Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 11, Number 2, October, 1938.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Immunization against Rinderpest, with Special Reference to the use of Dried Goat Spleen.

By G. PFAFF, Veterinary Research Laboratory, Insein, Burma.

CONTENTS.

I. Introduction.

In little more than two years rinderpest reduced the cattle population of the Cape of Good Hope by 35 per cent. In the eighteenth century the disease killed over thirty million cattle in Germany alone, and not until towards the close of the nineteenth century was it eradicated from the greater part of Europe, where for hundreds of years it had been the most serious plague of animals. In many parts of the world segregation of the healthy and slaughter of the sick, which had proved successful in Europe, is not practicable, and in Burma and India, and presumably also in many other tropical countries, rinderpest remains the most important disease of cattle.

During the last forty-five years immunization against rinderpest has claimed the attention of numerous workers and has been the subject of many publications. The object of this paper is to describe a dried vaccine which is cheap, safe and effective, and which in the past two years has been successfully used on over three quarters of a million cattle.

II. Immunization against Rinderpest.

(*a*) *Early attempts with Virus.*

From about 1754, attempts to protect cattle were made by inserting under their skin, material soaked in the nasal and ocular discharges of diseased animals. With fairly resistant cattle this method attained a certain measure of success, but in most parts of Europe it caused heavy losses. In 1872 the International Veterinary Conference considered that rinderpest should be dealt with by segregation and slaughter, and that immunization should not be attempted, thus indicating that little progress had been made in control methods since A.D. 69 when Columella, in describing a disease which appeared to be rinderpest, recommended the segregation of sick animals.

(*b*) *The use of Bile.*

The spread of the disease to Southern Africa in the nineties of the last century attracted many workers, and from that time dates the intensive study of the problem of immunization against rinderpest.

In the South African outbreak Koch (1897) and others [Edington (1899) Kolle and Turner (1898), Kohlstock (1897), and Theiler (1897)] confirmed the claims made by cattle owners for the protective value of the bile of infected animals. The value of bile was influenced by the stage at which it was collected, the blood content, and the temperature and delays to which it was exposed before use. The effectiveness of bile was limited to two days and Theiler considered the immunity it conferred did not last more than three months. Bile contained both antibodies and virus and by adding glycerine Edington (1899) probably killed the virus, thus converting the bile

method into passive immunization lasting only about ten days. Kohlstock (1897) recommended virulent blood about a fortnight after the bile. Bile was extensively used during the South African epidemic but towards the end was replaced by the serum alone and the serum-virus methods. Nevertheless, in December 1903 the first Pan-African Veterinary Conference resolved that failing an adequate supply of serum, pure bile should be used for stamping out outbreaks of rinderpest (Curson 1936).

(c) *The use of Serum.*

The protective value of serum from recovered animals had been noted by Semmer (1893). This was later confirmed by Koch (1897), Theiler, and others, and from that time until recently the production of immune and hyperimmune serum has been studied by many, and numerous publications have appeared on the subject. Hyperimmunization and other means of obtaining potent serum were adopted in nearly all countries. Some workers, however, maintained that the serum of recovered animals was as good as that supplied by hyperimmunized animals. Edwards (1925 and 1927c) affirmed that for the production of potent serum hyperimmunization was unnecessary and in India this process was finally abandoned in 1924. He showed that susceptible buffaloes given virus and simultaneously sufficient serum to suppress reaction developed a solid immunity but their serum had negligible protective properties. If the quantity of serum was sufficiently reduced to allow the animal to develop a mild but decided reaction, including a pronounced temperature reaction, the antibody content of the blood rose sharply on the 18th day, was sustained until the 36th day, and then gradually dropped until the 60th to the 90th day. No matter how severe the clinical reaction, the antibody content of the blood was not increased. Following the work of Madsen (1923) in Copenhagen, Edwards injected intravenously the chlorides of manganese, zinc, magnesium, barium, and copper, and pilocarpine and found that " the salting 2 hours before bleeding of the serum producers when they are still yielding a good serum seems to cause them to furnish a somewhat better serum; later when they are yielding a poor serum the salting does not increase materially the quality of the serum."

Serum protects for little more than 10 days and the cost of conferring this transient immunity should restrict its use to areas where the disease is not endemic and where cattle movements are under control, or for immunizing cattle passing through an infected area. Bederke (1931) reports it little used because of bad results and Delpy (1935a) rightly says that it is too expensive and the immunity conferred too transient. Nevertheless in Syria serum alone is said to be superior to serum-virus and to vaccine (Said 1934). There is still a considerable demand for serum, either alone or in combination with virus or vaccine. In the Punjab, for instance, in 1936-37 it was given to 58,917 animals either alone or with goat virus (Quirke 1937); and in Kenya in 1934 to 99,245 animals either alone or with virus (Brassey-Edwards 1936). The number of doses of serum issued from Muktesar during the year ending 31st March, 1937, was 1,227,628 (Taylor 1938).

(d) The Serum-virus Method.

The earliest forms of the serum-virus method appear to be those of Edington (1899) who gave glycerinated bile followed by blood virus, and Kohlstock (1897) who recommended virulent blood 2 weeks after bile. Before the end of the South African epidemic this was replaced by the serum and virulent blood method, and in 1898 Kolle and Turner recommended serum-virus simultaneously as the best method for the control of the disease. Much of the work after this related to increasing the antibody content of the serum, and has been briefly referred to in connection with the serum alone method.

The disadvantages of the serum-virus method are:—

1. the limited viability of the virus,
2. the spread of the disease by starting new centres of infection,
3. the losses due to protozoa injected with the virus, and
4. the high cost of serum.

Many workers have remarked on the limited viability of the virus. Hall (1933) considered that in blood the virus remained effective for only four hours, and Walker (1921) showed that although the virus after withdrawal from the animal could give rise to a reaction it did not necessarily confer immunity. In 1929 he recommended that virulent blood should not be used later than four hours after withdrawal from the virus producer. To overcome this disadvantage several procedures were tried. One was to obtain the virus from infected animals in the herd to be immunized. Another was to infect susceptible cattle or goats and despatch them to the scene of inoculation by train or lorry. Blood was sent out on ice, but even then it was not always satisfactory when used within 24 hours (Walker 1929). Edwards (1927a), seeking to overcome the disadvantage of limited viability by the application of the work of Kligler and Robertson (1922) on the spirochaete of relapsing fever, found that in blood diluted with an equal quantity of diluted horse serum (1:2) and kept in tubes sealed with paraffin wax the virus retained its potency for 10 days at 37° C. He, therefore, despatched the virus in bottles nearly filled with defibrinated blood and sealed with plugs of cotton wool soaked in paraffin wax. Thus packed the virus remained active for 8 days. Edwards remarks that "the original richness of the injected blood in virus is, however, a factor of considerable importance and sometimes the period of survival for this reason is likely to prove disappointing." Although it is not clear how the quantity of the virus influences the keeping quality, it appears from this remark that even under these conditions of despatch the virus did not always survive for 8 days.

The serum-virus method may set up new centres of infection by killing or causing severe reactions in inoculated animals and Cooper (1931b) showed that even cattle having a "blocked-out" reaction are capable of transmitting infection. Walker (1929) considered that cattle failing to react may contract rinderpest from reacting cattle. Since Edwards has shown that, provided active virus is used, non-reactors are immunized, failure to protect susceptible cattle must be due to the use of inactive virus.

In some countries the losses caused by protozoa injected with the virus at times reached 20 per cent. Daubney (1928) mentioned the loss of 50 per cent. from Babesidae in 18 cattle whose resistance to this parasite had been greatly reduced by short interval dipping. In India the serum-virus method is apt to cause a flare up of latent protozoa.

Although von Ostertag (1916) considered that in countries where protozoal diseases are endemic the cattle inoculated would be immune to those diseases and therefore the danger of spreading them could be disregarded, nearly all workers using the serum-virus method have found it advisable either to kill the protozoa in the virus or when necessary to treat animals after inoculation. Where large numbers of animals are dealt with, the disadvantages of post-injection treatment are obvious. Trypanosomes in the blood may be destroyed by tartar emetic (Carmichael 1926), but it has been pointed out that this destroys *T. vivax* but not *T. congolense*. Trypan blue is used to destroy Babesidae but instead of adding the drug to the blood after withdrawal it may be injected intravenously to the virus doner the day before bleeding (Conti 1933). Even though the virus is freed from contaminants it is apt to cause a flare up of latent infections and for this reason the serum-virus method remains one requiring observation and treatment of inoculated cattle.

To exclude protozoan contaminants, virus production in other animals was investigated. That sheep were susceptible was noted by Galambos in 1861 and later by Koch (1897), Rogers (1900) and Nicolle and Adil Bey (1902). Use was made of sheep by von Ostertag (1916) and Walker and Bradshaw (1925) who observed that they were not susceptible to the piroplasmoses of cattle.

Edwards in India successfully used goats to cleanse the blood of protozoa, with what far reaching results will be detailed later. Haddow, to overcome the rapid inactivation of the virus in blood, sent it out in the form of spleen pulp. In other countries goats have been tried with disappointing results (Kearney 1930).

The evidence on the duration of the immunity conferred by the serum-virus method is somewhat contradictory. Some regard it as life long, but Daubney (1930) is probably nearest the truth when he says that in animals guarded against all risks of reinfection the immunity begins to decline two to three years after the serum-virus inoculation, and that long immunity is due to symptomless reinfection.

Banerji (1933) and D'Costa and Balwant Singh (1933b) have shown that cattle virus and serum confers a longer immunity than goat virus and serum.

In Turkey the serum-virus method has been displaced by vaccine. Tahsin and Richter (1934) and Delpy (1935a) hold that it is too expensive and the losses caused by it too heavy. Nevertheless many still consider the serum-virus method to be the best. That it is still widely used even in countries where vaccine has been employed, is shown by the fact that of 173,881 animals immunized in Kenya in 1934, 98,373 received serum-virus (Brassey-Edwards 1936).

(*e*) *Vaccines prepared from Killed or Attenuated Virus.*

To overcome the disadvantages inherent in the serum-virus method of immunization, a vast amount of work has been done on the production of a vaccine from the virus killed or attenuated by exposure to chemicals, such as glycerine, formalin, phenol, toluol, and chloroform, either alone or in combination, one chemical being used to attenuate or kill the virus and the second added to preserve the vaccine.

The virus in the blood proving unsatisfactory for the preparation of a vaccine, various organs were examined, and as in the earlier work somewhat large doses of vaccine were found necessary, the practice was to incorporate in the vaccine as many organs as possible, mainly with the idea of increasing the bulk and hence the number of doses from each animal. Gradually the tendency grew to exclude the liver and other organs of low antigenic value and those liable to contamination, such as the mesenteric lymph glands, so that to-day many favour using only spleen. The aim of the research worker has been to prepare easily and quickly at low cost, a vaccine which would not transmit rinderpest or other infections nor cause undue local reaction, yet would immunize rapidly and for a long time and retain its immunizing properties when exposed to external temperature. After briefly reviewing the earlier work, the vaccines more commonly used to-day will be summarised in the light of these desiderata.

The satisfactory results with heated blood recorded by Gordziaklowski (1921) were not confirmed. Daubney (1928), Beaton (1931) and Hall (1933) failed to immunize with blood.

Some of the earliest work on the preparation of a vaccine was that of Kakizaki (1918) and his co-workers, who attenuated the virus with glycerine and added toluol and other preservatives. The exceptionally good results reported were queried by some investigators, but were later in part confirmed by Bennett (1936), who pointed out that Kakizaki used glycerine-water, whereas others had apparently used glycerine-saline.

In 1917 Boynton detailed the progress of his work, and in 1928 described the preparation of the vaccine finally evolved. He heated for three hours at 44° C. the minced lymph glands, spleen, liver, heart, kidneys and testicles to which had been added one-third of the weight of glycerine containing 0·5 per cent. phenol. Ageing or attenuation of the vaccine was effected by storage in the refrigerator for from one to six months. As the immunizing properties of the vaccine, which had to be determined, were lost soon after it was sufficiently attenuated, the material had to be used soon after the vaccine stage had been attained, and because of the poor keeping qualities no surplus stock could be kept on hand for use in an emergency. Some of the organs, notably the liver, were added simply to make up bulk and not for their antigenic properties. Boynton considered that the potency of the vaccine could be varied by the time and temperature at which it was stored. By killing the virus with chloroform, Kelser (1927) and Kelser *et al.* (1927) sought to overcome the drawback of having to attenuate the vaccine by storage and subsequently having to test it at intervals for attenuation

and potency. Allowing for the preparation of the pulp, and the storage to overcome the irritant effects of chloroform on subcutaneous injection, Kelser's vaccine was ready for use after three days. The spleen, liver, kidneys, testicles and various lymphatic glands were used. The dose given was three injections of roughly ten grams at weekly intervals. Rodier (1928) further altered the vaccine by using only spleen, lymph glands and tonsils, and giving only one injection. Kelser considered that cattle could be protected by a single dose of his vaccine, but that the more susceptible buffaloes required three or more injections; while Rodier, according to Kelser, showed that a single dose of vaccine prepared from lymph glands alone immunized buffaloes, and because of their greater susceptibility these animals were given half the dose necessary for cattle. This seems to indicate that Rodier was using an attenuated and not a dead virus.

The value of formalin in the preparation of rinderpest vaccine has been investigated by a number of workers, the earliest of whom appear to be Curasson and Delpy (1926), and Daubney (1928).

The work on Foot-and-Mouth Disease published by Vallée, Carré and Rinjard in 1926 induced Curasson and Delpy (1926) to use formalin to kill the virus of rinderpest and they produced a vaccine by submitting spleen pulp to the action of 1/250 formalin in normal saline for 48 hours at room temperature in the dark.

At the same time Daubney (1928) also influenced by the work of Vallée and his co-workers and by the reports of Bedson, Maitland and Burbury (1927), was investigating the value of formalinized spleen as a vaccine against rinderpest. He carried out extensive investigations and dealt with various aspects of the subject. His vaccine was prepared by mincing in a Latapie the spleen removed from infected animals bled to death under chloroform anaesthesia on the seventh day after inoculation with virulent blood. The pulp was mixed with Tyrode's solution containing 1/1,500 formalin, and allowed to stand for 72 hours at 5° C. The vaccine was given in doses of one gram and was said to retain its potency for seven days at 5° C. and for five days at room temperature. Daubney remarked that the preservation of antigenic properties was greatly influenced by the pH value of the vaccine. The vaccine was safe but to be effective had to be used soon after preparation.

Many publications have appeared on the subject of formalinized tissue vaccine. Investigators have varied slightly the concentration of formalin used and the period and conditions of storage. A solution of 0·01 per cent. formalin appears to attenuate the virus more rapidly than exposure to bodyheat, so that up to 97° F. the conditions of storage do not influence the rate of attenuation. Formalinized spleen pulp has been employed in doses of 1 gram (Daubney 1928); 8 grams (Hall 1930); and 20 grams (Jacotot 1929).

To increase the value of formalinized vaccine Prunier (1931) added castor oil and Curasson (1931) added tapioca.

Bennett (1936), following on the work of Kakizaki, prepared a vaccine by adding double the weight of 60 per cent. glycerine-water to minced spleens, the spleen being removed " from cattle plague patients at the height of an acute febrile reaction ", and storing for

three days at 37° C. or for three months at 0° C. The vaccine retained its immunizing properties for one month at 37° C. and for one year at 0° C., and in doses of one gram spleen pulp per 100 kilograms was claimed to immunize for about eight months, the immunity being developed within three days. The temperature of storage is important, as it is the temperature and not the glycerine which is said to attenuate the virus.

Boynton (1928) stressed the deleterious effect of impure glycerine, Daubney (1928) noted the deterioration of formalin by polymerization, and Walker (1929), who remarked on the variability of the antigenic value of spleens, advised that for vaccine preparation several spleens should be pooled and tested for potency before use.

Dried vaccines.—Andrievsky (1931) immunized cattle with a vaccine prepared from dried spleen and glands emulsified in formalin-saline. Curasson (1931) dried the tissues in vacuo over sulphuric acid and after storing at room temperature for a month the material was used in doses of 2, 4, 6 and 8 grams. Jacotot (1931) used dried spleen stored for a month at room temperature, and this in doses of one gram, without the addition of antiseptic, gave immunity in one month. Robles and Generoso (1930), working in the Philippine Islands, prepared a dried rinderpest vaccine from the pulp of spleen, lymph glands and tonsils by storing at 0° to 8° C. in a desiccator over calcium chloride. After 3 to 5 weeks drying it was used as a vaccine in doses of 1 gram. The method of washing and mincing was somewhat lengthy and tedious and the vaccine took at least three weeks to prepare. After 84 to 91 days in the ice chest it conferred only a very low degree of immunity, and became avirulent in less than seven days at room temperature. They say it is " impractical to send vaccine in fragment form to the field, since its preparation for use would necessarily be done under adverse field conditions involving too great a danger of contamination ".

In a second article (1931) the same workers record further progress and state that delay in drying seems to destroy potency. They claim that powdered rinderpest vaccine kept in the ice chest for 275 days suffered no loss in potency and that 30 days at room temperature did not destroy its immunizing powers, but that delayed drying appeared to be harmful to the immunizing value of the vaccine.

To prevent abscess formation formalin has been incorporated in dried vaccines, either before drying or when preparing the powder for injection. Andrievsky (1931) exposes dried spleen, tonsils and lymph glands to the action of formalin water for 48 hours immediately before injection. Delpy (1935a) prepares formalin vaccine and strains it through a coarse cloth, using both the filtrate and the solid residue which is first dried in electric stoves. 0·5 gram of the dried vaccine immunizes in 12 days.

The safety of vaccines, the retention of immunizing properties, the time taken to confer immunity, and the duration of immunity are of great practical importance.

It is claimed for all tissue vaccines that they are safe in that they do not transmit protozoa or other blood parasites. It is claimed, too, that unlike the serum-virus inoculation, tissue vaccines do not

cause severe reactions and so do not set up fresh centres of infection. Such claims should, however, be taken to refer only to the cattle on which the work was carried out and to the countries in which the work was done. It is conceivable that a vaccine harmless for fairly resistant cattle might prove dangerous for more susceptible animals, and there is evidence that this is the case. Daubney's formalin vaccine was perfectly safe immediately after preparation when used in East Africa on the cattle of that country, but Cooper (1931b) at Muktesar found that even four days after preparation it was still highly infective for hill cattle. In Indo-China formalin vaccine is used as it does not spread the disease (le Louet 1932), and there is no record from any other country of tissue vaccine spreading rinderpest. Although some vaccines are given in doses up to 60 c.cms. the local reaction causes no alarm, but vaccines containing chloroform and toluol are apt to cause oedema and abscess formation (Andrievsky 1931, and Granonillit and Do-van-Vien 1935).

Formalinized vaccine is said to retain its immunizing properties at room temperature for one month (Juge 1935); for one month even in the tropics (Curasson 1934); for five weeks (Missenard and Zylbertal 1934); for two months (Delpy 1935a); for ten months (Ismail and Zudhi 1933); for two months at 30° C. (Jacotot 1932); and when dried for six months (Jacotot 1932); and for one year (Delpy 1935a).

Immunity is established in 4 to 8 days (Jacotot 1932); 8 days (Curasson 1930, Ismail and Zudhi 1933, Delpy 1935b); 4 to 11 days (Daubney 1928); 10 to 12 days (Curasson 1936); 10 to 12 days in laboratory experiments and 20 days under field conditions (Juge 1935); 12 days (Delpy 1935a); and 15 days (Jacotot 1929).

To accelerate the onset of immunity the vaccine has been given intradermally, it being claimed that by this method the period is reduced to four days (Curasson and Zylbertal 1934, Ismail and Zudhi 1933). The latter authors recommend that 5 c.cms. of the vaccine be given intradermally into the the scrotum or lips of the vulva. Curasson (1930) finds that in infected herds, where the vaccine cannot be given subcutaneously, 10 c.cms. if given intradermally, produces immunity in 4 days. Jacotot and le Roux (1935), however, have found no advantage in giving the vaccine intradermally.

The duration of the immunity is said to be four months (Juge 1935); five to six months (Missenard and Zylbertal 1934); six months (Jacotot 1932, Curasson 1936); seven months (Ismail and Zudhi 1933); twelve months (Curasson 1930, Delpy 1935a); about two years, and five years when the vaccine is followed by active virus (Daubney 1934).

Bennett's glycerine vaccine retains its immunizing properties for thirty days at 37° C. and for one year at 0° C., and immunizes for eight months, the immunity being serviceable for all cattle and solid for most within three days, and solid for all in one week.

The cost of the vaccine is not easy to determine, but where the vaccine is prepared at the scene of the outbreak from a sick animal it is negligible. In Persia one calf produces about 200 doses of vaccine, at which rate it is considered cheap, and because of the

simplicity of manufacture it is used in that country (Delpy 1935a). Curasson (1936), however, considers that the cost of preparing formalin vaccine in a central laboratory would be prohibitive, and for that reason prepares it at the scene of the outbreak.

Obviously there is no great unanimity in the findings of those who have worked on tissue vaccines. It would seem that Bennett's glycerine vaccine is not influenced by the diluent, the rate of attenuation depending solely upon the temperature at which it is kept. All other chemically treated vaccines depend upon the action of the agent, and the best in a dose of 1 gram per 100 kilograms confer an immunity lasting little more than twelve months established in not less than one week. This refers only to fresh vaccine. Bennett's glycerine vaccine in a dose of 1 gram per 100 kilograms in three days confers an immunity which lasts somewhat less than a year. This also refers to vaccine stored at a low temperature as although Bennett claims that even after storage for 30 days at 37° C. the vaccine " produces a very serviceable immunity in all cattle and a solid immunity in most cattle within three days ", his protocols do not justify this. The immunity of only two cattle given vaccine stored for 3 days at 37° C. was tested 3 days after the injection of 1 gram per 100 kilograms and of these one died of rinderpest and the other had diarrhoea and ulcers in the mouth.

Daubney (1934) obtained excellent results by giving partially inactivated vaccine seven days after completely inactivated vaccine. He concluded that active or partially active spleen virus given after completely inactivated vaccine confers an immunity serviceable for more than five years. This method of immunization has at least some of the disadvantages associated with the use of virus in the serum-virus method.

Although tissue vaccines are now widely used, in assessing their value one should not lose sight of the fact that such a careful worker as Edwards (1927b) could not obtain satisfactory results with them, and at Muktesar this line of research was finally abandoned in 1932 after Haddow had found that formalinized vaccine was apt to cause fatal reactions and, when it was safe, the immunity conferred was unreliable. Moreover Mettam (1934) records that field results with formalin vaccine were so unsatisfactory that further experiments were carried out with other chemicals. He apparently had encouraging laboratory results with the addition of 1 per cent. sodium tauroglycocholate, normal saline, and 1/1,000 formalin to spleen pulp finely ground up with sand. One finds also that in 1934 in Kenya, where Daubney and others did a great deal of work on the production of formalinized tissue vaccine, of 173,881 cattle which received prophylactic rinderpest treatment, 65,607 received vaccine alone, 9,029 were given vaccine plus virus, 98,373 were given serum plus virus, and 872 received serum alone (Brassey-Edwards 1936).

(*f*) *Goat adapted virus.*

Apparently the earliest record of rinderpest in the goat was that of Jessen in 1836 (Flemming 1882). Others who drew attention to the susceptibility of this species were Chicoli (1863), Hallen (1871), and Topacio (1926). Koch (1897) observed no change in the virus

maintained in sheep, but noted some attenuation after five passages in goats, although even then the virus killed the susceptible South African cattle. Hall (1933) effected no attenuation after 27 passages in goats. Saunders and Ayyar (1936) showed that the virus attained a sufficient degree of attenuation for use as a vaccine only after about eighty passages in the goat. To fix the virus in goats Edwards (1927a) found it necessary to introduce " the bovine virus, by careful surgical operation, on the foetal side of the foetal envelopes in the pregnant goat ". Other workers have not had to resort to this. Hall (1933) could not maintain cattle virus in goats, losing one strain after 14 passages and another after 27 passages. To Edwards belongs the credit of realising the value of the goat for attenuating the virus for immunizing cattle. He in the first instance successfully used goats to overcome one of the disadvantages of the serum-virus method of immunization, namely, the injection of protozoa with the virus. Edwards reported on the value of goat virus alone, but did not recommend the entire abandonment of the serum-virus method, advising instead the replacement of serum alone by goat virus plus serum, and Cooper (1931), after showing that goat virus alone could be used, did not recommend it in view of the grave risks involved " for a tract in which the animals are considerably more susceptible is liable to be encountered suddenly at any time ". It should, however, be noted that according to Banerji (1933) Bliss in 1922 had recommended the use of goat virus in China, and in 1926 Topacio had pointed out the economy of this method.

Edwards (1930) observed at Karnal the survival of three bulls, which had been injected with goat blood virus alone by his colleague, Mr. Menon. Stirling (1932) noted this and also became acquainted with the fortunate error of his assistant, Mr. P. S. Nair, who while doing serum-virus inoculations gave five cattle goat virus and then found his supply of serum exhausted. All five cattle lived. These two cases and encouragement from Edwards led Stirling to carry out a small experiment with goat virus alone, and it is of interest to note that the four cattle on which the experiment was tried were obtained on loan from an Indian villager, after he " had been induced to make a written statement that the experiments would be carried out at his risk ". This small experiment was followed by the injection of 343 cattle and buffaloes, all of which survived the subsequent injection of bull virus. These appear to be the first experiments on the use of goat virus recorded in India.

Edwards (1927a) recommended field workers to prepare their own virus by the inoculation of two goats with Muktesar virus. Kerr and Menon (1934) investigated the value of the virus for buffaloes and for use in outbreaks. Stirling 1933 remarked on the value of the virus for cattle " with a relatively high degree of natural resistance " but still recommended the well-known serum-virus method for immunizing highly susceptible cattle in India and other countries. Stirling's method of preparing the virus was to bleed the infected goat on the fourth or fifth day. He considered female goats unsatisfactory as virus producers on account of the liability of pregnant goats to abort. Almost immediately workers were confronted with their first difficulty, which was to get active virus to the scene of inoculation. Kerr and Menon (1934) attempted to prepare

virus in the field by infecting virus producers at the scene of inoculation, but discarded this method because the technique was beyond the ability of field inoculators. They, therefore, concentrated on the use of spleen tissue vaccine, the spleen of reacting goats being cut into strips of about one gram each, and emulsified in saline immediately before injection by grinding in a mortar for half an hour, specially large needles being required for the inoculation. One goat produced between 2,000 and 2,500 doses, the dose being 0·01 gram of spleen. They injected large numbers of buffaloes and noted severe reactions only in those worked immediately after inoculation. To modify this the dose for buffaloes was reduced to half that for cattle. But even the use of spleen tissue did not overcome the limited viability of the virus, and Kerr (1935) records failures on two occasions due to the use of inactivated virus. One was due to the virus being used more than seven days after issue, and the other was during the very hot month of April. He noted specially that if his dose of 0·01 gram for cattle could be halved, there would be a very big saving. Kerr considered that increasing the concentration of the spleen to above one per cent. retarded or prevented immunization "probably due to the more ready diffusion of the thinner emulsion". He also considered that the virus lived longer in a saline solution weaker than normal saline, and finally used a half per cent. solution of sodium chloride for emulsifying his spleen tissue: Haddow (1936) found that the virus in tissue and tissue extracts remained active for 3 to 6 weeks at room temperature but for nothing like this at incubator temperature or even at 30° C. Haddow (1937) mentions that the rapid loss of viability of virus in hot weather is still "a cause of inconvenience" and adds that at room temperature the virus in spleen is viable for about a month but extracted with distilled water or normal saline becomes avirulent in about a week. As the temperature is such an important factor in the viability of the virus, it is unfortunate that the room temperature is not stated. Muktesar, at an elevation of about 7,000 feet, is comparatively cold, the variation in temperature being given by Lingard (1905) as from below 32° F. to 86° F. in the shade, and Ware (1933) states that "The climate of the place is for the most part of the year cold and the extreme temperatures range between 17·9° and 87·4° F." The time of the year, therefore, has an important bearing on Haddow's observation.

Haddow (1933) considers goat virus useful in imported animals, but in indigenous animals the virus resuscitates latent infection, which is as serious as conveyed transmission. The duration of the immunity conferred by goat virus vaccine is put at twelve months (Stirling 1933, Kerr 1935); and at something under three years (Edwards 1927a). On the time taken to develop immunity there is very little evidence—Stirling (1933) puts it at "about a week".

Vaccines made from cattle virus are safe only if the virus has been attenuated. Vaccines made from goat virus are effective only if the virus is fully potent. In spleen tissue goat virus is said to be viable for 30 days at 45° F. (Kerr and Menon 1934, Kerr 1935); for 6 days at room temperature (Kerr 1935); and for seven days at room temperature up to 85° F. (Kerr and Menon 1934). Haddow (1934) found that "at ordinary temperatures" at Muktesar spleen tissues and watery extracts of spleen tissues remained virulent for about a

month, and seemed to think that under hot weather conditions on the plains, the virus remained virulent for 15 days. Since Kerr and Menon (1934) failed to immunize with vaccine exposed to the plains temperature for six days, Haddow seems unduly optimistic.

III. Experimental work carried out at Insein.

A study of the literature on rinderpest was made during leave spent at Onderstepoort Laboratory, South Africa, in 1934 and again in 1937, and on my return to Insein in June, 1934, work was commenced by attempting to fix the virus neurotropically in white mice. At the same time the vaccine value of an emulsion of goat spleen in glycerine-saline was examined, and to determine the effect of storage on cattle virus the spleens of cattle were removed at the height of the thermal reaction and stored at 42° F. for further examination.

Attempts to fix the virus in mice on the lines of the work of Max Theiler (1930), Sawyer and Lloyd (1931) and Alexander (1933) were unsuccessful, and as the results with glycerine-saline emulsion of goat spleen were unsatisfactory, work on this was soon abandoned. Storage of cattle virus gave promising results and in March, 1935, it was possible to report to the Director of Veterinary Services, Burma, "there are reasons to believe, from the work done here, that simple storage will attenuate cattle virus sufficiently for it to be used as a vaccine." On the failure of glycerine-saline emulsion of goat spleen, work had in the meantime been commenced on a vaccine prepared from dried goat spleen. The results were so good that the efforts were concentrated on this aspect of the subject, and all other investigations on rinderpest discontinued. In March, 1938, however, a small experiment was done with Bennett's glycerine vaccine.

All experimental work was conducted at the Veterinary Research Laboratory, Insein, which is situated in the midst of a thickly populated area about eight miles from the centre of Rangoon. The grazing grounds, about fifteen acres in extent, provide grazing for less than six months in the year, and are used for all experimental animals. The cost of feeding is, therefore, high, and represents a large percentage of the very small amount granted for Veterinary Research. Under these circumstances experiments can be undertaken on only a very small scale and full use must be made of experimental animals. The experimental work was carried out as funds, animal accommodation, seasons, and numerous other duties would permit.

Rinderpest in goats.—In the earlier experiments, goats were obtained from the neighbourhood of the Laboratory, but with the use of large numbers it was necessary to get them from Upper Burma. As there appears to be no variation in the susceptibility of goats in Burma, in the experimental data their origin is not stated.

Rinderpest in goats has been described by Edwards (1927a), Stirling (1932), Beaton (1930), Cooper (1931), Hall (1933), D'Costa and Balwant Singh (1933), and Saunders and Ayyar (1936). Hall was unable to maintain cattle virus by passaging in goats, and thought if goats were to be used for virus production they would need to be specially bred and kept free from infection. D'Costa and Balwant Singh observed a breed susceptibility and noted that the

mortality, which varied with the season and the virulence of the virus, was from 70 per cent. to 100 per cent. Stirling described a jugular pulse, which, in his view, was all that need be looked for in a virus producing animal. He noted a mortality of only 15 per cent.

The lung lesion, which Hall (1933) does not consider a constant feature, has led to the disease being confused with contagious pleuropneumonia. Saunders and Ayyar (1936) passaged cattle virus 150 times through 300 goats and noted no change in the reaction in goats. Attenuation for cattle did not occur until after the 80th passage.

No natural infection in goats has been observed by me. At Insein the Muktesar virus has been passaged 302 times in 811 goats, obtained from widely scattered areas in Burma. No change in the reaction has been observed and the lung lesion has been a constant feature. Of the 811 goats, 23 died on the fourth day and all these had bronchopneumonia. As all the goats are temperatured for at least three weeks before being used for rinderpest purposes, and none are put into an experiment unless they have run a normal temperature for at least seven days, it seems likely that the pneumonia was that commonly associated with rinderpest. Small patches of early bronchopneumonia have been seen in several goats slaughtered for vaccine production on the 4th day after infection. In goats slaughtered for vaccine production there is always an enlargement of the spleen, which is due to the engorgement with blood and hypertrophy of the lymphoid follicles.

Of 162 goats in which the disease was allowed to run its course, 86 showed the initial rise in temperature on the 1st day after infection; 58 on the 2nd day; and 18 on the 3rd day. The peak temperature was reached in 3 animals on the 1st day; in 10 on the 2nd day; in 52 on the 3rd day; in 50 on the 4th day; in 16 on the 5th day; in 14 on the 6th day; in 10 on the 7th day; and in 7 of the 8th day. The rise in temperature after injection of the virus was between 1° and 1·9° F. in 9 goats; between 2° and 2·9° F. in 18 goats; between 3° and 3·9° F. in 67 goats; between 4° and 4·9° F. in 55 goats; and between 5° and 5·6° in 13 goats. Three goats showed an indefinite temperature reaction, and four reacted but survived. The other 155 died, the deaths occurring as follows:—4 on the 3rd day after infection, 9 on the 4th day, 13 on the 5th day, 13 on the 6th day, 20 on the 7th day, 15 on the 8th day, 19 on the 9th day, 11 on the 10th day, 9 on the 11th day, 2 on the 12th day, 2 on the 13th day, 5 on the 14th day, 1 on the 15th day, 3 on the 16th day, 2 on the 17th day, 2 on the 18th day, 1 on the 20th day, and 27 between the 21st and 86th day. Those which lived for some time became very emaciated and had extensive lesions of bronchopneumonia.

The cattle used in experiments.—In their resistance to rinderpest the cattle of Burma vary almost directly with the period that the areas from which they come have been free from the disease. In these experiments the term "Local cattle" refers to cattle bought on the Rangoon market, which is supplied from all parts of Burma. "Kyaukpyu cattle" are obtained from the Island of Ramree, off the west coast of Burma, and have been conveniently called "Kyaukpyu cattle" as they are purchased not far from Kyaukpyu and shipped from that port. They are small animals, seldom more

than two hundred pounds in weight, and very susceptible to rinderpest. In appearance and susceptibility to rinderpest they resemble the hill cattle used at the Muktesar Laboratory.

(*a*) *Experiments* 1 *and* 2 *with cattle virus.*

Experiment 1, *Table* 1.—*The passage of cattle virus in susceptible cattle.*—In all experiments from April, 1935, involving cattle virus for the testing of immunity or for any other purpose, the Kungyangon strain of virus was used. This was obtained from a natural outbreak of the disease in cattle in the Kungyangon village. It has been kept going on Kyaukpyu cattle which, as has been mentioned earlier on, have very little resistance to rinderpest. From Table 1, which gives details of the reactions of the cattle used for maintaining this virus, it will be noted that:—

(i) The initial rise in temperature was shown by—
16 cattle on the 2nd day,
39 cattle on the 3rd day,
4 cattle on the 4th day.

(ii) In the 18 cattle in which the disease was allowed to run its course, the peak temperature was reached—
on the 4th day by 6 cattle,
on the 5th day by 5 cattle,
on the 6th day by 5 cattle,
on the 8th day by 2 cattle.

(iii) The rise in temperature after injection of the virus was—
between 2° and 3° F. in 3 cattle,
between 3·1° and 4° F. in 25 cattle,
between 4·1° and 5° F. in 22 cattle,
between 5·1° and 6° F. in 9 cattle.
The figures include animals slaughtered on the 5th day.

(iv) Of 59 cattle—
1 recovered,
17 died,
38 were destroyed for conservation of the virus.

(v) Of the 18 animals in which the disease was allowed to run its course, 17, or nearly 95 per cent., died. These figures should be borne in mind when assessing the value of immunity tests in experiments to be described later.

Experiment 2, *Table* 2.—*The effect of storage at* 42° *F. on cattle virus in the form of powdered spleen dried in vacuo over calcium chloride.*—In this experiment two strains of cattle virus had to be used. Table 2 shows that after 100 days' storage the virus killed both animals injected, after 133 days' storage it killed one of two cattle, and after 177 days it killed none of the three animals injected. All the animals which survived the virus subsequently proved immune to fresh virulent blood. From this it seems that storage at

42° F. for about six months renders the virus safe and effective as a vaccine for susceptible cattle. Bennett (1936) found that spleen pulp in glycerine-water could be used as a vaccine after three months' storage at 0° C.

(b) Experiments 3 to 7 with goat adapted rinderpest virus in the form of an emulsion of spleen pulp in glycerine-saline.

In all these experiments by " glycerine-saline " is meant equal volumes of best quality glycerine and normal saline, and in all cases the spleen was removed from a goat on the fourth day after injection with rinderpest virus.

Experiment 3, Table 3.—The viability at 42° F.—The viability at 42° F. was determined on goats, the limit of the test being 231 days, at which period the virus was still viable. Virus stored up to 80 days provoked a rise in temperature which reached its peak on the 3rd, 4th or 5th day. After 102 days' storage both the initial rise in temperature and the peak temperature occurred later.

Experiment 4, Table 4.—The viability at 83° to 86° F.—The viability at 83° to 86° F. was tested after 3, 6 and 9 days' storage. All the goats reacted but although the initial rise occurred early the peak temperature was delayed, and this, and the fact that no goats died, seemed to indicate that at 83° to 86° F. glycerine-saline rapidly attenuated the virus. At this stage the use of goats as experimental animals was discontinued.

Experiment 5, Table 5.—The immunizing properties of varying quantities of a 4 per cent. emulsion of spleen.—The more resistant local cattle were used, and given doses of 1, 2, 3 and 4 c.cms. Of the three animals which gave no reaction to the vaccine, one died and two reacted very severely to the virus. Of the other eight cattle, which developed mild but delayed temperature reactions to the vaccine, virulent blood killed one, caused a severe reaction in four, a mild reaction in one, and had no effect on two. Four c.cms. of 4 per cent. emulsion of spleen gave no more protection than a quarter of that quantity. These results were unsatisfactory, but in the meantime experiment number 6 had been started.

Experiment 6, Table 6.—The duration of immunity conferred by an emulsion of spleen pulp in glycerine-saline.—Local and Kyaukpyu cattle were used. It was originally intended to test the immunity of a few animals at intervals over a period of two years, but after the unsatisfactory results obtained at the first immunity test another lot was tested immediately. No reaction to goat virus indicates either immunity to rinderpest or no protection from the goat virus. In other words, unless goat virus provokes a temperture reaction it fails to immunize. On this basis numbers 267 and 275 of the six cattle given vaccine stored for ten days at 42° F. may be disregarded. Of the other four in this group one gave a good temperature reaction to the vaccine and did not react to the virus, and three were killed by the virus. Of eight cattle given vaccine stored for 2 days at 42° F. and two days at 84° to 86° F., the virus killed

three and produced severe reactions in five. Vaccine stored for 2 days at 42° F. and 8 days at 84° to 86° F. protected one out of eight animals. The other seven were killed by the virus.

In addition to the 22 animals detailed in Table 6, ten other cattle were inoculated in this experiment. Two and a half months after they had been given vaccine, rinderpest broke out amongst them and killed seven. Before finally abandoning this line of investigation a last experiment was done.

Experiment 7, *Table* 7.—*The immunizing properties of* (*a*) 1 *per cent. emulsion in* 50 *per cent. glycerine-saline*, (*b*) 1 *per cent. emulsion in normal saline, after storage for* (i) *Five days at* 42° *F.* (ii) *Five days at* 82° *F.*—In this experiment the more resistant local cattle as well as the susceptible Kyaukpyu cattle were used. After five days at 42° F. both emulsions provoked temperature reactions, but only two of the eight animals showed no reaction to the subsequent injection of virulent blood. The vaccine stored for five days at 82° F. caused a temperature reaction in seven of the eight cattle. The eighth animal gave no reaction to virulent blood, so was presumably immune when it was given the vaccine. Of the seven reacting animals, four gave no reaction to virulent blood, two gave mild temperature reactions, and one reacted very severely.

The results obtained in these experiments showed that an emulsion of goat spleen in normal saline or in glycerine-saline had little protective value. It is possible that during the preparation of the vaccine the virus was attenuated, but careful checking up has failed to reveal any likely source of error. The goats which supplied the spleens also supplied blood for the passage of the virus, and for this purpose were bled immediately before being killed for removal of the spleen; so it may be concluded that the spleen on removal from the goat contained virulent virus. Walker (1929) pointed out the low antigenic value of certain spleens, but as three different spleens were used in these three experiments, it is unlikely that all three were low in antigenic value. Boynton (1928) remarked on the necessity of using only good quality glycerine in the preparation of rinderpest vaccine, as poor quality glycerine causes rapid deterioration of the virus. Although only glycerine of the best quality was used, it might have been affected by this tropical climate. It is, possible, however, that the virus, greatly attenuated for cattle by passage through goats, is still further weakened by glycerine. Whatever the reason, these experiments rule out the possibility of a satisfactory vaccine being prepared in this manner for use in Burma. Attention was therefore directed to the properties of virus in the form of dried spleen.

(*c*) *Experiments* 8 *to* 17 *with goat adapted rinderpest virus in the form of spleen powder dried in vacuo over calcium chloride.*

The goats supplying the spleen for use in these experiments were killed on the fourth day after injection with virus. The spleens were immediately minced in a Latapie and the pulp dried in a desiccator, in which it was kept at 42° F. for 24 hours. For each experiment the pooled material from not less than three spleens was used.

Experiment 8, *Table* 8.—*The viability at* 42° *F.*—The viability at 42° F. was determined at varying intervals up to 231 days. Disregarding for the moment the goat given material stored for 192 days, the reactions from virus stored up to 141 days were very similar to those provoked by fresh virus; the initial rise occurred on about the 3rd day and the peak temperature was reached on the 5th or 6th day. After 141 days' storage the initial rise was delayed and the peak temperature was reached much later. This delayed temperature reaction may be interpreted as indicating attenuation after about five months' storage. Similar results at the same temperature were obtained with goat virus in an emulsion of glycerine-saline, though in this case attenuation seemed to occur in less than four months, while cattle virus in the form of dried spleen became attenuated in just about the same time as dried goat virus (experiment 2). There are, therefore, grounds for thinking that although passage in goats has attenuated the virus for cattle, it has not changed its reaction to storage at 42° F.

Experiment 9, *Table* 9.—*The viability at* 85° *to* 89° *F.*—This was determined on goats and was unfortunately not carried to its conclusion. As far as it went the experiment showed that the virus survived 13 days at 85° to 89° F., and even after this period the initial rise in temperature and the time taken to reach the peak were well up to the average for fresh virus. Experiment 4 showed that at 83° to 86° F. glycerine-saline rapidly attenuated the virus. The conclusion was drawn that the virus is preserved longer in the form of dried powder than as an emulsion in glycerine-saline, and having arrived at this conclusion it was decided to investigate the immunizing properties of dried virus, and in this and all future experiments cattle were used.

Experiment 10, *Table* 10.—*The viability and immunizing properties of dried spleen virus emulsified in normal saline.*—Neither four days at 42° F. nor four days at 79° to 81° F.had any deleterious effect on the antigenic properties of the virus emulsified in normal saline. As storage for four days at 79° to 81° F. had no ill effect on the virus it seemed practicable to despatch vaccine in the form of dried spleen to be emulsified with normal saline on the day of injection.

Experiment 11, *Table* 11.—*The immunizing properties of dried spleen virus after storage for* (i) 5 *days at* 42° *F.*, (ii) 5 *days at* 82° *F.*—For each period of storage two Kyaukpyu and two local cattle were used, each receiving 0·005 gram of dried spleen. All reacted to the virus and all proved immune to virulent cattle virus. As these two small experiments had shown the undoubted antigenic properties of dried spleen, emulsified in normal saline, another small experiment was carried out to determine the effect of exposure to a temperature of 97° F.

Experiment 12, *Table* 12. *-The effect of storage at* 97° *F. on the viability and antigenic properties.*—Three cattle were given virus stored for 3 days and 3 cattle were given virus stored for 6 days. Three days at 97° F. had no deleterious effect on the virus, but after 6 days at 97° F. the effect was marked, only one of three cattle being immunized. Experiments had now shown that

very small quantities of the virus immunized and that a temperature of 97° F. rapidly destroyed the antigenic properties of the virus. Many parcels of vaccine despatched to the field would for hours be exposed to a temperature of 97° F. or higher, and as it had been shown that after 6 days at 97° F. the vaccine was unreliable as an immunizing agent there seemed to be no object in investigating the viability of the vaccine at room temperature, which would be in the neighbourdhaad of 85° F. It seemed more important to gain information on the optimum amount of virus required to confer immunity, and on the possibility of despatching vaccine packed in ice.

Experiment 13, *Table* 13.—*The optimum immunizing dose of dried spleen virus.*—Five pairs of cattle were given quantities of dried spleen decreasing from 0·1 to 0·0005 gram. All ten animals were immunized so that it was concluded that 0·0005 gram of dried spleen was sufficient to immunize cattle. Observations, detailed elsewhere in this paper, had been made on the rate of rise of temperature in ice-containing thermos jars. An experiment was now undertaken to determine the behaviour of the virus under what were considered adverse field conditions.

Experiment 14, *Table* 14.—*The effect of adverse field conditions on dried spleen virus.*—To reproduce adverse field conditions virus was packed in a thermos jar of ice and placed in an incubator at 97° F. Three days later the ice was replenished. The jar was replaced in the incubator, and the virus tested at intervals commencing with the sixth day, i.e., nine days after the thermos jar was first placed in the incubator. This procedure was adopted because most of the vaccine would be used later than three days after despatch from the Laboratory. More animals than necessary were put into this experiment, which was regarded as of considerable practical importance, because as it was desirable to use the same virus on all animals injections had to be continued until there was no doubt that the end point had been reached. The results show that six days after the addition of ice to the thermos jar the vaccine immunized all four cattle. After eight days, of four animals one was immunized, one was given no protection, and the other two were given some degree of immunity. After ten days, one of four animals was protected. Observations showed that in thermos jars containing ice and stored at 97° F. the temperature reached 97° F. after four days, so that actually this experiment confirmed the results obtained in experiment 12.

Experiment 15, *Table* 15.—*The duration of immunity.*—Before the optimum dose of vaccine had been determined an experiment to give information on the duration of immunity was commenced and for this purpose a dose of 0·01 gram of dried spleen was given. Local and Kyaukpyu cattle were used and their immunity tested in groups after 319 days, 429 days and 755 days. It should be noted that these animals were protected from exposure to the disease so that whatever immunity they had was conferred by the vaccine, and to be sure that this immunity was not reinforced by unnoticed natural infection, ten to twenty highly susceptible Kyaukpyu cattle were kept with the test herd, it being presumed that these susceptible animals would show the presence of any natural infection.

Of the ten animals in the 319 days group, one developed a rise in temperature commencing on the tenth day, and nine showed no reaction. Unfortunately no contral animals were included in this group, but as the virus was obtained from animal No. 527, Passage No. 45 in experiment 1, and dried spleen from this animal was used to carry on the strain on the 18th November, 1936, it may be presumed that the virus was active.

Of eight animals in the 429 days group, one developed a temperature reaction, the initial rise occurring on the 5th day. One of the other seven animals did not react to the vaccine and all proved immune when given virus.

Of six animals tested after 755 days the two Kyaukpyu animals showed mild temperature reactions, the initial rise occurring on the 6th day in one and on the 7th day in the other. The four local cattle were immune, though one of these did not react to the vaccine and so was probably immune when put into the experiment.

This experiment, which is still in progress, has so far shown that the immunity conferred by the vaccine lasts for twenty-five months.

Experiment 16, *Table* 16.—*The time taken to confer immunity.*—In an experiment carried out in September, 1936, it was shown that immunity was established within three days. A second experiment, done in February, 1938, may be conveniently described as part of the first.

It is hardly possible to summarise the thermal reactions to the vaccine as these were complicated by reactions to the virus. The charts show that virus, given before the temperature caused by the vaccine has returned to normal, provokes a second temperature reaction. Typical charts are shown in Figure 5.

Ten Kyaukpyu cattle were given vaccine and their immunity determined by the injection of cattle virus at intervals varying from twenty-four hours to eleven days atfer the vaccine. Of those tested in September, 1936, three to eleven days after inoculation, one of each pair was given 0·005 gram and the other 0·0025 gram of vaccine. The dose given to those tested in February, 1938, twenty-four to seventy-two hours after vaccination, was 0·0025 gram to one and 0·00025 gram to the other. Both animals in the twenty-four hours group reacted to the virus, one a little more severely than the other, but both recovered. In the thirty-six hours group only one developed mild diarrhoea. None of the animals in the other groups had either diarrhoea or mouth lesions, indicating that 0·00025 gram of dried goat spleen protects against virulent cattle virus injected forty-eight hours later. In the twenty-four hours group the animal which got 0·0025 gram of spleen has a less severe reaction to the virus than the one which got one-tenth that amount, while in the thirty-six hours group the animal with the smaller dose of vaccine reacted to the virus while the one which got the larger dose suffered no ill effects. The experiment shows that 0·00025 gram of dried spleen confers an immunity which is serviceable in twenty-four hours and absolute in forty-eight hours.

Experiment 17, *Table* 17.—*The immunizing value of dried spleen virus stored alternately for twelve hours at* 80° *F. and for twelve hours at* 97° *F.*—Experiment 12 had shown that after 72 hours at 97° F. the immunizing properties of dried spleen virus were unimpaired, and used after 144 hours at this temperature the virus immunized one of three cattle. After 13 days at 85° to 89° F. there was no change in the reaction it produced in goats, though it does not, of course, follow that it would still immunize cattle. It was of practical importance to find out for how long vaccine not kept on ice remained effective during the hot months, and for this purpose during the day it was kept in an incubator and during the night it was kept at room temperature, which did not fall below 80° F.

After storage under these conditions, for six, eight and ten days, the vaccine gave no immunity to one of two cattle in each group, and practically no immunity to the other animal in each group.

Obviously during the hot months vaccine cannot be effectively used six days after removal from cold storage. It seems that the attenuating effect of storage at 97° F. is not arrested by subsequent storage at 80° F.

(*d*) *Experiment* 18 *with Bennett's glycerine-water vaccine.*

The vaccine was prepared exactly as described by Bennett (1936), the spleen being minced in a Latapie pulper.

Experiment 18, *Table* 18.—*The safety and immunizing properties of Bennett's glycerine-water vaccine stored at* 37° *C.*—This experiment was undertaken before recommending this vaccine in the very few outlying areas of Burma where dried spleen vaccine can be used only with difficulty. Bennett found that spleen pulp in glycerine was safe after 3 months at 0° C. My experiment No. 2 showed that after 133 days at 5° C. (42° F.) the dried virus still killed cattle. As observations on Bennett's vaccine stored at 0° C. would take too long, the effect of storage at his second temperature, viz., 37° C., was examined. The vaccine was injected after exposure to 37° C. for four days, six days, fifteen days and twenty days, two cattle being used on each occasion. The Laboratory has no facilities for weighing live cattle. Several Kyaukpyu cattle in Experiment 1 were weighed after they had been slaughtered for removal of the spleen. They all weighed less than 200 lb., and as Bennett recommended a dose of 1 gram spleen pulp per 100 kilograms, all animals in this experiment were given 1 gram of spleen pulp.

Bennett found that his vaccine was safe for use after 3 days at 37° C. and " to produce solid immunity in all cattle, the limits of storage are not much more than one month at 37° C." (Bennett 1936.) Unfortunately this experiment did not confirm Bennett's findings. After only four days' storage the vaccine did not protect against 2 c.cms. virulent blood given 27 days later, both animals dying of rinderpest. Of the eight animals used, only two survived the injection of virulent blood—one in the six days group and one in the fifteen days group—but both had such severe reactions that it may be concluded that the vaccine conferred little, if any, immunity.

Bennett apparently does not state how he tested the immunity conferred by his vaccine. Possibly my test by injecting 2 c.cms. of virulent blood was far more severe than his, but it was the test used in all my experiments with goat adapted virus. It is possible, too, that my experimental animals were more susceptible than his and my virus more virulent than that used in his experiments.

(e) Observations on the rise of temperature in thermos jars containing ice.

The capacity of the thermos jars used is 1,500 c.cms. (roughly two and a half pints) and the price in Rangoon is three shillings and six pence each.

(i) A block of ice weighing 250 grams was put into each of six thermos jars. After 40 hours the temperature was 32° F. in No. 1 jar, after 64 hours it was 44° F. in No. 2 jar, and after 86 hours it was between 60° and 64° F. in the other four jars.

(ii) Various quantities of finely crushed ice were put into six thermos jars. Details of temperature rise and quantities of ice are as follows:—

Jar No.	Weight of Ice.	Temperature after 64 hours.	Temperature after 88 hours.
1	320 grams	33°F	—
2	330 grams	32°F	—
3	280 grams	—	64°F
4	355 grams	—	74°F
5	325 grams	—	59°F
6	330 grams	—	59°F

(iii) Twenty-four tubes of dried vaccine were put into each of three thermos jars, which were then filled with crushed ice (about 850 grams). At the end of 88 hours the temperatures recorded in the three jars were 59° F., 60° F., and 60° F.

(iv) Twelve thermos jars were filled with crushed ice (900 grams in each) and kept in an incubator at 97° F. The rise of temperature was as follows:—

Jar No.	Temperature recorded.	
	After.	Temperature.
1 and 2	24 hours	33°F
3 and 4	48 hours	33°F
5 and 6	72 hours	33°F
7	96 hours	67°F
8	96 hours	69°F
9, 10, 11 and 12	120 hours	97°F

These small experiments show that as a refrigerant crushed ice is as effective as a block of ice; and that the temperature in a quart thermos jar filled with crushed ice remains at 32° F. for at least 72 hours, and does not reach that of the surrounding air for at least four days.

(f) Observations on Dry Ice (Solid Carbon Dioxide).

Dry ice was obtained from Madras, packed in insulated leather containers and carried in the ship's cold store. During the 84 hours in transit the weight of the dry ice decreased from 114 lb. to 45 lb. It was then stored at 42° F., and at this temperature the daily loss was 10 lb., so that there was a gradual increase in the percentage loss. On exposure to room temperature, which varied from 78° to 83° F., the loss was very rapid. One pound of dry ice was packed in each of six thermos jars, which were tightly corked. Within 24 hours all the ice in all the jars had evaporated. Blocks of dry ice, weighing twelve ounces, were put in aluminium tins with screwed-on lids. All the ice evaporated within four hours.

As a refrigerant for the despatch of dried vaccine in Burma dry ice is useless.

IV. Application of Experimental Results.

(a) Preparation of dried goat spleen vaccine.

For the preparation of this vaccine the goat is killed on the fourth day after injection with goat adapted rinderpest virus, the spleen removed aseptically, finely ground and rapidly dried. The material, which is not tested for sterility or potency, is despatched in a thermos jar of ice, emulsified with normal saline immediately before injection, and used in doses of one gram for 400 cattle and one gram for 2,400 buffaloes. The vaccine is used as a prophylactic measure both in clean areas and in natural outbreaks of the disease. The preparation of the vaccine will be described in detail.

Goats are obtained from Upper Burma, where the supply is apparently inexhaustible, and delivered at the Laboratory at a cost of ten shillings each. They are not specially selected, though big, healthy-looking animals are taken for preference. It is not possible, even if it were desirable, to obtain only males, and no matter at what time of the year animals are purchased kidding is of daily occurrence. Pregnant goats used for vaccine production do not abort, and if they did it would not reduce their value. The spleens of female goats are slightly bigger than those of males, and the percentage of loss in drying is the same. The average weight of the spleen of the last 59 female goats used was 55·5 grams and that of the last 24 male goats was 49·9 grams. The loss in weight in drying was 76·5 per cent. for spleens of female goats and 77·1 per cent. for spleens of male goats.

The strain of goat virus originated from Muktesar and was kindly supplied by the Director of Veterinary Services, Madras, in December, 1934. At Insein it has been kept going in goats either

by direct blood inoculations on the day of bleeding, or by injection of blood or spleen pulp after storage at 42° F. for varying periods. In the earlier passages virus material from a single animal was on each occasion used, but latterly the pooled virus of the vaccine producers has been injected.

The reaction in goats has been dealt with elsewhere in this paper. The goats used here are so universally susceptible to rinderpest that it seems all may be used without reference to the temperature or other reaction. Of 615 infected for vaccine production, only 5 were discarded because of what appeared unsatisfactory temperature reactions.

The goat is bled to death and the spleen removed as quickly as possible through the left flank. The only treatment given to the skin is to clip the hair along the lines of incision and remove the loose hairs with a stiff brush. Using sterilized instruments the skin over the left flank is reflected and the abdominal contents allowed to drop down by an incision through the muscular coat in the mid-ventral line, the muscles in the flank being then incised without danger of puncturing the rumen. Lifting the ribs exposes the spleen, which is grasped with a pair of forceps and removed by snipping its attachments. A smear from each spleen is examined after staining for one minute by Giemsa, and the carcase is submitted to a cursory inspection. Of 610 spleens, ten were discarded because of fibrous peritonitis, and 14 because the goats had died of rinderpest before the 96th hour.

The spleens are minced in a Latapie pulper of 400 c.cms capacity. To preserve the cutting edges the knives are kept in 70 per cent. alcohol and washed in boiled water immediately before use the rest of the instrument being sterilized by boiling. The use of the Latapie pulper is a little wasteful in that some of the pulp is retained in the dead space between the piston and the grinding surface. Some pulp is also retained in the system of cutting edges, but to minimise the possibility of infection no attempt is made to salvage this material. The pulp is transferred to sterilized petri dishes through a short rubber tube attached to the outlet of the Latapie.

The pulp is dried in vacuo over calcium chloride, and for this purpose are used a Pfeiffer double stage vacuum pump, stout-walled desiccators, with an internal diameter of 9 inches and a depth of $8\frac{1}{2}$ inches, and calcium chloride of good quality. Rapid drying of the pulp is attained only by attention to details. The bottom of the desiccator is covered with lumpy calcium chloride and a little white vaseline is rubbed on the opposing surfaces of the lid and rim of the desiccator after they have been carefully cleaned. The lid is well seated on to the desiccator so as to give an even distribution of the layer of vaseline. The tubulure and stopcock are also carefully cleaned and vaselined, and the pump connected up with the desiccator by means of pressure tubing. Care is taken to keep the pump filled with an adequate supply of clean oil. These details have been enumerated as on the few occasions when assistants reported failure to dry the pulp rapidly or completely, this was due to neglect of one or more of the points mentioned. A petri dish containing the pulp from about one spleen is put into a desiccator and the pump

allowed to run for two or three minutes. The desiccator is placed in a refrigerator at 42° F., and after two hours the pulp is stirred to disturb the dry crust on the surface and to expose the moister material below. The air is again exhausted and the desiccator returned to the refrigerator. The following day the pulp is rapidly ground in a pestle and mortar, and transferred to drawn glass tubes, about 8 inches by $\frac{3}{4}$ inch, from which it is run into 2 inches by $\frac{1}{4}$ inch test tubes, which are closed with rubber corks and sealed by dipping in melted paraffin wax. This is done quickly so as not to heat the tube or its contents. The vaccine is stored in these tubes at 42° F. In emergencies the pulp is more rapidly dried by placing only small quantities in each desiccator and the vaccine issued within a few hours of the spleen being removed from the goat.

Vaccine is prepared weekly, the number of goats used being regulated by field requirements. This procedure was originally adopted as a matter of convenience but is now strictly adhered to because Haddow (1934) has shown that it is not possible to maintain the goat adapted virus for more than four generations if it is stored at 0° C. for more than ten days between each passage.

In the earlier experiments, to make the most of limited funds, use was made of whatever material was to hand. For this reason the tubes of vaccine were packed in the small aluminium tins used for photographic roll films. They were light and of a convenient size. As the tins of vaccine were packed in ice it was necessary to make them watertight, and for this purpose plasticine was used. With the adoption of the vaccine for routine inoculation in Burma it was possible to consider an improvement in the packing of the vaccine. More elaborate tins with screw caps and collars and rubber washers were devised and other methods of preventing the entrance of water were tried but the simple aluminium tin, with slip-on lid, which is made locally very cheaply, and the use of plasticine, remain the most satisfactory method of packing. A thermos jar, of two and a half pints capacity, is half filled with powdered ice, the tin of vaccine put on this ice, and the jar filled with powdered ice, which is rammed down firmly. The boxes containing the thermos jars receive rough handling in transport, and to guard against breakages powdered ice is used in preference to a single piece of ice. The cork of the thermos jar is sealed with a strip of plasticine, and the jar packed in a wooden box of such dimensions as to allow not less than four inches of sawdust, bran or paddy-husk between the jar and the box. The packing material acts as an insulator as well as a buffer against rough handling. To keep a check on thermos jars they are numbered and periodically tested.

It is usual for inoculators to arrive at their destination in the evening and commence work early the following morning. They are supplied with one hundred c.cms. and thirty c.cms. bottles, sodium chloride tablets, glass pestles and wedgewood mortars. Boiling cracks a glass mortar and melts the glue in the handle of a wedgewood pestle, but does not damage a glass pestle or a wedgewood mortar. Instruments and bottles are boiled the evening before inoculation, so that the following morning all instruments are sterilized but cold, there is no danger of killing the vaccine in a hot mortar, and there

is an ample supply of cold sterile water for the preparation of normal saline. By the adoption of these simple measures the procedure has been made very nearly fool proof, which is a very necessary precaution.

Each tube contains about 0·25 gram vaccine, which is sufficient for one hundred cattle. The powder is emptied into the mortar, saline added and the powder rubbed up into a paste. More saline is added and the powder is completely emulsified, the whole procedure taking not more than 30 seconds. The emulsion is poured into a 100 c.cms. bottle, which is filled up with normal saline and thoroughly shaken. For buffaloes five c.cms. of this vaccine is put into a 30 c.cms. bottle, which is then filled up with normal saline giving thirty doses of one c.cm. each. The vaccine is injected subcutaneously, after washing the skin with soap and water. The hair is not clipped and inoculators are advised not to use any disinfectant, though it is feared that many still cling to the old belief of the absolute necessity of scrubbing the site of injection with tincture of iodine or with one of the coal tar disinfectants. Inoculators are allowed to use the spleen powder up to thirty days after despatch from the Laboratory, provided the ice in the thermos jar is replenished every three days. They are not permitted to use it later than six days after ice was last added and vaccine not used on the day emulsified with normal saline must be discarded.

(*b*) *The use of the vaccine in the field.*

When first used in an area free from rinderpest for 30 years, 0·1 gram of the vaccine caused a mortality of about one per cent. in cattle and of about 20 per cent. in one lot of buffaloes. It was presumed that this heavy mortality was due to long freedom from the disease and the consequent lack of natural resistance. It was then thought that the dosage could with advantage be varied with the years of freedom from the disease, and for this reason all requisitions for vaccine were accompanied by a history of the disease in the area where the cattle were to be inoculated. Dosage was graded accordingly and used as follows:—

Years area free from Rinderpest.	*Doses of vaccine for cattle.*
Up to 2 years	200 doses per gram.
2 to 5 years	400 doses per gram.
6 to 9 years	600 doses per gram.
10 or longer	1,000 doses per gram.

A fairly extensive trial showed that the dose did not materially influence the reaction in cattle but that many buffaloes were killed by a dose which was safe for cattle. The dose was, therefore(fixed at one gram of powder for 400 cattle, and one gram for 2,400 buffaloes. Experiments have shown that one-tenth of the dose fixed for cattle is just as effective, but the main reason for fixing the dose at one gram for 400 cattle is that very rarely, if ever, is this number inoculated in one day. Each villager owns only a few animals, so that even if it were possible for inoculators to do more than 400 in one day, this number would not be available. Inoculators have

instructions to use vaccine on the day it is emulsified, which in practice means that it is used within six hours. The powder is not weighed but the amount is gauged fairly accurately by comparison with a weighed quantity in a similar tube. It would, however, be more difficult to gauge a smaller amount, so that all things considered it is deemed advisable to waste a little vaccine rather than reduce the dose to the limits indicated possible by experiments. The vaccine is now being used wherever it is possible to deliver it in a viable form, which excludes only a very few areas in Burma. It is used in infected herds during an outbreak, in herds where outbreaks are apt to occur, and to establish a zone of immunized cattle around an infected area. This, however, is outside my sphere of action, and will not be further commented upon. Details of the numbers of cattle and buffaloes inoculated with the vaccine are given in the table immediately below. The mortality said to have been caused by the vaccine is also shown. All these inoculations were carried out by officers of the

Month.	Cattle.		Buffaloes.	
	Number inoculated.	Deaths up to 30 days after inoculation.	Number inoculated.	Deaths up to 30 days after inoculation.
1935—October	827	3	47	1
November	1,144	2	147	1
December	702	3	251	45
1936—January	986	1	—	—
February	3,075	21	—	—
March	2,698	18	1,012	24
April	4,325	46	733	11
May	13,046	9	638	25
June	25,923	82	550	28
July	27,259	94	3,457	107
August	22,110	36	2,054	93
September	13,606	33	2,832	66
October	22,566	28	3,399	109
November	19,688	19	3,138	84
December	25,055	17	2,116	41
1937—January	28,429	16	1,214	12
February	38,720	32	3,340	61
March	52,872	53	4,768	98
April	30,046	41	3,035	79
May	43,166	65	2,257	149
June	27,628	24	1,158	15
July	24,487	14	1,036	16
August	15,501	23	985	9
September	27,712	24	1,946	14
October	46,392	88	4,013	106
November	41,183	57	3,192	44
December	37,502	31	3,502	58
1938—January	46,025	20	3,476	48
February	57,536	24	3,393	24
March	59,583	20	4,785	17
Total	759,792	944	62,474	1,385

Veterinary Department, many of whom are of limited education. Since in all parts of the country big numbers of cattle have been inoculated without any mortality it is reasonable to conclude that in nearly every case death after inoculation was not due to the vaccine, but to other diseases, or to poverty and neglect. Enquiry has shown that in some cases poverty-stricken animals, especially buffaloes, were turned out into the jungle after inoculation and neglected, many dying of starvation. Animals dying in infected herds were reported as having been killed by the vaccine, whereas many probably died of natural infection. Nevertheless the figures in the table include all deaths up to about 30 days after inoculation, and show a total mortality of 944 out of 759,792 cattle, and 1,385 out of 62,474 buffaloes. During the fifteen months ending 31st March, 1938, which excludes the heavy mortality when the vaccine was first introduced, of 576,782 cattle inoculated, 532 died, and of 42,100 buffaloes, 750 died. As the table is based on reports received from inoculators up to the 30th April, 1938, the figures are necessarily incomplete.

(*c*) *The advantages of the vaccine.*

The vaccine may reasonably be described as cheap, safe, and effective.

As at present used in Burma the vaccine is many times more expensive than it would be if used in the doses shown to be effective, but even so it is estimated that the cost, including goats, apparatus, packing and freight amounts to about four shillings a thousand doses. The preparation of a vaccine from cattle would be a hazardous undertaking in Burma, where the supply of susceptible cattle is limited to far away areas not free from natural outbreaks of the disease, so that at any moment one's supply might be cut off. Goats, on the other hand, are obtainable everywhere, and all seem equally susceptible to the disease. They are, moreover, cheap and the cost of their upkeep is small. The breakages of thermos jars, which is perhaps the main item in the cost of bottling and despatch, is surprisingly small, and has amounted to only 12 out of 1,507 sent out. There is a great saving in time and material in tubing the vaccine, as 100 doses can be put into a small agglutination tube in a few seconds, while the cost of transport on a box of vaccine from the Laboratory to the inoculators' headquarters averages about one shilling.

A gross mortality of less than one in a thousand cattle during the fifteen months ending 31st March, 1938, shows that the vaccine is safe. Repeated attempts at the Laboratory to transmit the disease to Kyaukpyu cattle by contact have failed, and field observations support these findings. In each of the Laboratory experiments ten Kyaukpyu cattle were mixed in a small loose box and five given goat virus. All the cattle were fed and watered from the same utensils, and although the inoculated cattle reacted to the virus and were kept in contact with the test cattle for 30 days these showed no rise in temperature and later reacted typically to the subcutaneous injection of goat virus.

With buffaloes the position is a little uncertain. Edwards (1927b) stated that in India the buffalo is not regarded as particularly susceptible. In the Philippines buffaloes are considered much more susceptible than cattle. In Burma the buffalo is extremely susceptible and in any outbreak of rinderpest a mortality of 100 per cent. is expected, leaving no recovered animals and consequently no animals with any acquired immunity. In the Philippines it was found necessary to give buffaloes three injections of chloroform vaccine, as the immunity developed from one injection was insufficient to protect them against exposure to natural infection. In Burma inoculators have reported that dried goat spleen in a dose of 1 gram for 2,400 buffaloes has at times caused severe reactions and a few deaths. Spread of the disease to uninoculated buffaloes by contact with inoculated buffaloes has also been reported. It is hoped that at Insein facilities will soon be available for work on buffaloes and that then the immunization of these animals will be further investigated.

The vaccine transmits no blood parasites and causes no local reaction. As only the spleen is used and this can be quickly and aseptically removed, there is little danger of contamination. It would be otherwise if tonsils and lymph glands were incorporated in the vaccine. Field work is sometimes performed under great difficulties, and it is conceivable that contamination might occur during emulsification of the vaccine in the mortar. On two occasions at the Laboratory vaccine was emulsified in a small cattle enclosure while many assistants intentionally created as much dust as possible, but even then the vaccine produced no local reaction. Emulsification may also be effected by prolonged shaking of the spleen powder in a bottle of normal saline. This is a longer and more tiring process which does not satisfactorily break up the spleen tissue, and Haddow (1934) has shown that watery extracts are much less virulent than tissues. The use of the mortar has no disadvantages and with it complete emulsification is effected in 30 seconds.

Experiments have shown that the vaccine confers immunity which is serviceable in 24 hours and solid in 48 hours, and endures for at least two years. As immunity is not lasting and as that conferred by vaccines can be reinforced by virus, it is reasonable to presume that loss of immunity is gradual and not sudden, and can be reinforced by exposure to natural outbreaks of the disease. In estimating the duration of immunity, animals which might have been exposed to natural infection should, therefore, be disregarded, and failure to do this has probably contributed to the disagreement on the subject of immunity conferred by tissue vaccines and by serum-virus. In my own work great care was taken to exclude any possibility of immunity being reinforced and experiments have shown that the immunity lasts for at least twenty-five months. Reports from the field confirm these Laboratory findings.

Since the vaccine is kept on ice its effectiveness is assured and one would expect field results to be in accordance with Laboratory experiments. In many countries ice would present an insurmountable difficulty but in Burma it is no handicap. It is available on trains, steamers and ferry boats. It is made in most of the smaller towns and procurable in many out of the way villages, the price per pound

varying from half a penny in the larger towns to two pence in the villages, though in a few distant villages it goes up to as much as sixpence. Figures 7, 8 and 9 give a good idea of the temperatures to which vaccine would be exposed if not packed on ice.

V. Discussion.

The term "strains" in connection with rinderpest virus has been used as a matter of convenience, and should not be taken to indicate the existence of a plurality of strains. The difference is one of virulence or degree. I have on several occasions failed to maintain by passage in susceptible cattle strains obtained from natural outbreaks of the disease. One strain of cattle virus was much less virulent for goats than the goat adapted virus which never kills our most susceptible cattle.

The cattle of Burma vary greatly in their susceptibility to rinderpest. Mention has also been made of this in other countries, and it is possible that this variation in susceptibility and the difference in virulence of strains account for much of the lack of uniformity reported in immunization against rinderpest. This is possibly the reason for my failure to confirm the results obtained by Bennett with his glycerine vaccine.

Although doubt has been cast on the living nature of at least some of the filterable viruses, there is some justification for speaking of "attenuation" and "killed" in connection with rinderpest virus. At any rate it is convenient to do so.

Curasson (1930) has condemned as useless vaccines prepared from attenuated virus. Although killed virus will immunize against such infections as herpes, psittacosis and distemper, it is open to doubt whether any really effective rinderpest vaccine does not contain living virus, however attenuated. In 1928 Daubney stated "Whether the reaction to inoculation with vaccines prepared in weak formalin depends upon the presence of a minimal dose of unchanged living virus, the size of the dose being the determining factor in the severity of the reaction, if any, provoked; or whether the virus is in any way progressively attenuated by the action of formalin without being actually killed, are problems that must await solution by further experiment". Edwards (1925) made a most interesting observation. He stored blood virus at body heat under paraffin. After three months the virus had become inert for cattle, but intense passage through rabbits brought back its original virulence for cattle. In view of the fact that Walker (1929) showed that in blood, virus becomes inert in a matter of hours, and that Edwards was unable to preserve the potency of blood virus for more than eight days, it would seem that when apparently dead, rinderpest virus may be only greatly attenuated. On the other hand Shilston (1917) noted that at Muktesar defibrinated blood remained virulent for 40 days at room temperature.

The most lasting immunity is that conferred by virulent cattle virus in combination with either immune serum or vaccine. It has been shown that the immunity conferred by goat virus and serum is

not as lasting as that conferred by cattle virus and serum. Investigators have searched for a vaccine to overcome the disadvantages of the serum-virus method, but while they have gained in cost and safety they have lost in duration of immunity. All vaccines prepared from cattle tissues are rendered safe by storage or by exposure to chemicals. Even when they have reached the safety stage they may still kill highly susceptible cattle, as was shown by Cooper (1931), indicating that they still contain active virus. In connection with tissue vaccines, "safety" is a purely relative term. After storage at 42° F. for five months even the most virulent cattle virus loses its power to kill susceptible cattle, and instead immunizes them. An infinitesimal quantity of fully potent virulent virus kills a susceptible animal but the more attenuated that virus becomes the greater the quantity required to produce a reaction. Some consider that rinderpest virus does not become attenuated and that the effect of such material is due to the survival in the injected material of minute quantities of virus, but considering the very minute quantities of virulent material required to kill a susceptible animal and the regularity with which reactions may be provoked by large quantities of "attenuated" virus, a more reasonable explanation is that all or nearly all the virus has become attenuated, and that none, or very little, has been killed. This subject is of more than academic interest as if it can be shown experimentally that tissue vaccines depend upon living but attenuated virus for their immunizing effect, the necessity for not allowing attenuation to go too far is obvious. There is no doubt that for goat adapted virus to immunize it must be fully potent. 0·01 gram of dried spleen is an unreliable immunizing agent after exposure for six days at 97° F. But even after seven days at this temperature 0·5 gram will immunize, indicating that a large quantity of attenuated virus may be successfully substituted for a small quantity of fully potent virus. Of great practical importance is the short time for which goat virus remains effective at a temperature of 97° F. A small preliminary experiment indicated that by exposure for twelve hours to 80° F. and 97° F. alternately the virus becomes attenuated as rapidly as it does on exposure to a constant temperature of 97° F. It is not known if the virus may be attenuated to a required degree by exposure to a temperature of 97° F. and then maintained at this strength by storage at about zero.

In Burma during the hot months of March, April and May vaccine despatched to the field is exposed to temperatures of 97° F. and more for many hours each day and in many parts during these months the temperature never drops below 85° F. After 6 days vaccine stored alternatively for twelve hours at 97° F. and for twelve hours at 80° F. failed to immunize in doses of 0·01 gram so that vaccine despatched from the Laboratory not packed on ice would be exposed to fairly high temperatures, and rapidly lose its immunizing properties. Only with vaccine kept on ice can one hope to reproduce Laboratory results. Fortunately in Burma ice is easily obtainable and for that reason keeping the vaccine on ice presents no difficulty. The only alternative to this method of despatch is for inoculators to prepare vaccine as required. Very few of our inoculators could be entrusted with this and the procedure would in any case meet with opposition as it would offend against the religious principles of the people.

VI. Summary.

(*a*) The various methods of immunizing against rinderpest are briefly summarised, the use of goat adapted virus being dealt with more fully.

(*b*) Experiments leading up to the production of a vaccine prepared from dried goat spleen are described.

(*c*) The dried vaccine is prepared from the spleen of a goat destroyed on the fourth day after injection with goat adapted virus. The spleen is minced in a Latapie and the pulp rapidly dried in vacuo over calcium chloride.

(*d*) Packed in a thermos jar of ice, which is replenished every third day, the vaccine is emulsified in normal saline immediately before use, and used in a dose of one gram for 400 cattle and one gram for 2,400 buffaloes, although experiments have shown that 0·00025 gram is equally effective for cattle.

(*e*) Immunity commences within twenty-four hours and is solid within forty-eight hours. It endures for at least 755 days, the limit to which it has so far been tested.

(*f*) 576,782 cattle and 42,100 buffaloes were inoculated during the fifteen months ending 31st March, 1938. The mortality in them was 0·092 per cent. in cattle and 1·78 per cent. in buffaloes. Complete reports are not yet available, but those received show that from October, 1935, to 31st March, 1938, 759,792 cattle and 62,474 buffaloes were inoculated.

(*g*) The opinion is expressed that to confer lasting immunity tissue vaccines must contain living virus.

Acknowledgements.

It is a pleasure to acknowledge my indebtedness to Mr. D. T. Mitchell, Director of Veterinary Services, Burma, for providing facilities for the experimental work, and for arranging the initial field trials; to Dr. P. J. du Toit, Director of Veterinary Services, Union of South Africa, for many favours granted during study leave spent at Onderstepoort; to my colleague, Mr M. H. V. Brown, for invaluable help in supervising experiments during my absences from the Laboratory; to my wife for drawing the figures; and to my assistant, U Tun Aung, for help in keeping experimental records and in training inoculators in the final preparation and use of the vaccine.

REFERENCES.

ALEXANDER, R. A. (1933). Preliminary note on the infection of white mice and guinea-pigs with the virus of horsesickness. *J.S. Afr. Vet. Med. Ass.*, Vol. 4, pp. 1-9.

ANDRIEVSKY (1931). Sur la préparation du vaccin contre la peste bovine et son application methodique. *Rev. gen. Med. Vet.*, Vol. 40, pp. 728-733. Abst. in *Vet Bull.* Welbridge, Vol. 2, p. 607.

BANERJI, P. C. (1933). The duration of immunity in calves inoculated by the goat virus cum serum method against rinderpest. *Indian J. Vet. Sci.*, Vol. 3, pp. 338-345.

BEATON, W. G. (1930). Rinderpest in goats in Nigeria. *J. Comp. Path.*, Vol. 43, pp. 301-307.

BEATON, W. G. (1931). The use of formalised virulent rinderpest blood as a vaccine. *Vet. J.*, Vol. 87, pp. 530-532.

BEDERKE, O. (1931). An der transkaukasischen Rinderpestgrenze. *Berl. tierarztl. Wschr.*, Vol. 47, pp. 186-187.

BEDSON, S. P., MAITLAND, H. B., AND BURBURY, Y. M. (1927). Further observations on foot-and-mouth disease. *J. Comp. Path.*, Vol. 40, p. 5.

BLISS, E. L. (1922). Reprint from *China Med. J.*, March, 1922. Cited by Banerji (1933).

BOYNTON, W. H. (1917). Note on the use of organ extracts in place of virulent blood in immunisation and hyperimmunisation against rinderpest. *Philipp. Agric. Rev.*, Vol. 10, pp. 448-455.

BOYNTON, W. H. (1928). Rinderpest, with special reference to its control by a new method of prophylactic treatment. *Philipp. J. Sci.*, Vol. 36, pp. 1-35.

BRASSEY-EDWARDS, H. H. (1936). *Rep. Dep. Agric., Kenya*, 1934, pp. 114-145. Abst. in *Vet. Bull.*, Weybridge, Vol. 8, p. 57.

CHICOLI (1863). Cited by Hutyra & Marek (1926). *Spec. Path. and Therap.* London.

CONTI, G. (1933). L'impiego del Trypanblau nella pratica della siero-vaccinazione contro la peste bovina. *Clin. Vet.*, Milan, Vol. 56, pp. 592-595. Abst. in *Vet Bull.*, Weybridge, Vol. 4, p. 346.

COOPER, H. (1931). *Ann. Rep. Inst. Vet. Res. Muktesar*, for year ending 31.3.1931. *Rep. of Vet. Res. Officer*, pp. 13-22.

CURASSON, G. (1930). La Prophylaxie de la Peste bovine. *Eleventh Inter. Vet. Congr.*, London, 1930.

CURASSON, G. (1931). Nouvelles recherches sur la peste bovine. *Bull. Acad. vet. de France*, Vol. 4, pp. 304-310.

CURASSON, G. (1934). La Peste bovine dans l'Afrique occidentale française pendant l'année 1933. *Bull. off. internat. Epiz.*, Vol. 9, pp. 2-12.

CURASSON, G. (1936). Sur les methodes d'immunisation a employer contre la peste et la peripneumonie bovines. *Conf. consult. Elevage Afrique, occ. française*, pp. 41-55 (see p. 53). Abst. in *Vet. Bull.*, Weybridge, Vol. 8, p. 25.

CURASSON, G., AND DELPY, L. (1926). Sur l'immunisation contre la peste bovine par le virus formolé. *Rec. Med. Vet.*, Vol. 102, p. 297.

CURASSON, G., AND ZYLBERTAL (1934). Sur la vaccination intradermique dans la peste bovine. *Bull. Acad. Vet. Fr.*, Vol. 7, pp. 265-267.

CURSON, H. H. (1936). Theiler and the rinderpest epizootic of 1896-1903. *J.S. Afr. Vet. Med. Ass.*, Vol. 7, pp. 187-203.

DAUBNEY, R. (1928). Observations on rinderpest. *J. Comp. Path.*, Vol. 31, pp. 228-248, and pp. 263-298.

DAUBNEY, R. (1930). *Ann. Rep. Dept. Agric. Kenya*, 1929, pp. 210-224. Nairobi.

DAUBNEY, R. (1934). *Ann. Rep. Dept. Agric. Kenya*, 1933, pp. 358-365. Nairobi.

D'COSTA, J., AND BALWANT SINGH (1933a). Rinderpest: Clinical syndrome in goats in India. *Indian. J. Vet. Sci.*, Vol. 3, pp. 122-128.

D'COSTA, J., AND BALWANT SINGH (1933b). The duration of immunity in calves inoculated by the bull virus cum serum method against rinderpest. *Indian. J. Vet. Sci.*, Vol. 3, pp. 131-148.

DELPY, W. L. (1935a). La peste bovine en Perse. *Rev. Gen. Med. Vet.*, Vol. 44, pp. 577-601. Abst. in *Vet. Bull.*, Weybridge, Vol. 6, p. 328.

DELPY, W. L. (1935b). Nouvelles modifications apportées a la methode de preparation du vaccin antipestique formole. Vaccin liquide et vaccin sec. *Bull. Acad. Vet. Fr.*, Vol. 8, pp. 444-449.

DELPY, W. L. (1936). Sur la preparation du vaccin antipestique déssèché hors du laboratoire, avec un materiel simplifié. *Rec. méd. vét. exot.*, Vol. 9, pp. 145-151. Abst. in *Vet. Bull.*, Weybridge, Vol. 7, p. 595.

EDINGTON, A. (1899). A retrospect of the rinderpest campaign in South Africa. *Lancet*, Vol. 1 of 1899, p. 357.

EDWARDS, J. T. (1925). *Ann. Rep. Inst. vet. Res.* Muktesar for two years ending 31.3.1924. Calcutta.

EDWARDS, J. T. (1926). *Ann. Rep. Inst. Vet. Res.* Muktesar for year ending 31.3.1925. Calcutta.

EDWARDS, J. T. (1927a). Rinderpest: Some properties of the virus and further indications for its employment in the serum-simultaneous method of protective inoculation. *Trans. Seventh Congress Far Eastern Ass. Trop. Med.*, Vol. 3, pp. 699-706.

EDWARDS, J. T. (1927b). *Ibid.*, p. 645. (Discussion on paper by Kelser *et al.*).

EDWARDS, J. T. (1927c). Rinderpest: Some points in immunity. *Ibid.*, pp. 707-717.

EDWARDS, J. T. (1928a). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1927. Calcutta.

EDWARDS, J. T. (1928b). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1928. Calcutta.

EDWARDS, J. T. (1930). The problem of rinderpest in India. *Imp. Inst. Agric., Pusa. Bull. No.* 199 (Govt. of India.) Calcutta.

FLEMMING, G. (1882). Animal plagues: their History, Nature, and Prevention. London.

GALAMBOS (1861). Cited by Hutyra and Marek (1926). *Spec. Path. and Therap.* London.

GORDZIAKLOWSKI (121). Note sur une nouvelle inoculation preventive contre la peste bovine. *Rec. méd. vét.*, Vol. 97, p. 115. Cited by Hall (1933).

GRANONHLLIT, F., AND DO-VAN-VIEN (1935). Note sur la reaction locale due aux injections de vaccin antipestique a pulpe (vaccin au toluène). *Rec. vét., Toulouse*, Vol. 87, pp. 621-624. Abst. in *Vet. Bull.*, Weybridge, Vol. 6, p. 509.

HADDOW, J. R. (1932). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1932. Rep. of the Serology Sect., pp. 22-28. Calcutta.

HADDOW, J. R. (1933). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1933. Rep. Serology Sect., pp. 20-26. Delhi.

HADDOW, J. R. (1934). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1934. Rep. Serology Sect., pp. 15-24. Delhi.

HADDOW, J. R. (1935). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1935. Rep. Serology Sect., pp. 16-23. Delhi.

HADDOW, J. R. (1937). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1936. Rep. Serology Sect., pp. 14-21. Delhi.

HALL, G. N. (1930). *Ann. Rep. Vety. Dept.*, Northern Provinces, Nigeria. 1929. Lagos.

HALL, G. N. (1933). Investigations on rinderpest immunization. Zurich.

HALLEN, J. H. B. (1871). Rep. Ind. Cattle Plagues, pp. 883-889.

ISMAIL, R., AND ZUDHI, M. (1933). Die Rinderpest in der Turkei Bekämpfungsmethoden und neue Versuche. *Arch. wiss. prakt. Tierhlk.*, Vol. 66, pp. 79-85.

JACOTOT, H. (1929). Sur la vaccination contre la peste bovine au moyen de pulpes organiques. *Bull. Soc. Path. Exot.*, Vol. 22, pp. 310-312.

JACOTOT, H. (1931). Recherches sur la vaccination contre la peste bovine. *Bull. Soc. Path. Exot.*, Vol. 24, pp. 789-793.

JACOTOT, H. (1932). Observations et recherches sur la peste bovine du bétail d'Indochine. *Arch. Inst. Pasteur d'Indochine*, Vol. 15, pp. 7-95.

JACOTOT, H., AND LE ROUX, G. (1935). La vaccination intradermique en matière de peste bovine. *Bull. Soc. Path. Exot.*, Vol. 28, pp. 753-762.

JUGE (1935). De la protection conferée au cheptel indochinois contre le peste bovine par le vaccin avirulent a base de pulpes d'organes. These; Docteur Veterinaire, Lyons, p. 88. Abst. in *Vet. Bull.*, Weybridge, Vol. 6, p. 39.

KAKIZAKI, C. (1918). Study on the glycerinated rinderpest vaccine. *Kitasato Arch. Exp. Med.*, Vol. 2, pp. 59-66. Cited by Bennett (1936).

KAKIZAKI, C., NAKANISHI, S., AND OIZUMI, T. (1926). Experimental studies on the prophylactic inoculation against rinderpest. 3rd Communication. *Jour. Jap. Socy. Vety. Sci.*, Vol. 5, pp. 221-280. Also Fourth Report, etc. (1927). Cited by Bennett (1936).

KEARNEY, W. (1930). Eleventh International Vety. Congress, London.

KELSER, R. A. (1927). A new vaccine for rinderpest immunisation. *Milit. Surgeon*, Vol. 61, p. 31. Abst. in *Trop. Vety. Bull.*, Vol. 16, pp. 33-34.

KELSER, R. A., YOUNGBERG, S., AND TAPACIO, T. (1927). An improved vaccine for immunization against rinderpest. *Trans. 7th Congress Far Eastern As. Trop. Med.*, Vol. 3, pp. 628-646.

KERR, P. J. (1935). The use of goat-tissue vaccine for the control of outbreaks of rinderpest in Bengal. *Indian J. Vet. Sci.*, Vol. 5, pp. 67-89.

KERR, P. J., AND MENON, M. B. (1934). Preliminary note on the use of goat tissue vaccine alone for the control of outbreaks of rinderpest. *Indian J. Vet. Sci.*, Vol. 4, pp. 75-80.

KOCH, R. (1897). Berichte des Herrn. Prof. Dr. Koch über sein Kimberley gemachten Versuche bezüglich Bekämpfung der Rinderpest. *Cent. f. Bak.*, Vol. 21, p. 526.

KOLLE, W., AND TURNER, G. (1898). Über Schutzimpfungen und Heilserum bei Rinderpest. *Zeit. f. Hyg.*, Vol. 29, p. 309.

KOHLSTOCK (1897). Die sanitären Massnahmen gegen die Rinderpest in Südwest Afrika. *Deutch. Kolonialblatt.*, Vol. 8, p. 22. Abst. in *Cent. f. Bakt.*, Vol. 22, p. 787.

LINGARD, A. (1905). Preparation of rinderpest protective serum. Calcutta.

LE LOUET, G. (1932). La Peste bovine en Indochine en 1931. *Bull. off. internat. Epiz.*, Vol. 6, pp. 449-460.

METTAM, R. W. M. (1934). *Ann. Rep. Vet. Dept., Uganda*, 1933. *Ann. Rep. Vet. Pathologist, Entebbe*, pp. 29-45.

MISSENARD AND ZYLBERTAL (1934). Quelques observations sur la peste bovine au Soudan. *Rec. Med. vet. Exot.*, Vol. 7, pp. 5-9. Abst. in *Vet. Bull.*, Weybridge, Vol. 5, p. 14.

OSTERTAG, R. VON (1916). Ueber Rinderpest. Ein Beitrag zum Stande und zur Bekämpfung der Tierseuchen in Deutsch-Ostafrika. *Zeit. f. Inf. u. Hyg. d. Haust.*, Vol. 18, p. 1. Cited by Hall (1933).

PRUNIER, R. (1931). Utilisation de l'huile de ricin dans le traitement et la prévention de la peste bovine. *Bull. de l'Acad. Vet.*, Vol. 6, p. 153.

QUIRKE, T. F. (1927). *Rep. civ. vet. Dep. Punjab for year* 1936-27. Lahore.

ROBLES, M. M., AND GENEROSO, J. D. (1930). A Preliminary report on dried rinderpest vaccine. *Philipp. J. Agric.*, Vol. 1, pp. 367-380.

ROBLES, M. M. AND GENEROSO, J. D. (1931). Progress report on dried rinderpest vaccine. *Philipp. J. Agric.*, Vol. 2, pp. 341-346.

RODIER, E. A. (1928). A single-injection method of immunization against rinderpest. *Philipp. J. Sci.*, Vol. 36, pp. 397-407.

SAID, A. (1934). La peste bovine en Syrie. *Rec. Med. Vet. Exot.*, Vol. 7, pp. 115-128.

SAUNDERS, P. T., AND AYYAR, K. K. (1936). An experimental study of rinderpest virus in goats in a series of 150 direct passages. *Indian J. Vet. Sci.*, Vol. 6, pp. 1-86.

SAWYER, W. A., AND LLOYD, W. (1931). The use of mice in immunity tests against yellow fever. *J. Exp. Med.*, Vol. 54, pp. 533-555.

SEMMER (1893). *Berliner Tierarzt. Woch.*, p. 590. Cited by Hutyra and Marek (1926).

SHILSTON, A. W. (1917). The vitality of the rinderpest virus outside the animal body under natural conditions. pp. 32. *Memoirs Dep. Agric. India, Vet. Series*, Vol. 3, No. 1. Calcutta.

STIRLING, R. F. (1932). Some experiments in rinderpest vaccination: Active immunisation of Indian plains cattle by inoculation with goat-adapted virus alone in field conditions. *Vet. J.*, Vol. 88, pp. 192-204.

STIRLING, R. F. (1933). Ibid. (Second Report.) *Vet. J.*, Vol. 89, pp. 290-306.

TAYLOR, W. (1938). *Ann. Rep. Inst. Vet. Res.*, Muktesar, for year ending 31.3.1937. Delhi.

TAHSIN, S., AND RICHTER, H. (1934). Ueber die Bekämpfung der Rinderpest in der Turkei. *Tierärztl. Rdsch.*, Vol. 40, pp. 537-539. Abst. in *Vet Bull.*, Weybridge, Vol. 5, p. 130.

THEILER, A. (1897). Rinderpest in Süd-Afrika. *Schweiz. Arch. f. Tierh.*, Vol. 39, p. 49.

THEILER, A. (1897). Experimentaluntersuchungen uber Rinderpest. *Schweiz. Arch. f. Tierh.*, Vol. 39, p. 193.

THEILER, A. (1898). Blutserum immuner Tiere im Kampfe gegen die Rinderpest. *Deutsch. tierärzt. Woch.*, Vol. 4, p. 205.

THEILER, A. (1904). Die Simultanimpfung gegen Rinderpest und ihre Gefahr. *Monatsch. f. prakt. Tierheilk.*, Vol. 16, p. 195.

THEILER, MAX (1930). Studies on the action of yellow fever in mice. *Ann. Trop. Med. Parasit.*, Vol. 24, pp. 249-292.

TOPACIA, T. (1926). *Philipp. Agric. Rev.*, Vol. 19, p. 4. Cited by Banerji (1933).

VALLEE, H., CARREE, H., AND RINJARD, P. (1926). Sur l'immunization antiaphtheuse par le virus formolé. *Rev. Gen. Med. Vet.*, Vol. 36, p. 129.

WALKER, J. (1921). Rinderpest immunization. *Dep. Agric., Kenya Colony, Bull. No.* 2.

WALKER, J. (1929). *Pan-African Vet. Conf.*, Pretoria, 1929. *Dept. Agric., Kenya, Bull. No.* 8.

WALKER, J., AND BRADSHAW, J. T. C. (1925). The utilisation of sheep for the supply of rinderpest virus free of *B. bigeminum* (redwater) and anaplasmosis (gallsickness) infections.

WARE, F. (1933). A description of the Imperial Institute of Veterinary Research Muktesar, and its sub-station. The Imperial Veterinary Serum Institute, Izatnagar. Delhi.

Rinderpest statistics for the colony of the Cape of Good Hope. Compiled by the Dept. of Agric., May, 1898. Capetown.

APPENDIX.

Tables 1 to 18.—Giving details of experiments described in the text.

NOTE.—1. In all tables (K) indicates a highly susceptible Kyaukpyu animal, and (L) indicates an animal bred in Burma, of unknown susceptibility, but usually more resistant to rinderpest than the Kyaukpyu cattle.

2. In tables 1 to 18 the temperature in all cases refers only to that recorded before eight o'clock in the morning, the evening temperature being probably accentuated by the heat of the day and for that reason being disregarded.

3. In immunity tests "Immune" indicates no reaction whatever.

TABLE 1. (EXPERIMENT 1.)

The passage of the Kungyangon strain of rinderpest cattle virus in Kyaukpyu cattle.

Passage Number.	ANIMAL.			INJECTION OF VIRUS.			REACTION.				FATE.	
	Number.	Age in years.	Sex.	Date Injected.	Material used. Blood.	Material used. Dried spleen powder.	Day of initial rise.	Day Peak reached	Peak temperature.	Rise in temperature.	Day died.	Day destroyed.
1	320	4	Cow. .	2/ 4/35	Blood from ox and buffalo with naturally acquired Rinderpest	—	3rd	8th	103·6°F.	3·4°F.	11th	—
2	278	1½	Cow...	11/ 4/35	Ex. 320 direct..	—	3rd	5th	104·2°F.	4°F.	—	8th
3	289	1½	Bull..	23/ 4/35	—	Ex. 278 stored for 4 days at 42°F.	3rd	5th	104°F.	4°F.	9th	—
4	298	2½	Bull...	28/ 4/35	Ex. 289 direct.	—	3rd	5th	104°F.	4°F.	9th	—
5	291	1	Bull...	3/ 5/35	Ex. 298 direct.	—	3rd	5th	105·4°F.	5·2°F.	—	5th
6	314	3	Cow. .	8/ 5/35	Ex. 291 direct.	—	2nd	6th	105·8°F.	5·4°F.	9th	—
7	309	2	Cow..	13/ 5/35	Ex. 314 direct.	—	2nd	5th	106°F.	5·8°F.	—	6th
8	274	1½	Cow...	18/ 5/35	Ex. 309 direct.	—	3rd	5th	105·2°F.	5°F.	—	6th
9	283	1	Bull...	23/ 5/35	Ex. 274 direct.	—	3rd	5th	102·6°F.	3°F.	10th	—
10	300	1	Bull...	28/ 5/35	Ex. 274 and 283 direct	—	3rd	4th	104·2°F.	4°F.	—	6th
11	311	1½	Cow...	2/ 6/35	Ex. 300 direct.	—	2nd	5th	104°F.	3·6°F.	8th	—
12	306	2	Cow. .	12/ 6/35	Ex. 311 direct.	—	3rd	5th	104·6°F.	4·6°F.	—	2nd
13	360	3½	Bull..	22/ 7/35	—	Ex. 306 stored for 39 days at 42°F.	2nd	6th	106°F.	6°F.	9th	—
14	348	3½	Bull...	27/ 7/35	Ex. 360 direct.	—	3rd	4th	103·8°F.	3·8°F.	7th	—
15	380	3	Bull...	1/ 8/35	Ex. 348 direct.	—	3rd	6th	105·4°F.	5·8°F.	9th	—
16	353	1	Cow...	11/ 8/35	Ex. 380 direct.	—	3rd	5th	105·6°F.	5·2°F.	—	5th
17	391	1	Bull...	16/ 8/35	Ex. 353 direct.	—	3rd	3rd	105°F.	5°F.	—	5th

TABLE 1—(*continued*).

Passage Number.	Animal.			Injection of Virus.			Reaction.				Fate.	
	Number.	Age in years.	Sex.	Date Injected.	Material used.		Day of initial rise.	Day Peak reached.	Peak temperature.	Rise in temperature.	Day died.	Day destroyed.
					Blood.	Dried spleen powder.						
18	352	2	Bull...	30/ 8/35	—	Spleen pulp ex. 391 in 50 per cent. Glycerine Saline stored for 9 days at 42°F.	3rd	4th	105°F.	4·6°F.	—	6th
19	374	2	Cow...	4/ 9/35	Ex. 352 direct.	—	3rd	6th	104·6°F.	3·4°F.	10th	—
20	383	1½	Bull...	9/ 9/35	Ex. 374 direct.	—	3rd	6th	105°F.	5°F	9th	—
21	371	4	Cow...	19/ 9/35	Ex. 383 direct.	—	3rd	5th	105·4°F.	4·8°F.	—	5th
22	351	1½	Cow...	1/11/35	—	Ex. 371 stored for 37 days at 42°F.	4th	4th	104·6°F.	3·6°F.	—	5th
23	390	3½	Cow...	17/11/35	—	Ex. 351 stored for 12 days at 42°F.	2nd	4th	105·4°F.	4·8°F.	8th	—
24	392	5	Cow...	22/11/35	Ex. 390 direct.	—	3rd	4th	103·4°F.	3·4°F	9th	—
25	463	5	Cow...	27/11/35	Ex. 392 direct.	—	3rd	4th	103·4°F.	2·4°F.	—	5th
26	474	1	Bull...	28/12/35	—	Ex. 463 stored for 29 days at 42°F.	2nd	5th	103·4°F.	4·4°F.	—	5th
27	456	4	Cow...	16/ 1/36	—	Ex. 474 stored for 17 days at 42°F.	3rd	5th	102·6°F.	3·6°F.	11th	—
28	464	2½	Cow...	21/ 1/36	Ex. 456 direct.	—	3rd	4th	103°F.	3°F.	10th	—
29	450	3	Cow...	26/ 1/36	Ex. 464 direct.	—	3rd	6th	104·8°F.	4·8°F.	—	6th
30	470	2	Cow...	15/ 2/36	—	Ex. 450 stored for 16 days at 42°F.	3rd	5th	104°F.	5°F.	—	5th
31	446	1	Cow...	24/ 2/36	—	Ex. 470 stored for 5 days at 42°F.	2nd	6th	105°F.	5·6°F.	—	6th
32	471	2	Bull...	15/ 3/36	—	Ex. 446 stored for 16 days at 42°F.	3rd	5th	103·4°F.	3·8°F.	—	5th
33	457	2½	Cow...	3/ 4/36	—	Ex. 471 stored for 15 days at 42°F.	3rd	4th	104·8°F.	3·8°F.	—	5th
34	475	2½	Cow...	22/ 4/36	—	Ex. 457 stored for 15 days at 42°F.	3rd	5th	104°F.	3·8°F.	—	5th
35	435	3½	Bull...	11/ 5/36	—	Ex. 475 stored for 15 days at 42°F.	3rd	5th	104·8°F.	3·6°F.	—	5th
36	443	5	Bull...	30/ 5/36	—	Ex. 435 stored for 15 days at 42°F.	3rd	5th	105·6°F.	4·6°F.	—	5th
37	499	3	Cow...	11/ 6/36	—	Ex. 443 stored for 15 days at 42°F.	3rd	4th	104·2°F.	3·2°F.	8th	—
38	514	1	Cow...	16/ 6/36	Ex. 499 direct.	—	3rd	4th	104·6°F.	3·6°F.	8th	—
39	516	2	Cow...	21/ 6/36	Ex. 514 direct.	—	3rd	3rd	103·8°F.	3·2°F.	—	5th

TABLE 1—(*continued*).

Passage Number.	Animal.			Injection of Virus.			Reaction.				Fate.	
	Number.	Age in years.	Sex.	Date Injected.	Material used.		Day of initial rise.	Day Peak reached.	Peak temperature.	Rise in temperature.	Day died.	Day destroyed.
					Blood.	Dried spleen powder.						
40	522	1½	Cow...	1/ 7/36	—	Ex. 516 stored for 6 days at 42°F.	3rd	5th	104·8°F.	4·6°F.	—	5th
41	525	2	Cow...	1/ 8/36	—	Ex. 522 stored for 17 days at 42°F.	4th	8th	105°F.	5°F.	—	Recovered.
42	531	1½	Cow...	6/ 8/36	Ex. 525 direct.	—	3rd	5th	105·2°F	5·2°F	—	5th
43	498	3	Cow...	2/ 9/36	—	Ex. 531 stored for 23 days at 42°F.	2nd	5th	104°F.	3·6°F.	—	5th
44	528	4	Cow...	24/ 9/36	—	Ex. 498 stored for 18 days at 42°F.	4th	5th	104·8°F	4·4°F.	—	5th
45	527	3½	Cow...	17/10/36	—	Ex. 528 stored for 19 days at 42°F.	4th	5th	104·4°F.	3·6°F.	—	5th
46	502	2	Cow...	18/11/36	—	Ex. 527 stored for 28 days at 42°F.	3rd	5th	105·6°F.	5·2°F.	—	5th
47	492	3	Bull...	12/12/36	—	Ex. 502 stored for 20 days at 42°F.	3rd	5th	104°F.	4°F.	—	5th
48	486	3	Cow...	16/ 1/37	—	Ex. 492 stored for 31 days at 42°F.	3rd	5th	103·2°F	4·2°F.	—	5th
49	532	2	Cow...	20/ 2/37	—	Ex. 486 stored for 31 days at 42°F.	3rd	5th	104°F.	3·6°F.	—	5th
50	534	5½ months	Heifer	19/ 3/37	—	Ex. 532 stored for 23 days at 42°F.	2nd	5th	104·6°F.	3·6°F.	—	5th
51	491	4	Cow...	17/ 4/37	—	Ex. 534 stored for 25 days at 42°F.	3rd	5th	104·6°F.	4·6°F.	—	5th
52	545	3½	Bull...	15/ 5/37	—	Ex. 491 stored for 24 days at 42°F.	2nd	5th	104·2°F.	4·4°F.	—	5th
53	550	2½	Cow...	21/ 6/37	—	Ex. 545 stored for 33 days at 42°F.	2nd	4th	104·2°F.	4·2°F.	—	5th
54	543	2	Bull...	21/ 7/37	—	Ex. 550 stored for 26 days at 42°F.	2nd	4th	104°F.	4°F.	—	5th
55	542	2	Bull...	23/ 8/37	—	Ex. 543 stored for 29 days at 42°F.	2nd	3rd	104°F.	4°F.	—	5th
56	555	3	Cow...	22/ 9/37	—	Ex. 542 stored for 26 days at 42°F.	2nd	5th	104·6°F.	4·6°F.	—	5th
57	546	3½	Bull...	25/10/37	—	Ex. 555 stored for 29 days at 42°F.	3rd	5th	105°F.	5°F.	—	5th
58	551	2	Cow...	27/11/37	—	Ex. 546 stored for 29 days at 42°F.	2nd	5th	104·8°F.	4·8°F.	—	5th
59	564	2½	Cow...	27/12/37	—	Ex. 551 stored for 26 days at 42°F.	2nd	5th	104°F.	4·2°F.	—	5th

TABLE 2. (EXPERIMENT 2.)

To determine the effect of storage at 42° F. on Rinderpest Virus (Cattle Strain) in the form of powdered spleen dried in Vacuo over Calcium Chloride.

Cattle Number.	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11	12
		Gram.	Days.								
366 (K)	5/8/35	0·01	77	5th	8th	105·6°F.	4·8°F.	Died on 11th day.	—	—	—
381 (K)	5/8/35	0·05	77	5th	8th	105°F.	3·8°F.	Died on 12th day.	—	—	—
358 (K)	27/7/35	0·01	100	9th	13th	104·4°F.	3·8°F.	Died on 16th day.	—	—	—
354 (K)	27/7/35	0·01	100	10th	12th	104·2°F.	2·2°F.	Died on 15th day.	—	—	—
385 (K)	28/9/35	0·01	133	4th	5th	103·8°F.	2·4°F.	Died on 11th day.	—	—	—
377 (K)	28/9/35	0·01	133	7th	9th	105·4°F.	4·2°F.	Recovered	2/12/35	2 c.cm.	Immune.
321 (K)	6/4/35	0·8	177	5th	8th	105°F.	4·4°F.	Recovered.....	28/ 4/35	5 c.cm.	Immune.
304 (K)	6/4/35	1·6	177	7th	8th	103·2°F.	1·2°F.	Recovered.....	28/ 4/35	5 c.cm.	Immune.
301 (K)	6/4/35	1·6	177	4th	8th	105°F.	4·2°F.	Recovered	28/ 4/35	5 c.cm.	Immune.
298 (K)	Control to	Nos. 321,	304 and 301	—	—	—	—	—	28/ 4/35	5 c.cm.	Died on 9th day.

NOTE.

The Virus used on Nos. 366, 381, 385 and 377 was derived from Cattle Bovine No. 309 (Kungyangon Strain).
The Virus used on Nos. 358 and 354 was derived from Cattle Bovine No. 309 (Kungyangon Strain).
The Virus used on Nos. 321, 304 and 301 was derived from Buffalo No. 33 (Moulmein Cattle Strain).

TABLE 3. (EXPERIMENT 3.)

To determine the viability at 42° F. of Goat Adapted Rinderpest Virus in the form of a 20 per cent. emulsion of spleen pulp in 50 per cent. Glycerine-Saline.

Goat Number.	Injection of Virus.			Reaction.				
	Date Injected.	Quantity of 20 Per cent. Spleen Emulsion.	Period Stored at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
255.....	17/12/34	2 c.cm.	20 days	2nd	3rd	105°F.	3·6°F.	Recovered.
273.....	6/ 1/35	2 c.cm.	40 days	3rd	4th	105°F.	3°F.	Recovered.
281.....	17/ 1/35	3 c.cm.	51 days	2nd	3rd	104°F.	2·2°F.	Died on 6th day.
289.....	25/ 1/35	2 c.cm.	60 days	2nd	5th	105·8°F.	4·2°F.	Recovered.
288.....	16/ 2/35	2 c.cm.	80 days	3rd	4th	102·8°F.	1·4°F.	Recovered.
306.....	14/ 3/35	2 c.cm.	102 days	8th	9th	101·4°F.	1·4°F.	Recovered.
353.....	2/ 4/35	2 c.cm.	121 days	16th	17th	104·8°F.	2·8°F.	Recovered.
332.....	22/ 4/35	2 c.cm.	141 days	3rd	8th	104·8°F.	2·8°F.	Recovered.
313.....	13/ 5/35	2 c.cm.	162 days	No distinct thermal reaction.				Died on 35th day
364.....	24/ 5/35	2 c.cm.	173 days	6th	10th	104·6°F.	2·6°F.	Died on 26th day.
370.....	14/ 6/35	2 c.cm.	193 days	5th	7th	105°F.	2·6°F.	Recovered.
375.....	2/ 7/35	2 c.cm.	211 days	10th	11th	104·6°F.	2·2°F.	Died on 24th day.
380.....	22/ 7/35	2 c.cm.	231 days	8th	9th	104·4°F.	1·2°F.	Recovered.

TABLE 4. (EXPERIMENT 4.)

To determine the viability at 83° to 86° F. of Goat Adapted Rinderpest Virus in the form of a 1 per cent. emulsion spleen pulp in 50 per cent. Glycerine-Saline.

Goat Number.	Injection of Virus.			Reaction.				
	Date Injected.	Quantity of 1 Per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Period Stored at 83° to 86°F. after two days at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
322.....	26/ 3/35	2 c.cm.	3 days	3rd	11th	104°F.	2·6°F.	Recovered.
323.....	26/ 3/35	2 c.cm.	3 days	2nd	14th	104·8°F.	4·2°F.	Recovered.
324.....	26/ 3/35	2 c.cm.	3 days	4th	9th	102·8°F.	2·4°F.	Recovered.
312.....	29/ 3/35	2 c.cm.	6 days	3rd	19th	104·4°F.	3·6°F.	Recovered.
329.....	29/ 3/35	2 c.cm.	6 days	2nd	13th	104·6°F.	2·2°F.	Recovered.
336.....	29/ 3/35	2 c.cm.	6 days	1st	3rd	102·4°F.	1·8°F.	Recovered.
328.....	1/ 4/35	2 c.cm.	9 days	4th	9th	104·6°F.	2·4°F.	Recovered.
346.....	1/ 4/35	2 c.cm.	9 days	9th	11th	103·2°F.	1·2°F.	Recovered.
340.....	1/ 4/35	2 c.cm.	9 days	3rd	16th	105·2°F.	3·2°F.	Recovered.

TABLE 5. (EXPERIMENT 5.)

To determine the immunizing properties of varying doses of Rinderpest Goat Spleen Vaccine in the form of a 4 per cent. emulsion of spleen pulp in 50 per cent. Glycerine-Saline after storage for four days at 42° F. followed by two days at 80° F.

Cattle Number.	Injection of Vaccine.		Reaction.					Immunity Test.		
	Date Injected.	Quantity of 4 per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11
245 (L)...	23/ 2/35	1 c.cm.	12th	13th	101·4°F.	1·6°F.	Recovered.	28/ 4/35	2 c.cm.	Rise in temperature from 100°F to 103·8°F. Diarrhoea Mouth lesions. Recovered.
242 (L). .	23/ 2/35	1 c.cm.	9th	13th	101·4°F.	1·4°F.	Recovered.	8/ 5/35	2 c.cm.	Rise in temperature from 100°F to 104·6°F. Diarrhoea. Severe mouth lesions. Recovered.
249 (L)..	23/ 2/35	1 c.cm.	12th	14th	102·8°F.	1·6°F.	Recovered.	8/ 5/35	2 c.cm.	Rise in temperature from 100°F to 102·4°F. Recovered.
254 (L)...	23/ 2/35	2 c.cm.	10th	11th	101·4°F.	1·2°F.	Recovered.	28/ 4/35	2 c.cm.	Rise in temperature from 100°F to 103·6°F. Diarrhoea. Mouth lessions. Recovered.
261 (L)...	23/ 2/35	2 c.cm.	6th	13th	101·2°F.	1·0°F.	Recovered.	8/ 5/35	2 c.cm.	Died of Rinderpest on 19th day.
271 (L)...	23/ 2/35	2 c.cm.	No	Reaction				8/ 5/35	2 c.cm.	Died of Rinderpest on 13th day.
246 (L)...	23/ 2/35	3 c.cm.	12th	13th	101·8°F.	1·8°F.	Recovered.	28/ 4/35	2 c.cm.	Rise in temperature from 100°F to 104·0°F. Diarrhoea. Severe mouth lesions. Recovered.
262 (L)...	23/ 2/35	3 c.cm.	No	Reaction				8/ 5/35	2 c.cm.	Rise in temperature from 101°F to 103·6°F. Diarrhoea. Severe mouth lesions. Recovered.
243 (L)...	23/ 2/35	3 c.cm.	12th	13th	102·0°F.	1·0°F.	Recovered.	8/ 5/35	2 c.cm.	Immune.
260 (L)...	23/ 2/35	4 c.cm.	10th	11th	101·6°F.	0·8°F.	Recovered.	28/ 4/35	2 c.cm.	Immune.
256 (L)...	23/ 5/35	4 c.cm.	No	Reaction				8/ 5/35	2 c.cm.	Rise in temperature from 100°F to 104·6°F. Diarrhoea. Severe mouth lesions. Recovered.
314 (K)..	Control							8/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.

TABLE 6. (EXPERIMENT 6.)

To determine the duration of immunity conferred by Rinderpest Goat Spleen Vaccine in the form of a 1 per cent. emulsion of spleen pulp in 50 per cent. Glycerine Saline after storage for—

(1) 10 days at 42° F.
(2) 2 days at 42° F. followed by 2 days at 84° to 86° F.
(3) 2 days at 42° followed by 8 days at 84° to 86° F.

Cattle Number.	Injection of Vaccine.			Reaction.					Immunity Test.		
	Date Injected.	Quantity of 1 per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
240 (L)...	31/ 3/35	2 c.cm.	10 days at 42°F.	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
248 (L)...	31/ 3/35	2 c.cm.	,,	3rd	4th	105·6°F.	4·6°F.	Recovered.	18/ 5/35	2 c.cm.	Immune.
267 (L)...	31/ 3/35	2 c.cm.	,,	No Reaction.					18/ 5/35	2 c.cm.	Immune.
275 (K)...	31/ 3/35	2 c.cm.	,,	No Reaction.					18/ 5/35	2 c.cm.	Immune.
282 (K)...	31/ 3/35	2 c.cm.	,,	6th	6th	103·2°F.	2·2°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
302 (K)...	31/ 3/35	2 c.cm.	,,	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
263 (L)...	25/ 3/35	2 c.cm.	2 days at 42°F. followed by 2 days at 84° to 86°F.	2nd	4th	102·8°F.	2·4°F.	Recovered.	8/ 5/35	2 c.cm.	Severe temperature reaction with Diarrhoea and mouth lesions. Recovered.
247 (L)...	25/ 3/35	2 c.cm.	,,	No Reaction.					8/ 5/35	2 c.cm.	,,
255 (L)...	25/ 3/35	2 c.cm.	,,	No Reaction.					18/ 5/35	2 c.cm.	,,
264 (L)...	25/ 3/35	2 c.cm.	,,	3rd	5th	101·8°F.	1·8°F.	Recovered.	18/ 5/35	2 c.cm.	,,
288 (K)...	25/ 3/35	2 c.cm.	,,	No Reaction.					8/ 5/35	2 c.cm.	,,
319 (K)...	25/ 3/35	2 c.cm.	,,	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 10th day.
299 (K)...	25/ 3/35	2 c.cm.	,,	3rd	6th	100·8°F.	1·8°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
303 (K)...	25/ 3/35	2 c.cm.	,,	No Reaction.					18/5/ 35	2 c.cm.	Died of Rinderpest on 9th day.

TABLE 6—(*continued*).

Cattle Number.	Injection of Vaccine.			Reaction.					Immunity Test.		
	Date Injected.	Quantity of 1 per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
253 (L)...	31/ 3/35	2 c.cm.	2 days at 42°F. followed by 8 days at 84° to 86°F.	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 10th day.
265 (L)...	31/ 3/35	2 c.cm.	,,	3rd	3rd	104·0°F.	4·0°F.	Recovered.	8/ 5/35	2 c.cm.	Temperature reaction only. Recovered.
270 (L)...	31/ 3/35	2 c.cm.	,,	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
251 (L)...	31/ 3/35	2 c.cm.	,,	3rd	6th	102·2°F.	2·0°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 8th day.
316 (K)...	31/ 3/35	2 c.cm.	,,	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
310 (K)...	31/ 3/35	2 c.cm.	,,	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
273 (K)...	31/ 3/35	2 c.cm.	,,	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
308 (K)...	18/ 5/35	2 c.cm.	,,	1st	2nd	103·6°F.	2·6°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 11th day.

TABLE 7. (EXPERIMENT 7.)

To determine the immunizing properties of Rinderpest Goat Spleen Vaccine in the form of—

(a) 1 per cent. emulsion in 50 per cent. Glycerine Saline. *(b) 1 per cent. emulsion in Normal Saline.*

After Storage (1) for 5 days at 42° F.
(2) for 5 days at 82° F.

Cattle Number.	Injection of Vaccine.			Reaction.					Immunity Test.		
	Date Injected.	Form of Vaccine.	Quantity.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
399 (L).	19/8/35	1 per cent. emulsion in 50 per cent. Glycerine-Saline after storage for 5 days at 42° F.	2 c.cm.			No definite temperature reaction.			9/9/35	2 c.cm.	Slight temperature reaction. Recovered.
402 (L).	19/8/35	,, ,,	2 c.cm.	3rd	4th	105·0°F.	3·0°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 99·2°F. to 101·4°F. Recovered.
395 (K)	19/8/35	,, ,,	2 c.cm.	3rd	5th	103·6°F.	2·4°F.	Recovered.	9/9/35	2 c.cm.	Mild temperature reaction. Recovered.
357 (K)	19/8/35	,, ,,	2 c.cm.	2nd	3rd	105·4°F.	4·6°F.	Recovered.	9/9/35	2 c.cm.	Immune.
408 (L).	19/8/35	1 per cent. emulsion in Normal Saline after storage for 5 days at 42°F.	2 c.cm.	1st	4th	106·0°F.	4·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
400 (L).	19/8/35	,, ,,	2 c.cm.	6th	7th	102·2°F.	1·2°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 100°F. to 103°F. Recovered.
389 (K)	19/8/35	,, ,,	2 c.cm.	3rd	6th	105·0°F.	4·0°F.	Recovered.	9/9/35	2 c.cm.	Mild temperature reaction. Recovered.
355 (K)	19/8/35	,, ,,	2 c.cm.	2nd	3rd	104·8°F.	3·2°F.	Recovered.	9/9/35	2 c.cm.	Mild temperature reaction. Recovered.
404 (L).	19/8/35	1 per cent. emulsion in 50 per cent. Glycerine-Saline after storage for 5 days at 82°F.	2 c.cm.	3rd	5th	102·4°F.	1·2°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 99°F. to 105·4°F. Diarrhoea. Severe mouth lesions. Recovered.
409 (L).	19/8/35	,, ,,	2 c.cm.			Mild temperature reaction.			9/9/35	2 c.cm.	Rise in temperature from 99·4°F. to 101·8°F. Recovered.
378 (K)	19/8/35	,, ,,	2 c.cm.	3rd	5th	105·6°F.	5·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
356 (K)	19/8/35	,, ,,	2 c.cm.	3rd	6th	104·6°F.	4·6°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 99·8°F. to 101·6°F. Recovered.
398 (L).	19/8/35	1 per cent. emulsion in Normal Saline after storage for 5 days at 82°F.	2 c.cm.	3rd	4th	103·6°F.	2·4°F.	Recovered.	9/9/35	2 c.cm.	Immune.
407 (L).	19/8/35	,, ,,	2 c.cm.	3rd	5th	105·4°F.	3·8°F.	Recovered.	9/9/35	2 c.cm.	Immune.
373 (K)	19/8/35	,, ,,	2 c.cm.	3rd	5th	104·4°F.	3·4°F.	Recovered.	9/9/35	2 c.cm.	Immune.
375 (K)	19/8/35	,, ,,	2 c.cm.			No reaction.			9/9/35	2 c.cm.	No reaction.
412 (L).	Control.								9/9/35	2 c.cm.	Rise in temperature from 101°F. to 103·2°F. on 7th day. Recovered.
383 (K)	Control.								9/9/35	2 c.cm.	Rise in temperature from 100°F. to 105°F. Diarrhoea. Mouth lesions. Died of Rinderpest on 9th day.

TABLE 8. (EXPERIMENT 8.)

To determine the viability at 42° F. of Goat Adapted Rinderpest Virus in the form of Powdered Spleen dried in vacuo over Calcium Chloride.

Goat Number.	Injection of Virus.			Reaction.				
	Date Injected.	Weight of Powdered Spleen.	Period Stored at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
257.....	17/12/34	0·2 gram	20 days	2nd	3rd	104·6°F.	3°F.	Recovered.
274.....	6/ 1/35	0·2 gram	40 days	3rd	3rd	105·2°F.	3·4°F.	Recovered.
287.....	25/ 1/35	0·1 gram	60 days	2nd	7th	106°F.	5°F.	Died on 12th day.
297.....	16/ 2/35	0·1 gram	80 days	2nd	3rd	105°F.	3°F.	Died on 29th day.
303.....	14/ 3/35	0·1 gram	102 days	3rd	3rd	103·8°F.	1·8°F.	Died on 11th day.
335.....	1/ 4/35	0·01 gram	120 days	4th	6th	105°F.	3°F.	Recovered.
337.....	1/ 4/35	0·1 gram	120 days	2nd	5th	105·2°F.	4·2°F.	Died on 7th day.
357.....	22/ 4/35	0·1 gram	141 days	3rd	7th	105°F.	2°F.	Died on 11th day.
355.....	14/ 5/35	0·1 gram	163 days	6th	15th	104·8°F.	2·6°F.	Died on 17th day.
369.....	12/ 6/35	0·1 gram	192 days	1st	4th	106·2°F.	4·2°F.	Recovered.
356.....	2/ 7/35	0·1 gram	211 days	6th	10th	105°F.	4·2°F.	Died on 17th day.
379.....	22/ 7/35	0·1 gram	231 days	8th	18th	105°F.	2·6°F.	Recovered.

TABLE 9. (EXPERIMENT 9.)

To determine the viability at 85° to 89° F. of Goat Adapted Rinderpest Virus in the form of powdered spleen dried in vacuo over Calcium Chloride.

Goat Number.	Injection of Virus.			Reaction.				
	Date Injected.	Weight of Powdered Spleen.	Period Stored at 85° to 89°F. after two days at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
317.....	11/ 4/35	0·01 gram	3 days	3rd	5th	103°F.	1·4°F.	Died on 16th day.
348.....	11/ 4/35	0·005 gram	3 days	4th	12th	104·4°F.	2·2°F.	Recovered.
352.....	14/ 4/35	0·005 gram	6 days	3rd	3rd	106°F.	3·4°F.	Died on 26th day.
349.....	14/ 4/35	0·01 gram	6 days	3rd	3rd	106°F.	4°F.	Died on 10th day.
351.....	14/ 4/35	0·005 gram	6 days	2nd	5th	104°F.	2°F.	Recovered.
333.....	14/ 4/35	0·01 gram	6 days	2nd	3rd	105°F.	3·2°F.	Died on 6th day.
350.....	18/ 4/35	0·01 gram	10 days	2nd	3rd	104°F.	0·8°F.	Died on 9th day.
326.....	18/ 4/35	0·01 gram	10 days	2nd	3rd	106°F.	3°F.	Recovered.
308.....	21/ 4/35	0·01 gram	13 days	2nd	7th	104°F.	3°F.	Recovered.
342.....	21/ 4/35	0·01 gram	13 days	Temperature	reaction	indefinite.		Died on 36th day.

TABLE 10. (EXPERIMENT 10.)

To determine the viability and immunizing properties of Goat Spleen Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride and then made into an emulsion with Normal Saline and stored for (a) 0 days, (b) 4 days at 42° F., (c) 4 days at 79° to 81° F.

Cattle	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Day of Initial Rise.	Day Peak Reached	Peak temperature.	Rise in temperature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11	12
367 (K)..	27/ 7/35	0·1 gram	0 days.....	2nd	3rd	104·8°F.	4·8°F.	Recovered.	16/ 8/35	2 c.cm.	Immune.
350 (K)..	27/ 7/35	0·1 gram	0 days.....	2nd	3rd	103°F.	3°F.	Recovered.	16/ 8/35	2 c.cm.	Immune.
394 (K)..	5/ 8/35	0·02 gram	4 days at 42°F.	5th	7th	105·2°F.	5·2°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
345 (L)..	5/ 8/35	0·02 gram	4 days at 42°F.	No reaction.					9/ 9/35	2 c.cm.	Immune.
349 (K)..	5/ 8/35	0·02 gram	4 days at 79° to 81°F.	4th	5th	105°F.	5°F.	Died on 35th day	—	—	Died of intercurrent disease.
346 (L)..	5/ 8/35	0·02 gram	4 days at 79° to 81°F.	9th	13th	105°F.	4°F.	Recovered	9/ 9/35	2 c.cm.	Immune.
391 (K)..	Control to Nos. 367 and 350.								16/ 8/35	2 c.cm.	Temperature rise of 5°F. Destroyed on 5th day for removal of spleen.
383 (K)..	Control to Nos. 394, 345 and 346.								9/ 9/35	2 c.cm.	Died of Rinderpest on 9th day.

TABLE 11. (EXPERIMENT 11.)

To determine the immunizing properties of Rinderpest Goat Spleen Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride after storage for (1) *5 days at 42° F.*, (2) *5 days at 82° F.*

Cattle Number.	Injection of Vaccine.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
401 (L)..	19/ 8/35	0·005 gram	5 days at 42°F.	3rd	4th	104·0°F.	2·6°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
406 (L)..	19/ 8/35	0·005 gram	,, ,,	3rd	5th	104·4°F.	3·0°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
387 (K)..	19/ 8/35	0·005 gram	,, ,,	3rd	5th	105·0°F.	3·0°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
362 (K)..	19/ 8/35	0·005 gram	,, ,,	3rd	5th	105·2°F.	4·0°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
405 (L)..	19/ 8/35	0·005 gram	5 days at 82°F.	5th	6th	103·2°F.	2·0°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
403 (L)..	19/ 8/35	0·005 gram	,, ,,	3rd	5th	102·6°F.	0·8°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
361 (K)..	19/ 8/35	0·005 gram	,, ,,	3rd	6th	105·4°F.	5·4°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
384 (K)..	19/ 8/35	0·005 gram	,, ,,	3rd	3rd	105·2°F.	3·6°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
412 (L)..	Control.								9 /9/35	2 c.cm.	Rise in temperature from 101°F. to 103·2°F. on 7th day. Recovered.
383 (K)..	Control.								9/ 9/35	2 c.cm.	Died of Rinderpest on 9th day.

TABLE 12. (EXPERIMENT 12.)

To determine the effect of storage at 97° F. on the viability and immunizing properties of Goat Adapted Rinderpest Virus in the form of powdered spleen dried in vacuo over Calcium Chloride.

Cattle Number.	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Period Stored at 97°F after four days at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11	12
441 (K)..	12/12/35	0·005 gram	3 days	2nd	9th	103°F.	3°F.	Recovered.	26/ 1/36	2 c.cm.	Immune.
440 (K)..	12/12/35	0·01 gram	3 days	2nd	5th	105°F.	5°F.	Recovered.	26/ 1/36	2 c.cm.	Immune.
434 (K)..	12/12/35	0·01 gram	3 days	2nd	4th	102·6°F.	3°F.	Recovered.	26/ 1/36	2 c.cm.	Immune.
439 (K)..	15/12/35	0·005 gram	6 days	3rd	4th	101°F.	1·4°F.	Recovered.	26/ 1/36	2 c.cm.	Rise in temperature of 4·2°F. Diarrhoea. Severe mouth lesions Recovered.
479 (K)..	12/12/35	0·01 gram	6 days	4th	6th	103°F.	3°F.	Recovered.	26/ 1/36	2 c.cm.	Immune.
467 (K)..	12/12/35	0·01 gram	6 days	Mild indefinite temperature reaction.					26/ 1/36	2 c.cm.	Died of Rinderpest on 13th day.
450 (K)..	Control.								26/ 1/36	2 c.cm.	Rise in temperature of 4·8°F. Peak reached on 6th day. Destroyed on 6th day for removal of spleen.
477 (K)..	Control.								26/ 1/36	2 c.cm.	Rise in temperature of 4·6°F. Diarrhoea. Severe mouth lesions. Recovered.

TABLE 13. (EXPERIMENT 13.)

To determine the optimum dose of Goat Adapted Rinderpest Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride.

Cattle Number.	Injection of Vaccine.		Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11
476 (K)..	3/1/36	0·1 gram	3rd	5th	103°F.	3·6°F.	Recovered	26/1/36	2 c.cm.	Immune.
460 (K)..	3/1/36	0·1 gram	3rd	3rd	103·2°F.	4·2°F.	Recovered.	26/1/36	2 c.cm.	Immune.
436 (K)..	3/1/36	0·01 gram	4th	7th	101·4°F.	2·4°F.	Recovered	26/1/36	2 c.cm.	Immune.
433 (K)..	3/1/36	0·01 gram	4th	5th	102·4°F.	3·4°F.	Recovered.	26/1/36	2 c.cm.	Immune.
438 (K)..	3/1/36	0·005 gram	Indefinite temperature reaction.					26/1/36	2 c.cm.	Immune.
468 (K)..	3/1/36	0·005 gram	3rd	6th	103·2°F.	4·2°F.	Recovered	26/1/36	2 c.cm.	Immune.
442 (K)..	3/1/36	0·001 gram	4th	6th	103·6°F.	4·2°F.	Recovered.	26/1/36	2 c.cm.	Immune.
482 (K)	3/1/36	0·001 gram	4th	6th	102·4°F.	3°F.	Recovered.	26/1/36	2 c.cm.	Immune.
437 (K)..	3/1/36	0·0005 gram	5th	7th	102·0°F.	3·0°F.	Recovered	26/1/36	2 c.cm.	Immune.
481 (K)..	3/1/36	0·0005 gram	5th	6th	102·0°F.	3·0°F.	Recovered.	26/1/36	2 c.cm.	Immune.
450 (K)..	Control.							26/1/36	2 c.cm.	Rise in temperature of 4·8°F. Peak reached on 6th day. Destroyed on 6th day for removal of spleen.
477 (K)..	Control.							26/1/36	2 c.cm.	Rise in temperature of 4·6°F. Diarrhoea. Severe mouth lesions. Recovered.

TABLE 14. (EXPERIMENT 14.)

To determine the viability, under adverse field conditions, of Goat Adapted Rinderpest Virus in the form of powdered goat spleen dried in vacuo over Calcium Chloride.

Method.—The virus was packed in ice in a Thermos Jar which was stored at 99° F. The ice was replenished on the fourth day (when the temperature in the jar was 56° F.) and the flask replaced in the incubator at 97° F. The testing of the virus was commenced on the 6th day after ice was last put in the flask, i.e. on the 9th day after it was first packed in the Thermos Jar.

Cattle Number.	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Number of days Thermos Stored at 97°F after last filled with Ice.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
513 (K)..	10/7/36	0·01 gram	Six.....	4th	5th	105·4°F.	5·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
518 (K)..	10/7/36	0·01 gram	Six.....	3rd	5th	103·6°F.	3·4°F.	Recovered.	11/8/36	2 c.cm.	Immune.
521 (K)..	10/7/36	0·001 gram	Six.....	3rd	6th	103·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
505 (K)..	10/7/36	0·001 gram	Six.....	3rd	5th	103·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
509 (K)..	12/7/36	0·01 gram	Eight.....	4th	4th	104·4°F.	4·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
517 (K)..	12/7/36	0·01 gram	Eight.....	3rd	4th	102·2°F.	2·2°F.	Recovered.	11/8/36	2 c.cm.	Died of Rinderpest on 9th day.
512 (K)..	12/7/36	0·001 gram	Eight.....	3rd	7th	104·0°F.	3·8°F.	Recovered.	11/8/36	2 c.cm.	Mild temperature reaction. Recovered.
523 (K)..	12/7/36	0·001 gram	Eight.....		No reaction.				11/8/36	2 c.cm.	Severe temperature reaction with diarrhoea and mouth lessions. Recovered.
515 (K)..	14/7/36	0·01 gram	Ten.....		No reaction.						Severe temperature reaction with diarrhoea and mouth lessions. Recovered.

TABLE 14—*(continued)*.

Cattle Number.	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Number of days Thermos Stored at 97°F after last filled with Ice.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
520 (K)..	14/7/36	0·01 gram	Ten.....	3rd	7th	104·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
511 (K)..	14/7/36	0·001 gram	Ten.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 11th day.
524 (K)..	14/7/36	0·001 gram	Ten.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 9th day.
500 (K)..	16/7/36	0·01 gram	Twelve.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 10th day.
507 (K)..	16/7/36	0·01 gram	Twelve.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 9th day.
489 (K)..	16/7/36	0·001 gram	Twelve.....		No	reaction.			11/8/36	2 c.cm.	Severe temperature reaction with diarrhoea and mouth lesions. Recovered.
508 (K)..	16/7/36	0·001 gram	Twelve.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 12th day.
493 (K)..	18/7/36	0·01 gram	Fourteen.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 12th day.

TABLE 14—(*continued*).

Cattle Number.	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Number of days Thermos Stored at 97°F after last filled with Ice.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
496 (K)..	18/7/36	0·01 gram	Fourteen.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 12th day.
490 (K)..	18/7/36	0·001 gram	Fourteen.....		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 10th day.
495 (K)..	18/7/36	0·001 gram	Fourteen.....		No	reaction.			11/8/36	2 c.cm.	Severe temperature reaction with diarrhoea and mouth lesions. Recovered.
506 (K)..	10/7/36	0·01 gram	Not stored in Thermos Jar. Control for stored virus	3rd	6th	103·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
510 (K)..	10/7/36	0·001 gram	,,	3rd	6th	102·0°F.	2·4°F.	Recovered.	11/8/36	2 c.cm.	Immune.
508 (K)..	Control	for virus used in Immunity Test.							11/8/36	2 c.cm.	Died of Rinderpest on 13th day.
488 (K)..	Control	for virus used in Immunity Test.							11/8/36	2 c.cm.	Died of Rinderpest on 10th day.

TABLE 15. (EXPERIMENT 15.)

To determine the duration of immunity conferred by Rinderpest Goat Spleen Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride.

Cattle Number.	Injection of Vaccine.		Reaction.					Immunity Test.			
	Date Injected.	Weight of Dried Spleen.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Period Since Injection of Vaccine.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
453 (K)..	9/12/35	0·01 gram	3rd	5th	104·0°F.	4·2°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
445 (K)..	9/12/35	0·01 gram	2nd	5th	103·4°F.	3·4°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
480 (K)..	9/12/35	0·01 gram	3rd	5th	102·4°F.	2·6°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
444 (K)..	9/12/35	0·01 gram	3rd	5th	104·4°F.	5·4°F.	Recovered.	22/10/36	319 days	2 c.cm.	Initial rise in temperature on 10th day. Rise of 4·6°F. Recovered.
428 (L)..	9/12/35	0·01 gram	4th	7th	101·8°F.	2·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
429 (L)..	9/12/35	0·01 gram	4th	7th	101·8°F.	2·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
423 (L)..	9/12/35	0·01 gram	5th	7th	101·8°F.	1·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
422 (L)..	9/12/35	0·01 gram	3rd	6th	102·4°F.	3·0°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
419 (L)..	9/12/35	0·01 gram	3rd	5th	103·4°F.	4·0°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
418 (L)..	9/12/35	0·01 gram	3rd	6th	100·8°F.	1·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
469 (K)..	9/12/35	0·01 gram	3rd	5th	103·4°F.	4·4°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
458 (K)..	9/12/35	0·01 gram	3rd	3rd	103·0°F.	3·0°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
449 (K)..	9/12/35	0·01 gram	2nd	4th	104·2°F.	4·0°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Initial rise in temperature on 5th day. Rise of 3·0°F. Recovered.
432 (L)..	9/12/35	0·01 gram	3rd	8th	102·2°F.	2·2°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
430 (L)..	9/12/35	0·01 gram	5th	5th	103·0°F.	3·0°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
414 (L)..	9/12/35	0·01 gram	3rd	5th	104·4°F.	4·4°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.

TABLE 15—(*continued*).

Cattle Number.	Injection of Vaccine.		Reaction.					Immunity Test.			
	Date Injected.	Weight of Dried Spleen.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Period Since Injection of Vaccine.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
413 (L)..	9/12/35	0·01 gram	3rd	6th	104·0°F.	4·4°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
427 (L)..	9/12/35	0·01 gram		No	reaction.			20/ 5/37	429 days	2 c.cm.	Immune.
547 (K)..	Control.							20/ 5/37		2 c.cm.	Died of Rinderpest on 8th day.
544 (K)..	Control.							20/ 5/37		2 c.cm.	Died of Rinderpest on 8th day.
462 (K)..	9/12/35	0·01 gram	3rd	5th	104·0°F.	4·0°F.	Recovered.	1/ 1/38	755 days	2 c.cm.	Initial rise in temperature on 6th day. Rise of 2·0°F. Recovered.
472 (K)..	9/12/35	0·01 gram	3rd	5th	103·6°F.	4·2°F.	Recovered.	1/ 1/38	755 days	2 c.cm.	Initial rise in temperature on 7th day. Rise of 2·0°F. Recovered.
335 (L)..	9/12/35	0·01 gram	3rd	5th	100·8°F.	2·0°F.	Recovered.	1/ 1/38	755 days	2 c.cm.	Immune.
324 (L)..	9/12/35	0·01 gram		No	reaction.			1/ 1/38	755 days	2 c.cm.	Immune.
267 (L)..	9/12/35	0·01 gram	3rd	5th	100·0°F.	2·0°F.	Recovered.	4/ 1/38	755 days	2 c.cm.	Immune.
337 (L)..	9/12/35	0·01 gram	3rd	4th	100·2°F.	1·2°F.	Recovered.	4/ 1/38	755 days	2 c.cm.	Immune.
582 (K)..	Control.							4/ 1/38		2 c.cm.	Died of Rinderpest on 8th day.
562 (K)..	Control.							4/ 1/38		2 c.cm.	Rise in temperature of 4·6°F. Severe mouth lesions. Recovered

TABLE 16. (EXPERIMENT 16.)

To determine the time taken to develop immunity.

Cattle Number.	Injection of Vaccine.		Injection of Virus.		
	Date Injected.	Quantity of Dried Goat Spleen.	Interval after Injection of Vaccine.	Quantity of Dried Bovine Spleen.	Result.
1	2	3	4	5	6
583 (K)	5/2/38	0·0025 gram	24 hours	0·01 gram	Diarrhoea. Recovered.
558 (K)	5/2/38	0·00025 gram	24 hours	0·01 gram	A few small ulcers in mouth. Recovered.
580 (K)	5/2/38	0·0025 gram	36 hours	0·01 gram	No reaction. Immune.
571 (K)	5/2/38	0·00025 gram	36 hours	0·01 gram	Diarrhoea. Recovered.
557 (K)	5/2/38	0·0025 gram	48 hours	0·01 gram	No reaction. Immune.
586 (K)	5/2/38	0·00025 gram	48 hours	0·01 gram	No reaction. Immune.
567 (K)	5/2/38	0·0025 gram	60 hours	0·01 gram	No reaction. Immune.
577 (K)	5/2/38	0·00025 gram	60 hours	0·01 gram	No reaction. Immune.
576 (K)	5/2/38	0·0025 gram	72 hours	0·01 gram	No reaction. Immune.
560 (K)	5/2/38	0·00025 gram	72 hours	0·01 gram	No reaction. Immune.
569 (K)	Control.		Injected on 7/2/38.	0·01 gram	Died of Rinderpest on 9th day.
494 (K)	8/9/36	0·005 gram	3 days	0·01 gram	No reaction. Immune.
487 (K)	8/9/36	0·0025 gram	3 days	0·01 gram	No reaction. Immune.
485 (K)	8/9/36	0·005 gram	5 days	0·01 gram	No reaction. Immune.
533 (K)	8/9/36	0·0025 gram	5 days	0·01 gram	No reaction. Immune.
526 (K)	8/9/36	0·005 gram	7 days	0·01 gram	No reaction. Immune.
529 (K)	8/9/36	0·0025 gram	7 days	0·01 gram	No reaction. Immune.
519 (K)	8/9/36	0·005 gram	9 days	0·01 gram	No reaction. Immune.
484 (K)	8/9/36	0·0025 gram	9 days	0·01 gram	No reaction. Immune.
497 (K)	8/9/36	0·005 gram	11 days	0·01 gram	No reaction. Immune.
504 (K)	8/9/36	0·0025 gram	11 days	0·01 gram	No reaction. Immune.
501 (K)	Control.		Injected on 19/9/36.	0·01 gram	Died of Rinderpest on 14th day.

Table 17. (Experiment 17.)

To determine the immunizing value of Goat Adapted Rinderpest virus in the form of powdered spleen dried in vacuo over Calcium Chloride and stored alternately for 12 hours at 80° F. and for 12 hours at 97° F.

Cattle Number.	Injection of Vaccine.			Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7
581 (K)	21/3/38	0·0025 gram	6 days	21/4/38	2 c.cm.	Pronounced temperature reaction. Recovered.
578 (K)	21/3/38	0·0025 gram	6 days	21/4/38	2 c.cm.	Died of Rinderpest on 13th day.
574 (K)	23/3/38	0·0025 gram	8 days	21/4/38	2 c.cm.	Died of Rinderpest on 9th day.
579 (K)	23/3/38	0·0025 gram	8 days	21/4/38	2 c.cm.	Diarrhoea. Mouth lesions. Recovered.
566 (K)	25/3/38	0·0025 gram	10 days	21/4/38	2 c.cm.	Died of Rinderpest on 9th day.
570 (K)	25/3/38	0·0025 gram	10 days	21/4/38	2 c.cm.	Diarrhoea. Mouth lesions. Recovered.
568 (K)	27/3/38	0·0025 gram	12 days	21/4/38	2 c.cm.	Died of Rinderpest on 14th day.
569 (K)	27/3/38	0·0025 gram	12 days	21/4/38	2 c.cm.	Died of Rinderpest on 14th day.
587 (K)	28/3/38	0·0025 gram	Vaccine stored for 14 days at 42°F.	21/4/38	2 c.cm.	Immune.
594 (K)	28/3/38	Control 0·0025 gram		21/4/38	2 c.cm.	Immune.
603 (K)	Virus control			21/4/38	2 c.cm.	Died of Rinderpest on 10th day.

Table 18. (Experiment 18.)

To determine the effect of storage at 37° C. on Bennett's Glycerine-Vaccine.

Cattle Number.	Injection of Vaccine.			Immunity Test.		
	Date Injected.	Weight of Spleen Pulp Injected.	Period Stored at 37°C.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7
559 (K)	25/3/38	1 gram	4 days	21/4/38	2 c.cm.	Died of Rinderpest on 11th day.
584 (K)	25/3/38	1 gram	4 days	21/4/38	2 c.cm.	Died of Rinderpest on 13th day.
588 (K)	27/3/38	1 gram	6 days	21/4/38	3 c.cm.	Diarrhoea. Mouth lesions. Recovered.
593 (K)	27/3/38	1 gram	6 days	21/4/38	2 c.cm.	Died of Rinderpest on 11th day.
597 (K)	5/4/38	1 gram	15 days	21/4/38	2 c.cm.	Diarrhoea. Mouth lesions. Recovered.
595 (K)	5/4/38	1 gram	15 days	21/4/38	2 c.cm.	Died of Rinderpest on 10th day.
600 (K)	10/4/38	1 gram	20 days	21/4/38	2 c.cm.	Died of Rinderpest on 11th day.
598 (K)	10/4/38	1 gram	20 days	21/4/38	2 c.cm.	Died of Rinderpest on 9th day.

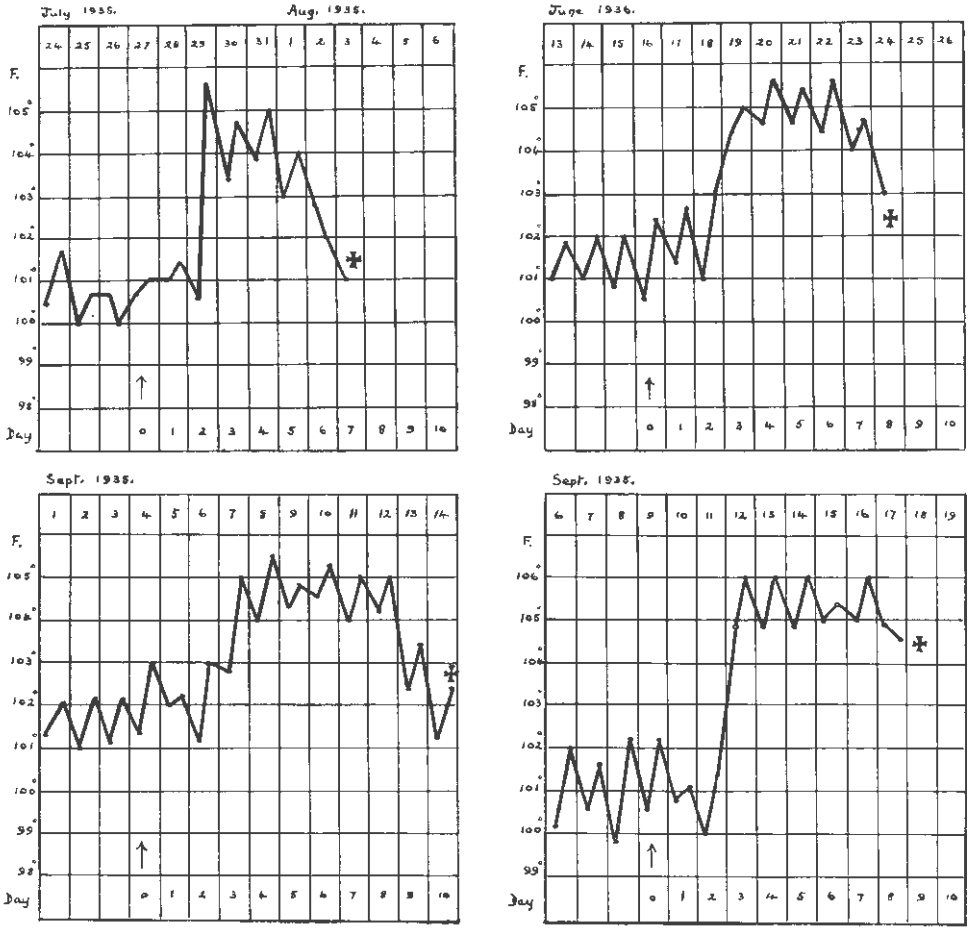

Figure 1.—Four typical temperature charts of cattle used in the passage of the Kungyangon cattle virus.

↑ = Injection of virus. † = Died of rinderpest.

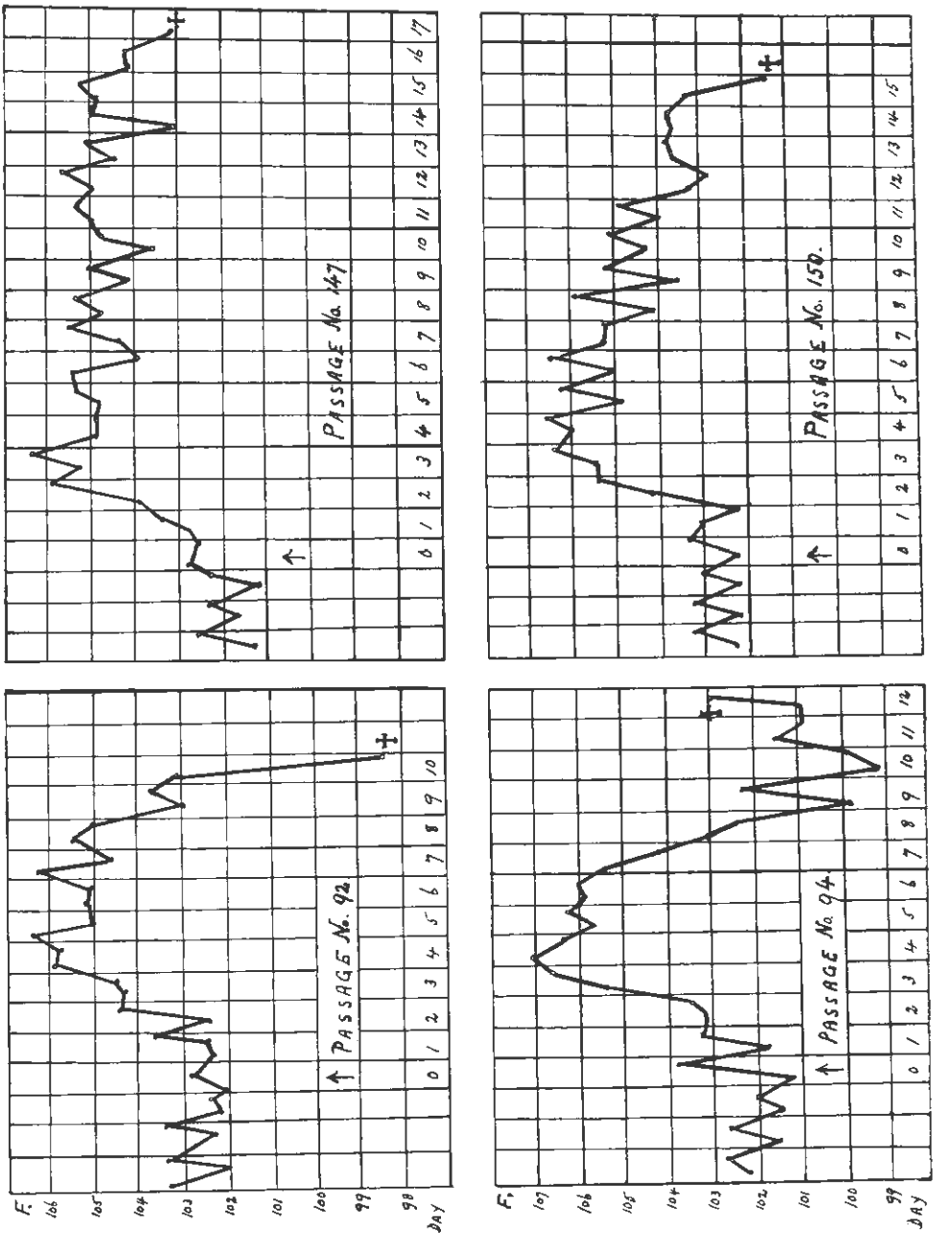

Figure 2.—Four typical temperature charts of goats used in the passage of the goat adapted rinderpest virus.

↑ Injection of virus. † Died of rinderpest.

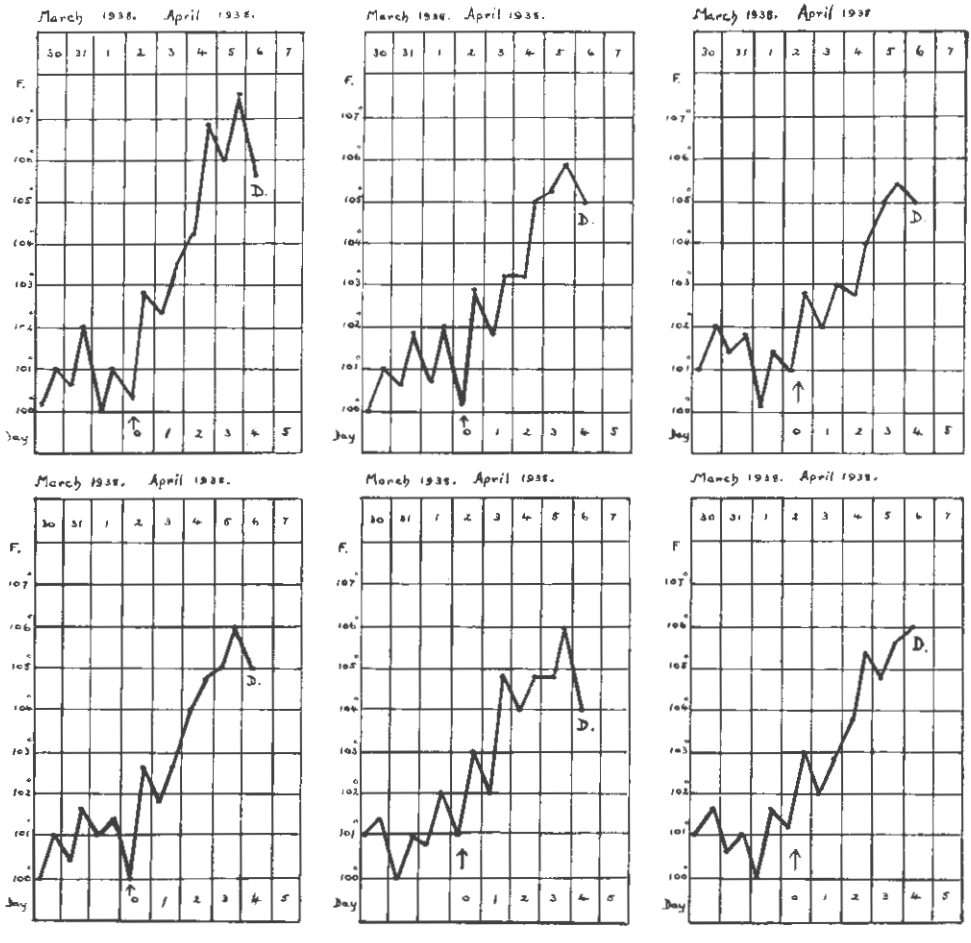

Figure 3. -Six typical temperature charts of a batch of goats used in the preparation of dried goat spleen vaccine.

↑ = Injection of virus. D = Destroyed.

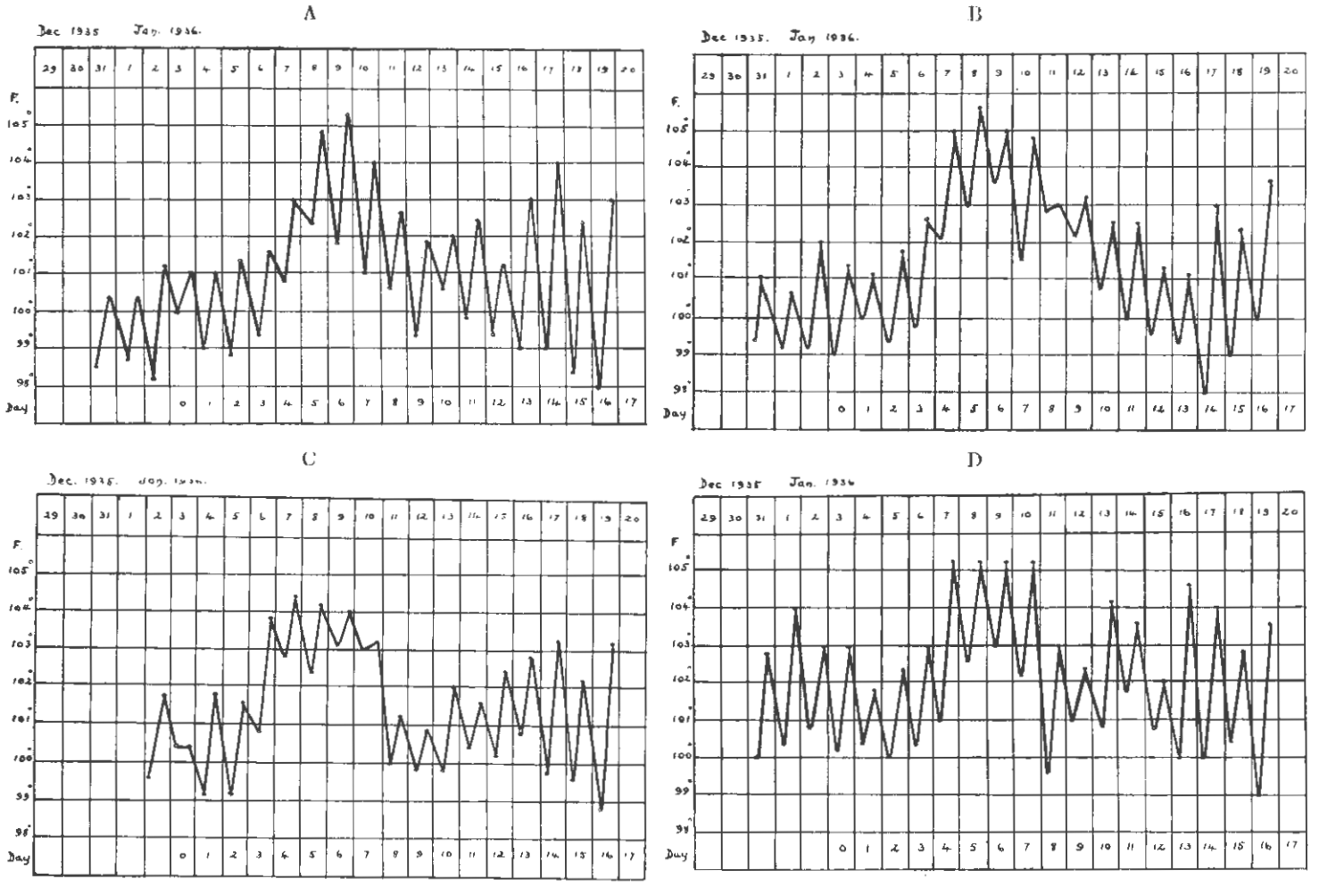

Figure 1. Four typical temperature charts of Kyaukpyu cattle injected with dried goat spleen vaccine.

A = Bovine 433. Injected 0·01 gr. dried vaccine.
B Bovine 442. Injected 0·001 gr. dried vaccine.
C Bovine 468. Injected 0·005 gr. dried vaccine.
D Bovine 481. Injected 0·0005 gr. dried vaccine.

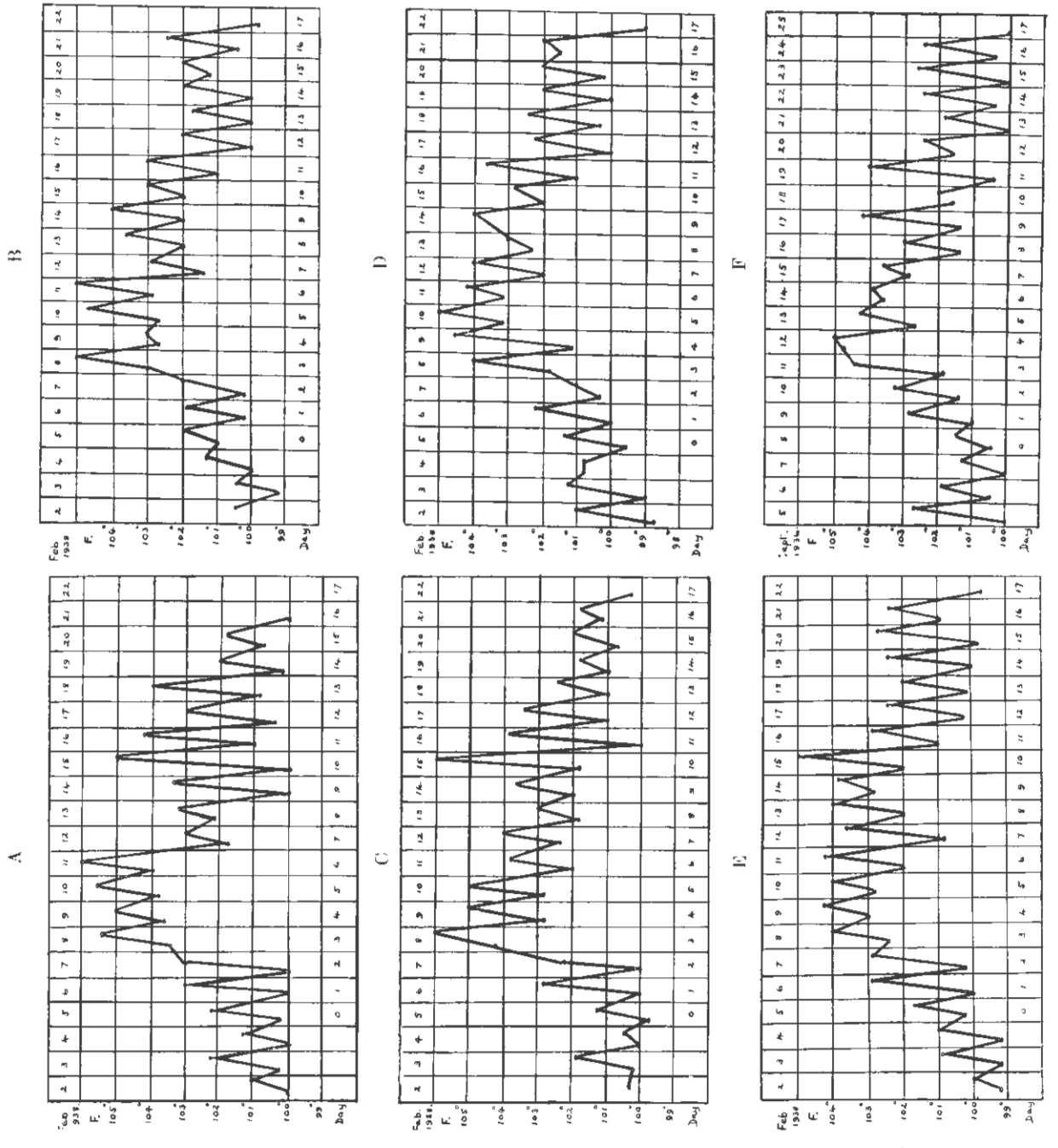

Figure 5.—Temperature charts of six Kyaukpyu cattle given cattle virus soon after the injection of dried goat spleen vaccine to determine the onset of immunity (Expt. 16).

A = Bovine 583. Virus given 24 hours after vaccine.
B = Bovine 567. Virus given 60 hours after vaccine.
C = Bovine 580. Virus given 36 hours after vaccine.
D = Bovine 576. Virus given 72 hours after vaccine.
E = Bovine 557. Virus given 48 hours after vaccine.
F = Bovine 485. Virus given 120 hours after vaccine.

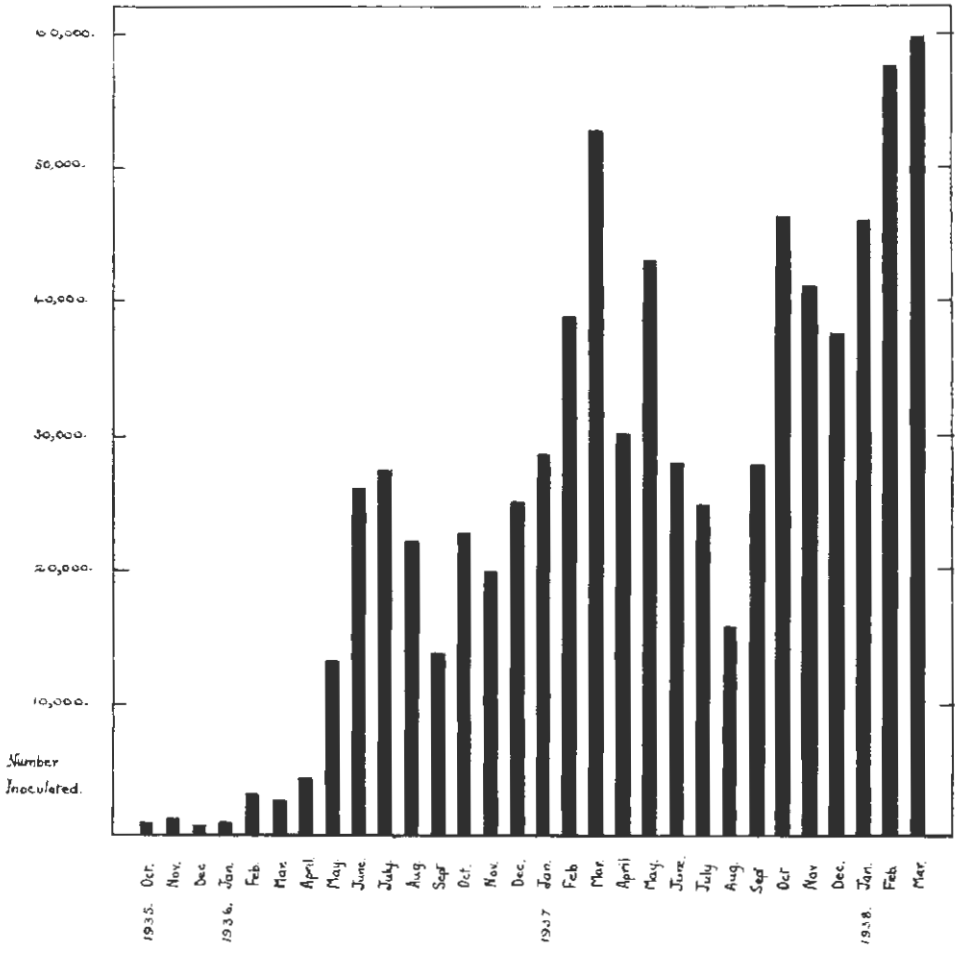

Figure 6.—Number of cattle inoculated with dried goat spleen vaccine from October, 1935, to March, 1938.

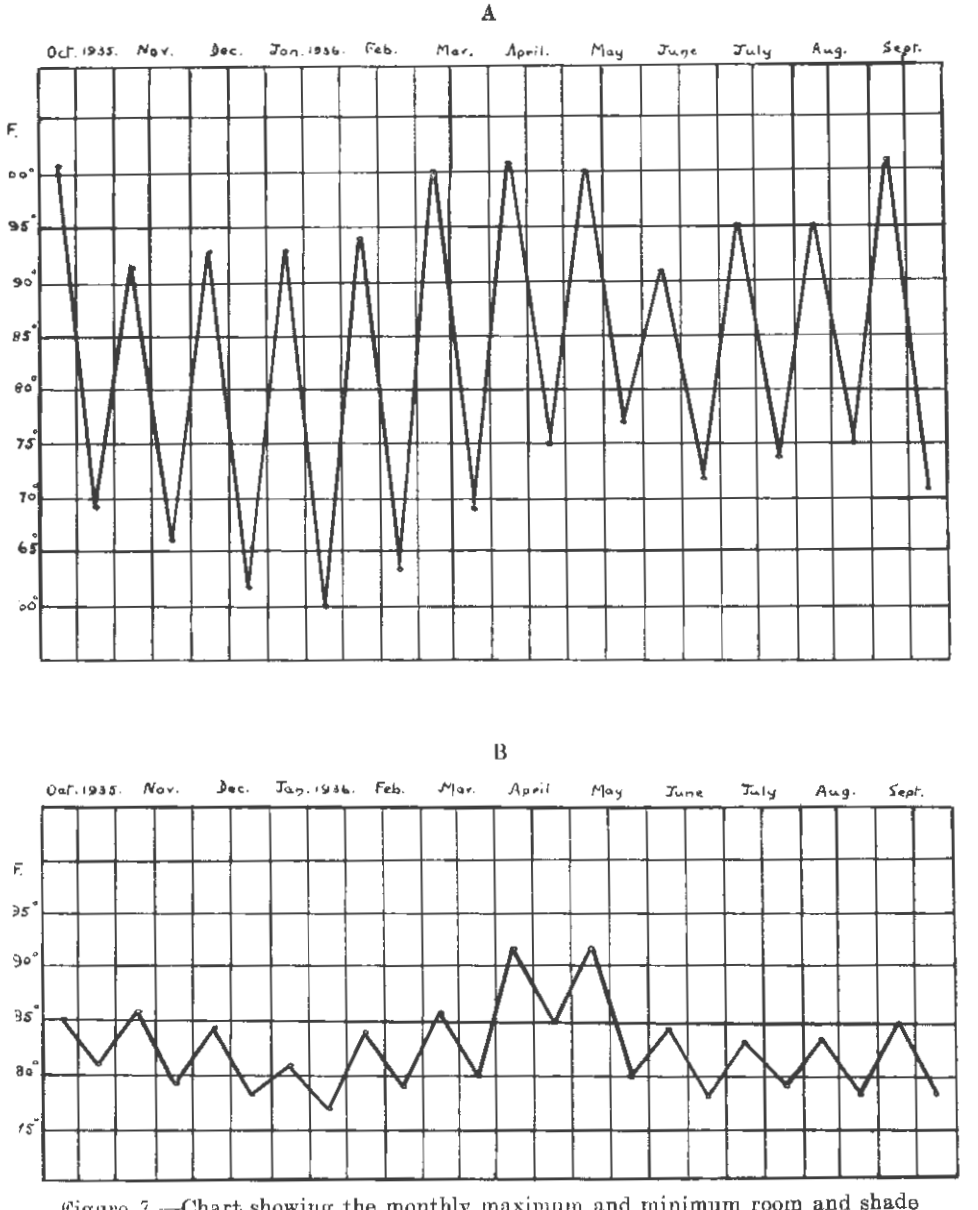

Figure 7.—Chart showing the monthly maximum and minimum room and shade temperatures at the Veterinary Research Laboratory, Insein, over one year.

A = Shade temperature.
B = Room temperature

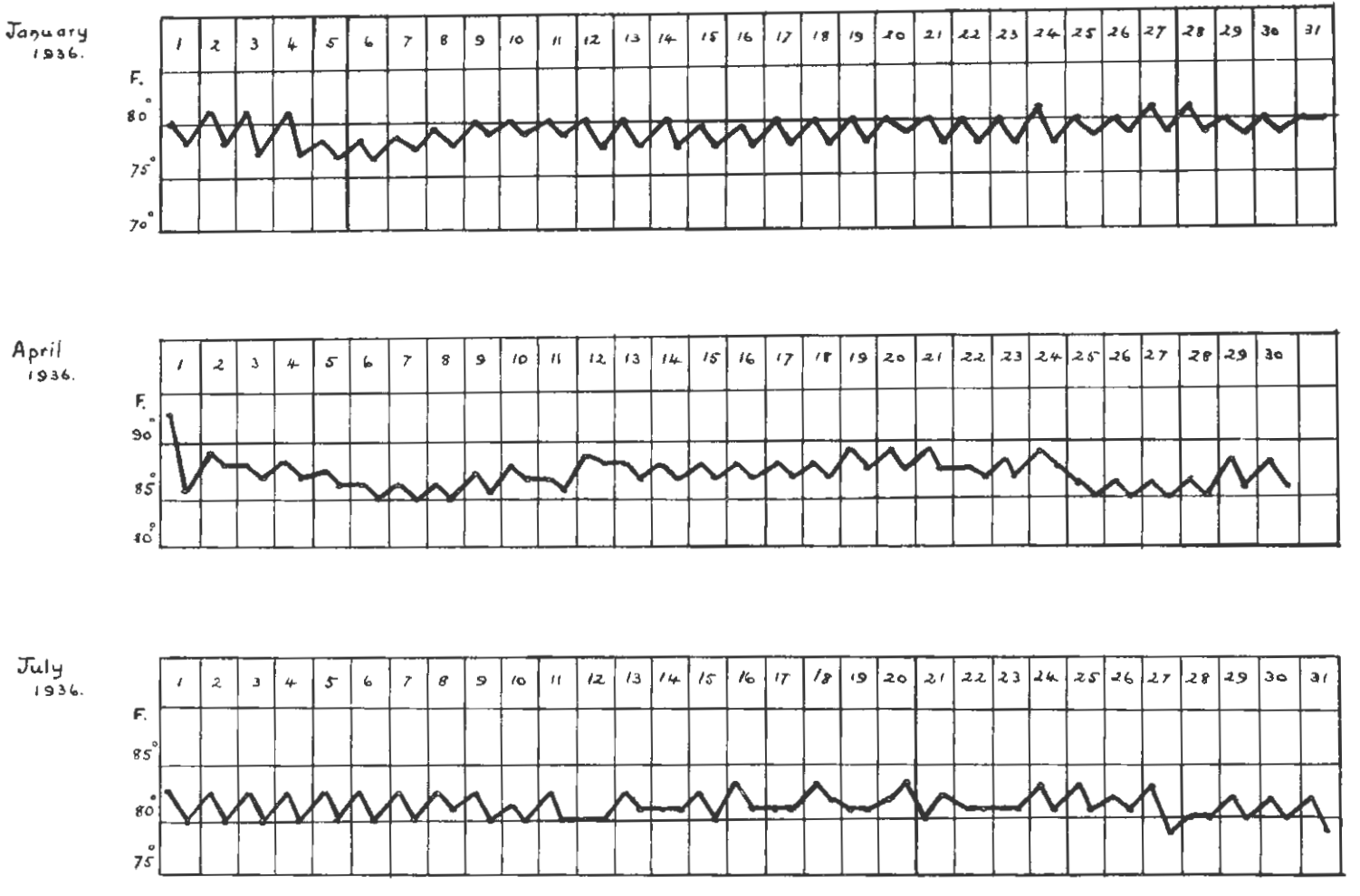

Figure 8. - Chart showing the daily maximum and minimum room temperatures at the Veterinary Research Laboratory, Insein, in January (cold weather), April (hot weather), and July (rainy weather).

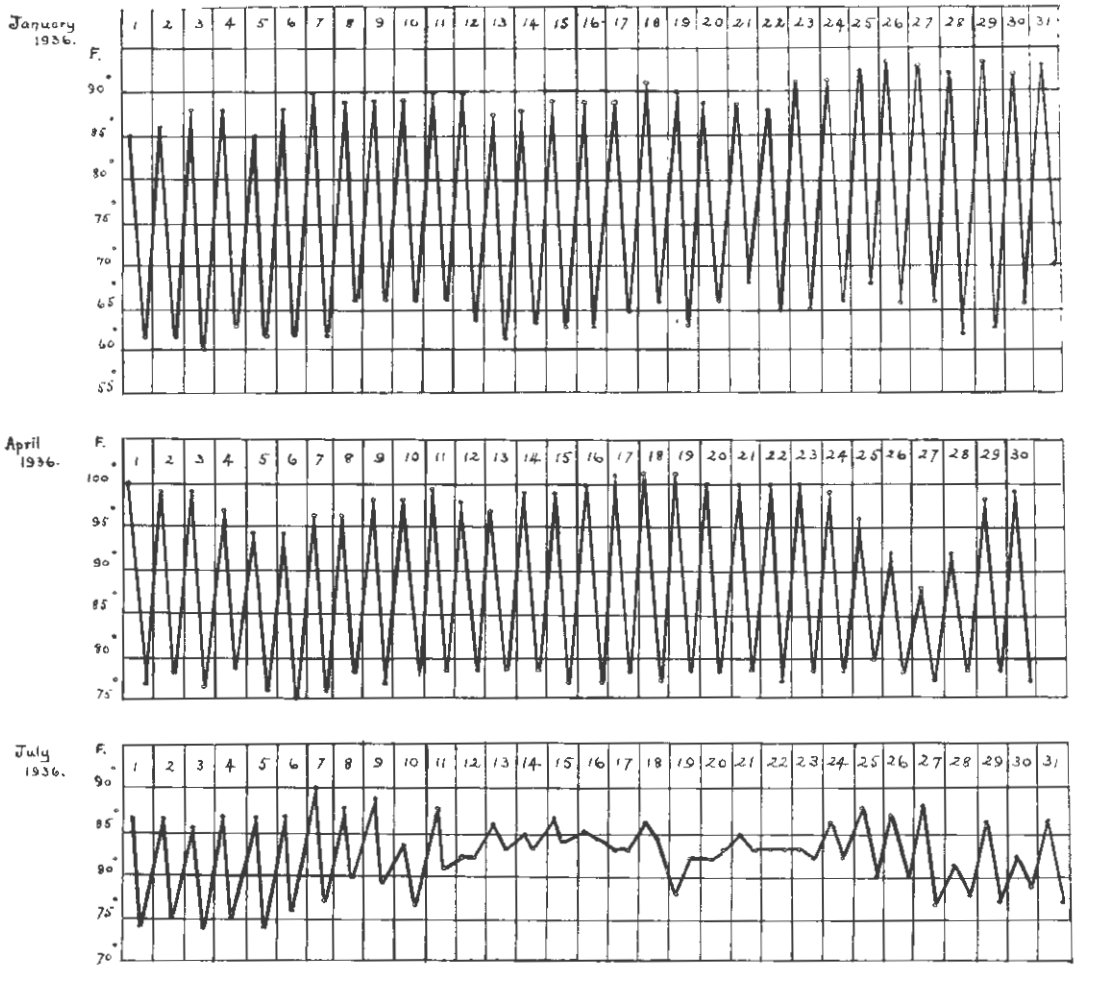

Figure 9.—Chart showing the daily maximum and minimum shade temperatures at the Veterinary Research Laboratory, Insein, in January (cold weather), April (hot weather), and July (rainy weather).