importance utterly, since we are able to isolate its most precious constituent, free from all the drawbacks of the crude fat. Eucerin wax, finally, is not an ointment base at all, but the long-sought-for body, by means of which we are able to render any stable fat into an ideal ointment base.

THE PRODUCTION OF VACCINE.*

W. F. ELGIN, M. D.

I shall discuss the question of vaccination tonight largely from the laboratory side, leaving for the gentlemen who follow me, its practical application. But to make a connected story for the benefit of those present who may not be familiar with the subject, I shall call attention to a few historical facts in reference to smallpox, the ravages of the disease, and the earlier methods employed to secure protection.

It is thought by some that the great plague of Athens referred to in the History of the Peloponnesian War, was smallpox. It was probably unknown in China prior to the twelfth century, B. C., but is said to have prevailed in India at a much earlier date.

Rhazes, an Arabian physician who died in 932, A. D., seems to have been the first to give a succinct account of the disease.

According to some authorities, the disease was probably unknown in Europe prior to the sixth century; others claim a much more remote antiquity. Smallpox appears to have been introduced into Mexico by the Spaniards in 1520, and is said to have destroyed 3,500,000 people; in some places whole tribes were wiped out.

In 1707, smallpox was introduced into Iceland, where it apparently had never existed before. Eighteen thousand out of 50,000 died of the disease.

From the fifteenth to the seventeenth century, it was practically universally present in most of the large cities over the known world. The epidemology resembled measles of the present day. No class of society was exempt. The king on his throne and the peasant in his hut were equally liable to the contagion. The question asked in the industrial world was "Have you had your smallpox?" just as today we ask "Have you been vaccinated?"

There was a high smallpox death rate in all large cities at that time. Dr. Farr estimated deaths from variola in the eighteenth century at about 4000 per million. Sir Lyon Playfair's estimate for all England was 3000 deaths per million, and it is calculated that about one-twelfth of the total mortality from all causes was due to smallpox. Smallpox was preëminently a disease of childhood. In Berlin from 1758-74 6000 deaths were reported, and only about 45 over 15 years of age, or one in 147 deaths.

Facts of this character could be presented almost without number, showing

*Report of a lecture delivered to the Philadelphia Branch, May 7, 1912.

first, the enormous loss of life due to the disease; second, that the disease was very largely confined to children.

In discussing the disease at the present time, previous conditions must be borne in mind or we will fail to realize that some potent factor is responsible for what would otherwise be a marvelous change in the epidemology of the disease.

It would be interesting at this point to enter into a discussion of the question of immunity underlying the whole subject of vaccination which was first exemplified in the attempt to prevent smallpox; but time does not permit. Suffice it to say that various theories have been advanced by Pasteur, Chauveau, Metchnikoff, Bucher, Nutall, Behring, Roux, Ehrlich, Wright, Wasserman and others, each in turn being in a measure superseded by the others, but all throwing light on this vexed question, until at the present time we are in a position to say that acquired immunity *to a contagious disease* depends upon a more or less profound impression made upon a higher organism by products thrown off by the micro-organisms, causing a specific disease. These products are proteids chemically, and the action appears to be a physiologic-chemical one, whereby the exciting poison, or antigen, stimulates the cells of the host to an antagonistic reaction. This reaction is largely specific and is directed to the protection of the host from the specific disease engendered by the invading microbe. In the light of this definition of "immunity", I will briefly sketch the methods employed to immunize against smallpox.

Inoculation.—In 1717 Lady Mary Worley Montague, wife of the British ambassador to Turkey, wrote a letter to a friend in England, describing the method of conferring artificial immunity used in Turkey known as "protection by inoculation," being nothing more or less than taking smallpox virus from a diseased person suffering with a mild type, *and inoculating* into a well person, thus conferring upon him a slight attack of the disease. This method continued in use in England for a number of years and spread over the most of Europe. It was never popular, however, with the peasant class. It was open to the objection in that while it conferred immunity, patients sometimes died. It was likewise probably one of the most potent factors in spreading the disease, since a person contracting smallpox by inoculation was just as liable to spread the contagion as one who took it in the natural way. It was prohibited in England by act of parliament in 1840.

Introduction of Cow-pox.—In the early part of the seventeenth century, several observers noted a tradition in the dairy districts to the effect that persons who had become infected with a localized skin eruption known as "cow-pox" from milking or handling cattle, were immune to smallpox.

Dr. Fewster, of Gloucester, in 1765 seems to have sent a paper to the Medical Society of London, indicating that inoculation failed in persons previously affected with cow-pox.

In 1774, a farmer, Benjamin Jetsy, of Dorsetshire, vaccinated his wife and two sons with this virus. Fifteen years later, one of the sons was inoculated with smallpox virus with negative result. This tradition seems also to have been known in Germany, Italy, the South of France, Holland, and elsewhere,

but nothing of a practical nature was accomplished until Edward Jenner took up the question and made a scientific study of it.

DR. EDWARD JENNER,
Discoverer of Vaccination.

Jenner studied medicine under the celebrated John Hunter, of London, and began practice near Berkley, where he came in contact with this tradition. So impressed was he that he made a number of experiments, inoculating people who had had cow-pox with smallpox, and finally on the fourteenth day of May, 1796 (known as the birthday of vaccination) he inoculated a boy—James Phipps—with a sore from the hand of a milkmaid who had accidently been infected with cow-pox. A successful vaccination resulted, and a few weeks later he inoculated the same boy with fresh smallpox lymph, without results. So thoroughly was he satisfied with the results of these experiments, that he reported them to the Royal Society in June, 1798, in a paper entitled, "An Inquiry into the Cause and Effects of the Variolæ Vaccinæ, a Disease Discovered in some of the Western Counties of England, particularly Gloucestershire, and known by the name of Cow-pox."

This method of protection was tried out in a number of hospitals. Thus in 1802 Dr. Woodville reported that he vaccinated 7500, about one-half of whom were afterwards inoculated with smallpox without results.

On October 31, 1802, King Frederick William of Germany stated that the Medical College had reported 17,741 carefully observed vaccinations, of which 8000 were subsequently tested by inoculation.

Dr. Waterhouse, of Boston, received a small supply of virus from Jenner, and vaccination began in this country in 1802.

Early Method of Propagating and Preparing Virus.—As it is well known, the early methods of obtaining virus was known as the "arm to arm" method; that is, a child in healthy condition was vaccinated, and on about the eighth (?) day when the vesicle was considered "ripe," a small part of the clear lymph was taken, and put up in capillary tubes or charged on ivory points for the routine vaccination of that particular district. This method was used in England until about 1898, when it was largely superseded by the glycerinated virus.

In this country we first used what is known as the "scab method." A child was vaccinated and when the scab dropped off, it was wrapped up in beeswax or other impervious material until required. It is reported that this virus could be kept for some time, even as long as several months, and still remain active. Objections to these methods were raised by anti-vaccinationists and others, because of the possibility of conveying some disease other than vaccinia. A few cases of syphilis were undoubtedly transmitted in this way.

In 1842 Negri seems to have modified the method introduced by Galbiati of inoculating the virus from one calf to another to obtain material for human vaccination.

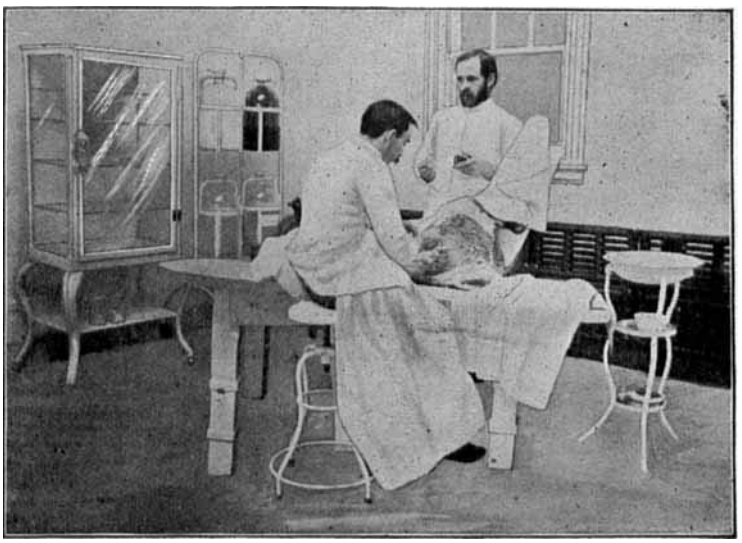
In 1870 Dr. Martin, of Boston, introduced this method in the United States. Crusts taken from the calf were then used. After this goose quills roughened on an emery-wheel were dipped into the pock on the animal and these were employed for the purpose of vaccinating.

This was followed some years later by the ivory points. These were made from ivory chips, a by-product of ivory manufacturers. The objection to all of these forms was that the virus was always contaminated.

My first experience when beginning the work some twenty years ago may be briefly summarized as follows:

Stables—rough sheds, white-washed once or twice a year, with animal refuse over everything. Corn stalks and manure from six to eighteen inches deep.

Animals—small calves, inoculated in small squares on the abdomen, and crusts removed on third or fourth day, amid fœcal accumulation, and filth with prac-



A Modern Vaccine Laboratory.

tically no attention to cleanliness. This underlying pus was charged on ivory points with soiled brushes and hands; in fact the whole picture was unthinkable when viewed from the standpoint of our present knowledge.

Insofar as I have been able to learn, these were the normal conditions at practically all vaccine plants at that time.

Virus had to be removed on the third to fifth day or the putrifying odor became offensive. Necrosis of tissue only was noted, and because this necrotic process was more rapid than vaccinia and usually entirely overshadowed it, the normal lesions of vaccinia were rarely observed, and we did not know at times whether vaccinia was present or not.

In 1898 Monckton Copeman delivered his Milroy Lectures, dealing with vaccine virus, and the results of some experiments with the admixture of glycerin with the virus, showing how virus was purified by this method.

Copeman was, however, not the fiirst to mix glycerin with vaccine virus.

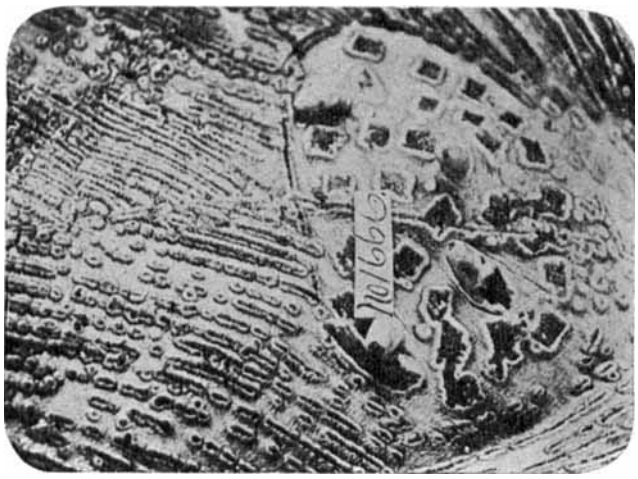
Müller of Berlin had done this for quite a while in order to increase the bulk of the virus and to act as a preservative. He did not seem to appreciate, however, the purifying effect of this method.

Koke reported somewhere about 1887 that the number of extraneous organisms decreased in the glycerinated lymph. He seems to have missed the importance of this statement because he stopped just at this point.

On a visit to Rome some years ago, I called the attention of Prof. Leoni to these facts and he stated that he had begun a line of investigations in December, 1888, looking to the purification of virus by this method, and had published his results in 1890, about one year before Copeman's publication.

Investigations of these observers and others prove that when from 50 to 60% of glycerin is added to the virus taken from the animal and allowed to remain under certain conditions for varying periods of time, a large reduction of non-spore forming germs can be noted from week to week.

The Reasons for Using Glycerin with Vaccine Virus.—You will note from what has already been said that at the present time glycerin and water are



Developed Vesicles on Vaccinated Calf.

mixed with the virus in about the proportion of 60% for the dual purpose of first, as a preservative; second, for purification.

As previously noted, this work was described by Copeman in his Milroy Lectures in 1898, and I immediately began investigating the properties of glycerin with virus; it was only after two years work that I could convince myself that virus submitted to this treatment would remain active the length of time described by Copeman; and in fact, after we had begun the work at Glenolden, several facts developed for which we could not account on the theory that glycerin preserved the vaccine at the same time destroying bacteria.

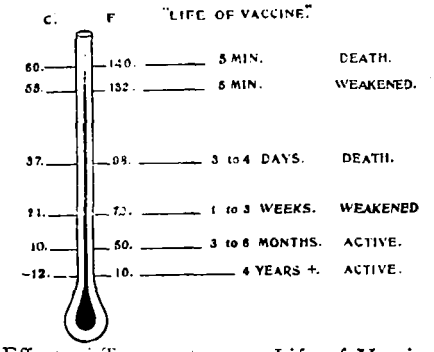
During the summer numerous complaints were reported where the vaccine did not give the expected results. Further experiments demonstrated the fact that heat was an important factor, causing the destruction of bacteria and at the same time destroying the virulence of the vaccine. In other words, the temperature of

the ice chest where the material was stored was one of the most important facts to be considered.

You will note the curved line marking the point of destruction of germ life crosses the life-line of vaccine in the summer season. In other words, virus sent out during this time of year will not stay active as long as the germ content. At the time this experiment was made, we kept virus in this laboratory under ordinary ice-chest temperature, and found that the germ life and the life of the virus were practically destroyed. The only way that we have been able to overcome this during this season of the year, and at the same time prepare a safe virus, is not to undertake to destroy germ life, but to test for the virulence of the germ life from week to week until it is no longer pathogenic.

Please remember that these experiments were made before the introduction of the cold storage method of keeping virus.

The experiments showing the importance of cold storage to vaccine production may best be illustrated in this slide.



Effect of Temperature on Life of Vaccine.

Taking the same virus and subjecting it to different temperatures, we have found that 60° C. killed virus in 5 minutes.

55° C. weakened it so we can note degeneration in the vesicles produced.

At 37° C. the virus will last from 3 to 4 days.

At 21° C., which we regard as practically room temperature, from 1 to 3 weeks showed deterioration of the product.

At 10° C., which is the usual ice-chest temperature, the virus will stand for three months or longer, occasionally as long as a year.

At —12° C. we have kept it four years, and it remained in good condition after being removed and subjected to ordinary ice-chest temperature. You can now understand the importance of this second factor to the preservation of virus.

This work was done at our laboratory after encountering the difficulty with the method discussed by Copeman in applying it to conditions of extreme summer heat, long shipping distances, long dating. Experiments were begun in 1899 and the first report regarding this was made at the American Public Health Association in 1900.

Shortly after this Blaxall began investigation, and reported to the Local Government Board and confirmed it in 1907; however, I am sorry to say, without any recognition of the work done in America.

Tubes of virus were placed at the temperatures noted before, and removed at stated periods, the virus plated and the number of colonies noted. From this you will see that the temperature of 60 degrees killed the non-spore contained bacteria in 30 minutes.

A temperature of 55° C. left less than 69 colonies.

A temperature of 37°, after an exposure of 3 days, left only one colony.

A temperature of 21° C. exposure of 5 weeks, before it was cleaned up plating, week by week.

A temperature of 10° C., exposure 9 weeks, before bacteria were entirely cleaned out.

At —12° C. we have never been able to clean up bacteria—in fact the virulence of staphylococcus was isolated from vaccine after four years and inoculated into a rabbit, causing the death of the animal.

As has been observed from slides previously shown, it is impossible to destroy all of the non-spore bacteria in glycerinated virus, because of the close relation between the life of the virus and the life of bacteria, in other words, because the virus itself is so near the death point when bacteria are eliminated as to be practically worthless when it leaves the laboratory and reaches the hands of the consumer.

For quite a while we did not recognize this, and as a result, numerous complaints were registered against the virus during the summer season, when it was extremely important that active vaccine should be in the hands of those having the field work to do. This method was then abandoned, and in its place we determined to attentuate the pathogenic contaminating organisms rather than attempt their total destruction. This method may be described as follows:

"Virus is put in the ice-chest at a temperature approximately 12° C. and held there until such a time that it is believed the contaminated organisms are rendered harmless, although not necessarily destroyed. Large numbers of them, however, are destroyed as shown by plate culture taken from day to day. Probably the more active ones remain, but these lose their virulence before the testing point is reached. At this time plate cultures are made and staphylococci and streptococci isolated; bouillon cultures are made of these and kept in the incubator for 24 hours and then injected intravenously into a rabbit in quantities of 1 c. c. to each 1000 grams of body weight of the animal.

These animals are kept under observation for a week, the weight taken every other day, and at the end of this time they are killed and cultures made. If any lesion is noted that may be caused by these organisms the virus is again placed in the ice-chest at the temperature of 12° C. for a week longer, and the process repeated. If any other suspicious organism is found, similar methods are used to determine its pathogenicity.

Anaerobes.—As is well known to the most of you, the tests which I have already suggested are only possible with organisms which grow in the air. Any anærobic organism which might be in the virus could not be determined by this method. Because of the fact that tetanus has followed the use of vaccine very careful testing is done to determine the absence of this organism.

Tetanus being a very powerful toxine producer, the test is comparatively easy. Our method is as follows:

10 c. c. of the virus to be tested is placed on bouillon in a modified Smith tube, allowed to grow for 10 days at incubator temperature, at the end of which period a culture is made on a slide for microscopic investigation. The culture is then filtered in order that the germ life may be taken out while the toxin itself passes through the filter.

5 c. c. of this filtrate is inoculated into a guinea pig and the animal is kept under observation, and must remain alive for 14 days before the experiment is considered satisfactory.

In addition to this 2 c. c. of the crude virus is injected into a guinea pig which is also kept under observation for at least 10 days to see if anything develops other than vaccinia. This work with slight variation as to technique, I have reasons to believe is carried on by all laboratories propagating vaccine virus, and you can readily recognize its importance when you recall the fact that for the past 10 years an occasional report of tetanus following vaccination has been noted from year to year. It is natural to suppose that the virus was responsible for this condition, and yet the investigations made by the number of laboratories in the last 10 years, both experimental and as a routine procedure, have failed to determine the presence of the tetnaus germ in any sample examined in this country, as reported by the Committee on Vaccine at the American Public Health Association meeting in Richmond in '08. At that time there had been 5,800 tests made and possibly the number by this time is nearly double.

The only time that the tetanus germ has been reported in vaccine virus was by Carini of Berne, and the possible case of Dr. Robt. N. Willson of Philadelphia. Dr. Willson, however, is not sure that he found the organism.

Attention is simply called to this matter because of its importance to persons responsible for vaccinating and the after care of the vesicle. Tetanus is probably more prevalent in the eastern part of the United States than in former years. It is possible that the wound may be contaminated with the dust from the streets, in fact any wound whether from vaccination or not may be contaminated and tetanus follow.

In concluding this section, I wish to make some practical suggestions to those handling and using virus that may be of value.

The United States Government requires a date to be placed on biological products giving the supposed minimum of the length of activity. From what has been said you can readily see that this is entirely problematical in a given sample, because no one knows the temperature conditions through which it is required to pass before it reaches the hands of the consumer, nor can we say how it is going to be stored when in the local drug stores.

You have seen that it can be killed very quickly by high temperatures, while below 0° C. it remains active for an undetermined period.

Therefore vaccine should never be kept in stock in a drug store (except in very small quantities) or any other place outside of the laboratory except below freezing point, which is usually impossible except in the dead of winter. The rule is that virus should never be kept in the summer season for local distribution, excepting it is to be used immediately.

Order in small quantities and order often and better results will be obtained.

Again I wish to call your attention to an error made by ordering routine vaccination for Public School purposes in September.

We are compelled to put up the virus in the summer months and ship it in the heat of August and September, and as a result it is frequently worthless when used.

The Doctor, if conscientious, has to do his work over several times before getting the desired results, or the patient has to go to school vaccinated but not protected, because the virus did not take.

Immunity Conferred by Vaccination.—In the first place permit me to call attention to the fact that the Local Government Board of Great Britain require three separate and distinct vaccinations and claims it takes at least one-half square inch of vaccinated surface to afford complete and lasting immunity.

It is the custom of the United States to vaccinate at but one point; frequently this is sufficiently large to cover the requirements previously indicated.

Supposing that a person has been vaccinated to the point of saturation then the question is asked—how long will this protect against small-pox? We must not forget in attempting to answer this question, there are no two persons equally susceptible to any disease; in other words that the amount of natural immunity varies with the individual.

We must be guided by some rule in our work and in order to establish some basis for comparison, I have devised this chart.

You see, it stated in almost all literature on the question, that when a person is vaccinated in infancy and again at 10 or 12 years of age, the protection usually stands during life.

How true this may be in reference to smallpox, I do not know, but I know it is not true in reference to vaccination. Over and over again I have seen employes in our laboratory get a vaccine infection in almost any part of their body which happened to be scratched and was exposed when handling the virus, which vaccination may be repeated in three months' time, or in a year or longer.

I have seen some individuals who seem to be impervious to the disease altogether, yet if they continued handling the vaccine year after year a time will come when the natural immunity is at low ebb and the person will take the infection.

PRECAUTIONS TO BE OBSERVED IN STORING VACCINES FOR DISTRIBUTION.*

W. L. CLIFFE.

The part assigned to me in the discussion of this evening relates entirely to the functions of the retail pharmacist as a distributor of these products upon the prescription or requisition of the medical practitioner.

As a distributor of Biologic Preparations the retail pharmacist has a much more important part than he is generally credited with or even than the majority of pharmacists appreciate. Owing to the high grade of technical skill and the large

*Read before the Philadelphia Branch, May 7, 1912.