity for a year or longer, an average lymph, however, cannot be depended upon to remain fully potent longer than two months, and one of inferior quality still less. It is in this respect of range of activity that the determination of the quality of the lymph at the outset is a matter of such importance. It must be understood that the human subject is, as far as is known, the most susceptible animal to vaccinia, and that the transference of vaccine vesicles from the calf to the human subject, with or without glycerination, even if the calf vesicles are not of the first quality, will produce a human vaccinal manifestation typical in every respect, and therefore in all probability possessing full immunizing powers, provided that the calf lymph be used within its period of full activity. In calf lymphs of poor quality the duration of potency may be very short, in some cases hardly compatible with the complete effects of glycerination. Such lymphs need to be recognized at the outset and discarded.

Issue and Results.

Some of the more recent figures of the issue and results of glycerinated calf lymph prepared in these laboratories may be of interest, especially in relation to the current outbreak of small-pox. During the year ending March 31st, 1902, sufficient lymph has been issued for 974,595 vaccinations and revaccinations. The maximum amount dispatched in one week was 80,000, in the third week of January, and during the month of January 220,000 tubes were sent out. For the quarter ending December 31st, 1901, lymph was used for 261,044 cases, and showed a case success of 97.9 per cent., and an insertion success of 93.0 per cent. These cases are made up of:

	Cases.	Case Success.	Insertion Success.
		Per cent.	Per cent.
Primary	126,203	98.6	94.0
Revaccinations ...	134,835	97.2	92.0

SMALL-POX IN GLASGOW—1900-1902.

By J. C. McVAIL, M.D.,

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THE Medical Officer of Health of Glasgow, Dr. A. K. Chalmers, has issued a very valuable and interesting report on the recent prevalence of small-pox in that city. In noticing it we shall not attempt to touch on all the questions discussed by Dr. Chalmers, but shall refer only to certain of its more striking features.

Small-pox was introduced into Glasgow in April, 1900, and what Dr. Chalmers calls a pre-epidemic period continued until the end of that year. The total cases in this period were 397. Then, from the beginning of January, 1901, until the middle of July of the same year, there was an epidemic period in which the total cases amounted to 1,389. From the middle of July until November there was no case of small-pox in Glasgow. Next, from November, 1901, up to the beginning of May, 1902, there has been what he describes as a period of recrudescence, the total cases in this period having been 469. No doubt even since the beginning of May there have been a few cases not included in the Report, but Dr. Chalmers has done wisely in not further delaying its issue.

Recent Revaccination in Glasgow.

It is well known that in Glasgow during the period reported on there has been a very large amount of revaccination with the purpose of preventing the spread of the disease. The total population over 5 years of age is set down as 675,887, and the total revaccinations performed from the beginning of 1901 up till May 3rd, 1902, are recorded as 404,855. All but about 8,000 of these were done in the year 1901, and the rest have been done in the first four months of 1902.¹ It is obvious that these data provide with regard to the value of recent revaccination a test which has never been equalled in this country in the extent of the population dealt with in a well-defined area in a short and definite time.

The question is: What has been the influence of these 400,000 revaccinations in the prevention of small-pox? There have been two great groups of population—one the Not Recently Revaccinated, and the other the Recently Revaccinated. To avoid needless detail Dr. Chalmers takes the whole population of Glasgow over 5 years of age at the beginning of the year 1901 as not recently revaccinated. In Table VIII, quoted below, he shows in successive fortnights from January, 1901, how this population gradually diminished in amount through transference to the group of recently revaccinated, until by May 3rd, 1902, the 675,887 had been reduced to 271,032. *Pari passu* he shows how the recently revaccinated whom he regards as *nil* at the beginning of 1901 have steadily mounted fortnight by fortnight until they reach the grand total of 404,855 on May 3rd, 1902. These are the populations dealt with, and now we come to the behaviour of small-pox towards them. Here also the facts are given fortnight by fortnight. The total cases (including for convenience some children under 5) from the beginning of January, 1901, till May 3rd, 1902, were 1,858. How many of these 1,858 cases occurred in the Not Recently Revaccinated and how many, on the other hand, were found in the population recently revaccinated? In the first fortnight, when the whole population is regarded as not recently revaccinated, of course all the small-pox occurred in that group. The cases were 23. By the second fortnight 1,071 people had been revaccinated. Of 350 cases of small-pox in that fortnight, all belonged to the first or greater group, and none to the 1,071 recently revaccinated. That might be accidental owing to the smallness of the latter group. In the next fortnight the recently revaccinated population amounted to 4,862, and the small-pox cases in Glasgow were 202. None of the 202 cases occurred among the 4,862. Possibly that also might be held to be a mere coincidence. By this time revaccination was going on apace, and in the fortnight ending February 23rd the recently revaccinated population amounted to 41,674. A fortnight later it had gone up to 119,326, and in yet another fortnight to 157,461. During those six weeks the total number of cases of small-pox was 587. How many of them were among the recently revaccinated?

The answer is. Not one.

The whole 587 belonged to those who had not yet accepted the protection offered to them. Still following Dr. Chalmers's table we find that revaccination continued to spread, so that by April 6th the recently revaccinated numbered 201,193, by April 20th, 246,831; by May 4th, 291,596; by May 18th, 309,762; and by June 1st, 323,254. During this time small-pox was on the decline, the cases fortnight by fortnight being 92, 67, 28, 18, and 11, a total of 216. How many of these were among the now very large group of the recently revaccinated?

The answer again is, Not one.

From the beginning of June, 1901, onwards to the end of the year, there were very few cases of small-pox, and this fact corresponds generally with what is known of seasonal influence. The seasonal curves for London show that the first five months of the year are, outstandingly, the time for small-pox. From the beginning of June up to the middle of July there were only 11 cases in Glasgow, and these terminated the epidemic period. Once more the whole 11 belonged to the population who had not accepted revaccination. By this time that population was in a decided minority, as on July 13th the recently revaccinated numbered 394,020, and the not recently revaccinated were reduced to 218,867.

The great fact established by these very striking figures is

¹ Besides the above there were 8,001 revaccinations in 1900. *Vide infra.*