Vaccination in the practice as a substitute for simultaneous inoculation will be considered when further data on duration of immunity is available. Further research is necessary to determine whether cattle treated with spleen may be sucessfully immunized later by simultaneous inoculation.

(16) Inactivated spleen extract is still potent 3 months after preparation. In view of its keeping properties, a large supply could be prepared and stored.

(17) Variations occur in potency of spleens. The pulp of several spleens should be pooled to obtain an extract of high potency, and each lot of prepared extract tested on its potency prior to issue.

(18) Further work is necessary to determine whether spleen-pulp inactivated by a few days at incubator temperature (37° C.) is as effective as spleen-pulp treated with various chemicals, such as toluol, chloroform, formalin; if so, the addition of an antiseptic which would sterilize the extract of bacterial and protozoal infections without affecting its potency would only be necessary.

(19) Experiments show that redwater infection is not transmissible by inactivated spleen prepared from a beast reacting to redwater, but further work is necessary to determine the effect of the various chemicals which have been used for making extracts, e.g. toluol, chloroform, formalin on anaplasma parasites and period contact required for these to sterilize the extract of anaplasma infections.

Paper No. 6.

PROPHYLACTIC VACCINATION AGAINST RINDERPEST.

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Researches directed towards the preparation of a satisfactory immunogen from virus-containing tissues have, in recent years, been stimulated by certain defects in both the serum and serum-virus methods of immunization. These defects were discussed in the attached paper, and it is not proposed to do more than mention them here as an indication of the need for further research into methods of immunization.

The serum-alone method suffers from a grave disadvantage in the shortness of duration of the immunity conferred, and in the consequent trouble and expense involved by the repeated inoculations that are necessary to maintain animals in an immune state during an epizootic.

The serum-virus method, which is adopted by most countries where the disease is enzootic, involves the creation of new centres of infection where immunization is being carried out. The relative importance of this factor depends partly upon the dose and titre of the serum used in the inoculation, and partly also upon the

thoroughness and efficacy with which veterinary police control can be exercised. This is perhaps the most fundamental of the defects of the serum-virus method from the point of view of the state veterinarian or the economist, and it throws doubt upon the possibility of eradicating the disease from an enzootic area by this method. In practice the results of the serum-virus method, in this respect, no doubt largely depend upon the efficiency with which control of movement can be exercised. Thus in post-war Egypt, in the hands of Piot Bey, the method proved entirely successful; while in Eritrea the adoption of the serum-virus method led to considerable controversy, and it was maintained by some authorities (Ferraro and Zonchello), that outbreaks arising as a result of double inoculations were responsible for the continued persistence of the disease in the territory.

The serum-virus method labours under further disabilities which concern (*a*) the virus, and (*b*) the serum. With regard to the provision of suitable virus the first difficulty encountered is due to the poor—or perhaps it would be better to say uncertain—viability of stored virus, which necessitates the provision of virus-producing infected animals at the inoculating centres. A second difficulty arises in connection with the transmission by virus, in the form of virulent blood, of blood-borne infectious diseases; this point is discussed more fully in the previous paper, but it might be pointed out here that this is a difficulty which is certain to become progressively more acute as the general standard of stock-farming in a country improves. So far as the serum is concerned, it is the writer's opinion that the methods employed at present in the production of hyper-immune serum are not by any means fully understood, and the results of experimental work carried out recently cast considerable doubt on the value of the so-called hyper-immunizing process.

It will be evident then that there is ample need for some improvement upon the older methods, and success is most likely to come in the form of a virus-vaccine which will be free from the risk of producing rinderpest either in the inoculated or in in-contact animals, and which will not transmit protozoan or other infectious blood-borne diseases. Several important papers have been published quite recently on the subject of prophylactic vaccination. The paper of the Japanese workers (Kakizaki, Nakanishi and Oizumi) is briefly discussed in a footnote in the accompanying paper, while those of Boynton (1928) and Kelser (1928) came to hand after the manuscript was in press.

The earliest attempts at prophylactic vaccination, if we disregard the ancient setoning and the now discarded bile inoculation, were probably those of Kakizaki (1918), who stated that he successfully attenuated virus in blood and spleen tissue by the action of glycerine. The glycerinated blood or spleen proved completely avirulent when held at temperatures below zero for eighty days, and spleen tissue treated in this manner was claimed to retain some antigenic property for periods in excess of a year. The experiments initiated by Kakizaki culminated in the Report III of Kakizaki, Nakanishi and Oizumi (1926). Good results are here recorded from the use of spleen and lymphatic gland vaccines prepared by the action of glycerine and toluol, 10 per cent., for periods of from seven to ten days at 37° C. There was a noticeable decrease in antigenic value when the

period of incubation was extended to ten days or more and, in fact, in a subsequent experiment vaccine retained at 37° C. for ten days failed to afford any protection. The vaccine prepared by incubation for seven days was, however, not always completely inactivated, and there seems to be an insufficient margin between the period necessary for inactivation and that at which the preparation commences to deteriorate. Apparently throughout this series of experiments glycerine was employed for the attenuation or inactivation of the virus and toluol was usually added as a preservative; iodine and eucalyptol are said to be roughly as efficacious as toluol in the preservation of glycerinized vaccine, but ether was unsatisfactory. The account of the experiments is somewhat involved and rather difficult to follow, and it is noteworthy that no controls for the virulence of the virus or the susceptibility of experimental animals were included. Numerous instances of the keeping properties of the vaccine are given—it is stated that the vaccine will keep up to three and a half years at room temperature—but there are no experimental data as to the duration of the immunity in vaccinated animals.

Certain practical experiences with the vaccine are cited at the end of the article, but the record is singularly poor in detail. As an example one may quote experience 2, in which it is stated that 7,098 cattle were vaccinated in September, 1924. In April, 1925, the district in which the vaccinations had been carried out was invaded by rinderpest, of which there were 49 cases in the villages in question. When the outbreak had completely subsided in April, 1925, "among the vaccinated cattle there was only one infected, which had an abscess in the injected site, while the remaining 7,097 were perfectly protected from this disease." A truly remarkable result, implying as it does that during seven months there was no mortality from any cause among 7,098 cattle.

Undoubtedly the record of these experiments is of some value to other workers, but the experiments are insufficiently controlled and the results must await further confirmatory evidence.

A second centre of research into rinderpest vaccination has been the Laboratory of the Insular Bureau of Agriculture, Philippine Islands. As long ago as 1917 Boynton was investigating the virulence of extracts of organs in rinderpest, and was preparing these extracts in 0.5 per cent. phenol. Boynton demonstrated the virulence of extracts of liver, voluntary and involuntary muscle, spleen and lymphatic tissue, but failed either to immunize animals without the production of an attack of the disease, or to minimize the severity of the disease set up. He suggested the use of these extracts in place of virulent blood for immunization by the serum-virus method. In 1928 Boynton published an account of a method of prophylactic vaccination, developed no doubt from his earlier experiments on the virulence of organs. When the tissue extracts referred to above were held for prolonged periods it was found that after a certain time virulence was lost, although antigenic properties were still retained in the case of certain organs. It was next decided to add glycerine to the carbolized extracts, and a further modification was introduced by preliminary heating of the tissues at 44° C. for three hours.

Boynton's final method of preparation was to take lymph glands, spleen, liver, heart, kidneys and testicles, and after washing to place them in 5 per cent. phenol for ten minutes. The tissues were next washed with sterile water, and passed first through a meat grinder and then through a special mill which pulped them so finely that they could then be passed through a twelve-mesh sieve. The ground tissue was finally mixed with one-third its weight of pure neutral glycerine containing 0.5 per cent. phenol. Apparently the blood of the vaccine producers was also added. The emulsion was then heated at 42° C. for three hours, and finally bottled and stored at cold-room temperature. Boynton states that liver, kidney, heart and blood do not constitute suitable antigen either separately or together, but that when added to lymphatic tissues and spleen they form a vehicle which increases the yield of vaccine to a marked degree; by the addition of these tissues the average virus animal furnishes approximately six litres of vaccine. It is difficult to follow the reasoning here unless it is supposed that the dilution of lymphatic and spleen pulp by the inert tissues is not followed by a decrease in the potency index of the vaccine; in fact, blood has been discarded as worthless by Kelser in later improvements on Boynton's technique.

According to Boynton "variations in the strength" of the vaccine can be produced by varying the temperature at which the preparation is heated. Heating at 41.5° C. for three hours produces a vaccine that will give rise to more or less severe reactions in inoculated animals, if it is used immediately after preparation, but which will gradually become attenuated by cold-storage for periods of three or four months. On the other hand, a vaccine heated for three hours at 44° to 45° C. is likely to have lost its potency, whereas an intermediate but slightly higher temperature than that recommended, viz., 43.5° to 44° C. gives a product that may be used soon after preparation but which rapidly loses its potency; larger doses of this preparation are required. The vaccine must be tested for potency before issue and retested after holding in cold storage for any length of time. The duration of the immunity conferred by the vaccine is not definitely known, but Boynton concludes from field observations—of which no details are given—that it may last three years or longer. He recommends the re-inoculation of adult cattle at two-year intervals.

Kelser, Youngberg, and Topacio (1928) point out that Boynton's preparation suffers from the great disadvantage that it must be aged in the refrigerator for periods varying from one to as much as six or seven months before it can be used without danger of producing rinderpest, and that during the ageing process it is necessary to test it by inoculation at frequent intervals. Once it has become avirulent it must be used within a relatively short time since it rapidly loses its antigenic potency, in some cases becoming completely inert within five or six weeks. In some instances a vaccine which at one ageing test produced rinderpest had entirely lost both its virulence and its antigenic potency before the next test was carried out.

An effort was therefore made by Kelser, Youngberg, and Topacio to evolve a method of preparation which would not be open to these serious objections, and they have been able to produce a potent vaccine that can be used within two or three hours of preparation,

and which retains its potency in the refrigerator for at least a year. Preliminary notices of this work were published during 1927.

Kelser's method is to rinse organs in 5 per cent. carbolic acid for fifteen minutes, wash in water, mince in a coarse mincer and store overnight at 2° C. The following day the tissue is finally ground and is passed through a forty-mesh sieve. 1 c.c. of sterile salt solution is added for every gramme of tissue, and finally sufficient chloroform to make an 0.75 per cent. concentration. The vaccine is bottled with the usual precautions to avoid contamination of the mouths of the bottles and is stored for at least 48 hours. The storage is advisable on account of the irritant effect of chloroform if the preparation is injected earlier. Although blood was at first used in the preparation of the vaccine it was later discarded and saline substituted. The tissues used are spleen, liver, and lymph glands (not including the mesenteric glands). Kidney, and mesenteric glands—although it is stated that they furnish a good antigen—are not used, on account of the frequency with which bacterial contamination is present. According to Kelser's experiments liver tissue prepared by his method furnishes a satisfactory antigen; particulars are given of two animals, numbers 6,343 and 6,344, which were inoculated with three doses each of 10 c.c. of liver-vaccine at weekly intervals. Fifteen days after the vaccination was completed these animals were tested by the inoculation of virulent blood and showed no reaction whatever. Boynton, the Japanese workers, and the present writer have independently reached the conclusion that liver by itself does not furnish a satisfactory antigen, and additional experiments with liver vaccines prepared both by the method of the present writer and by Kelser's method are cited below.

Kelser and his collaborators recommend the injection of three doses of 15 to 20 c.c. each at weekly intervals as a routine method; this amount corresponds roughly to a total of from 28 to 37.5 grammes of infected tissue. When a single injection of vaccine was tested on two cattle it was found that a dose of 25 c.c.—roughly 15.5 grammes of tissue—protected to the point of allowing a slight reaction when the animal was inoculated with virulent blood 14 days later. The second animal which received a dose of 15 c.c.—rather more than 9 grammes of tissue—developed acute rinderpest when tested and would have died, but was killed to conserve tissues. The vaccine used here contained blood, and Kelser reasonably concludes that it was only some 75 per cent. as efficacious as the final vaccine preparation. Vaccine was found to retain its potency in cold store for periods of at least one year, and further data are promised with regard to this point. Rodier (1928) has carried out field experiments upon the possibility of immunizing by the single dose method with Kelser's vaccine. In a number of controlled observations he was able to demonstrate complete protection with single doses, ranging from 15 c.c. to a minimal amount of 4 c.c., when the immunity test was carried out ten to fifteen days after vaccination. It is noteworthy that liver was not used in Rodier's vaccine, but only lymph glands, tonsils and spleen. Again no data are available as to the duration of the immunity.

On reading the reports of these workers together with the brief record of one test of vaccination by Curasson and Delpy, one is struck

by the effort they have made to obtain the greatest possible amount of information from comparatively few animals in the experiments, by the general tendency to reduce the number of animals in each experiment and by the lack of definite observations on the duration of the immunity. One fully appreciates the circumstances that have forced workers into this position. The chief factors are perhaps the high cost and the difficulty in obtaining an adequate supply of certainly susceptible experimental animals, added to which there are incidental difficulties with regard to housing, isolation and feeding; indeed the present writer's own observations bear evidence of the same desire to cover much ground with few experiments, but on certain points perhaps they offer more definite data, particularly with reference to certain field observations on the duration of immunity.

In general, recent experiments of the present writer have been concerned with the confirmation and extension of the conclusions reached in the attached paper, and the findings will be collected and published in due course. It is only necessary to state here that no contradictory results have been obtained, and to mention one or two relevant details.

In the first place attention has already been drawn to the fact that Kelser considers liver a suitable antigenic tissue. As this was in direct opposition to the conclusions reached by the writer, by Boynton and the Japanese workers, tests were carried out with liver vaccines prepared both by the writer's methods and by Kelser's technique. Table I shows the results obtained in one of these tests, completely confirming the earlier conclusion that liver does not furnish a satisfactory antigen.

TABLE I.

Vaccine.	Number.	Dose of Tissue.	Reaction to Vaccination.	Interval before Test.	Result of Test.	Remarks.
Kelser's Liver Vaccine.....	X3519	25*g*	Nil..... ..	8 days..	Rinderpest.....	Confluent stomatitis, etc. Severe reaction 7 days.
„ „	X3562	12·5*g*	„	„ ..	„	Confluent stomatitis, etc. Severe reaction. Bled to death for virus.
„ „	X3546	6·25*g*	„	„ ..	„	Confluent stomatitis, etc. Severe reaction 9 days.
„ „	X3547	3*g*	„	„ ..	„	Confluent stomatitis, etc., Severe reaction. Bled to death for virus.
„ „	X3522	1·5*g*	„	„ ..	„	„ „ „

TABLE I.—*Contd.*

Vaccine.	Number.	Dose of Tissue.	Reaction to Vaccination.	Interval before Test.	Result of Test.	Remarks.
Formalized Liver Vaccine 1/1,000	X3545	8*g*	Nil.....	8 days..	Rinderpest.....	Confluent stomatitis, diarrhoea. Severe reaction 9 days.
„ „ „	X3558	4*g*	„	„ ..	„	Confluent stomatitis, etc., diarrhoea. Bled to death for virus.
Kelser's Spleen Vaccine.....	X3517	25*g*	„	10 days.	Immune.	
„ „	X3559	12·5*g*	„	„ ..	„	
„ „	X3532	6·25*g*	„	„ ..	„	
„ „	X3524	3*g*	„	„ ..	Temperature reaction.	Reaction lasting 6 days. No other symptoms.
Formalized Spleen Vaccine 1/1,000	X3529	1*g*	„	8 days..	„ „	Two morning temperatures of 104° and one of 104·4° were registered on the 5th, 6th and 7th days. No other symptoms.
„ „ „	X3523	1*g*	„	„ ..	„ „	One morning temperature of 103° and one of 103·4° on the 6th and 7th days. No other symptoms.
„ „ „	X3565	0·5*g*	„	„ ..	„ „	Three morning temperatures over 103° on the 5th, 6th, and 7th days. No other symptoms.
„ „ „	X3561	0·5*g*	„	„ ..	„ „	Two morning temperatures over 103° on the 5th and 6th days. No other symptoms.

A second detail concerns possible variations in the strength of formaldehyde in commercial formalin solutions. When the experiments were resumed after the writer's return from leave, unexpected temperature reactions were observed in animals inoculated with vaccines that had been prepared at the higher concentrations of formalin, viz., 1 in 1,000, 1 in 1,500. The presence of a quantity of polymetized deposit at the bottom of the formalin bottles attracted suspicion to the reagent.

TABLE II.

VACCINE.	Number.	Dose of Tissue.	Reaction to Vaccination.	Interval before Test.	Result of Test.	Remarks.
Spleen Vaccine Formalized 1/1,500 (untitrated polymerized sample)	X3548	2*g*	Temperature reaction	23 days.	Immune.....	Temperature reaction to vaccination on the 8–11th days. No symptoms.
,, ,, ,,	X3534	1*g*	,,	,,	,, ...	Temperature reaction to vaccination on the 10–13th days. No symptoms.
,, ,, ,,	X3489	1*g*	,,	,,	,, ...	Temperature reaction to vaccination on the 16th day. No symptoms.
,, ,, ,,	X3538	0·5*g*	,,	,,	,, ...	Temperature reaction to vaccination on the 9th and 10th days. No symptoms.
Spleen Vaccine Formalized 1/1,000 (untitrated polymerized sample)	X3488	2*g*	Nil.....	,,	,,	
,, ,, ,,	X3500	1*g*	,, ...	,,	Temperature reaction	Temperature reaction 5th to 8th days. No other symptoms.
,, ,, ,,	*X3516	1*g*	Temperature reaction	,,	Immune.....	Temperature reaction to vaccination 10–14th days. No symptoms.
,, ,, ,,	X3555	0·5*g*	,,	,,	,,	One morning temperature of 103–2° on the 10th day.
VACCINES PREPARED IN STANDARDIZED FORMALIN.						
Spleen Vaccine Formalized 1/3,000.....	X3702	4*g*	Temperature reaction	18 days	Immune.....	Temperature reaction to vaccination 6th to 12th days. No symptoms.
,, ,, ,, ...	X3726	1*g*	,,	,,	,, ...	,, ,, ,,
Spleen Vaccine Formalized 1/2,000.....	X3763	4*g*	,,	,,	,, ...	Temperature reaction to vaccination 7th to 11th days. No symptoms.
,, ,, ,, ...	X3766	1*g*	,,	,,	,, ...	Temperature reaction to vaccination 15th to 17th days. No symptoms.
Spleen Vaccine Formalized 1/1,500.....	X3714	4*g*	Nil.....	,,	,, ...	
,, ,, ,, ...	X3692	1*g*	,, ...	,,	,, ...	
Spleen Vaccine Formalized 1/1,000.....	X3728	4*g*	,, ...	,,	,, ...	
,, ,, ,, ...	X3718	1*g*	,, ...	,,	,, ...	

* Reaction confirmed as Rinderpest by sub-inoculation of blood to X3770.

It was concluded from the incidence of reactions among the animals inoculated with vaccines prepared in graded concentrations of formalin—see Table II—that if the explanation was to be found in deterioration of the formalin, then this particular sample was approximately 50 per cent. of the normal strength. Titration by the officially recognized American method gave a reading of 19.3 per cent. for this sample in place of the normal 38 to 40 per cent. Adjustment of the amounts added to vaccines to compensate for this loss immediately restored the vaccine to its normal behaviour. It is advisable then to titrate samples of formalin from time to time, to filter off any polymer that may be deposited, and to adjust the strength at which vaccines are prepared to compensate for any loss that may have taken place.

Field Inoculations.

On the graphs attached to this paper details are given of certain field trials with different vaccines carried out during December, 1927, and January, 1928. The animals inoculated on farm B were carefully marked with distinctive brands according to the type of vaccine used. It will be recalled that four types of vaccine were employed on this farm. Recently samples have been drawn from lots (1), (2), and (3) for the purpose of testing by contact exposure to rinderpest and by inoculation of virulent blood. No tests have been carried out on animals of lot (4) on account of the fact that these animals were in contact with the reacting animals of lot (1) for a period of roughly one month before they themselves were vaccinated. In the circumstances it is of course possible that some of the animals of this lot may have been immunized naturally before vaccination and therefore no useful purpose would be served by the testing.

Ninety animals from lots (1), (2), and (3) were dispatched to the laboratory for testing; no selection was exercised in the sampling except that an effort was made to obtain numbers roughly proportional to the numbers in each batch. All the animals were subjected to an inoculation with 5 c.c. of citrated virulent blood, corresponding roughly to some 7,000 minimal infective doses of virus, and the inoculations were carried out in heavily-infected buildings. In addition the first five animals of each batch were exposed to contact infection for a period before the test inoculation. Reference will be made to this contact exposure when dealing with the results of the tests. For convenience the test inoculations were carried out in three separate batches, and in each case the results were controlled by the inoculation of susceptible animals which were used on the 7th day as virus producers, 14 of these controls were utilized in the three inoculations and in no case was there a failure to infect. The longest period of incubation was 3 days and the average 2.4 days. A composite chart of these control animals is appended, together with the charts of all the test animals that showed any reaction whatsoever. For the purpose of this test any test-animal showing a single morning temperature of 103° or over, during the period from the 2nd to the 15th days, is classed as exhibiting a temperature reaction if the pyrexia is entirely unaccompanied by other symptoms. Any animal which, either in addition to or without a temperature reaction, displays any symptoms that may possibly be associated with rinderpest is classed as having suffered from rinderpest, even though the symptoms may have been confined to a watery lacrymation lasting a few hours only. When the reactions are judged by these criteria it will be found that

the animals classed as having undergone temperature reactions cannot be detected by the most careful clinical examination, excluding of course the taking of temperatures.

TABLE III.

VACCINE TYPE (1).		VACCINE TYPE (2)		VACCINE TYPE (3).	
Total 29		Total 24		Total 37	
3335		3340		3337	
3336		41	Temperature reaction	39	
43				39	
45		42	„	47	
48		44	„	49	
		46			
3598				3600	
99		3609		01	
3602		14		03	
04		21	Rinderpest	05	
08		23		06	Temperature reaction
10	Temperature reaction	25			
		29	Temperature reaction	07	
11				12	
13		35		22	
15		36		26	
16		37		28	
17		41		30	
18		43	Rinderpest	34	
19		45		38	
20	Temperature reaction	46	Temperature reaction	39	
				40	
24		48		42	
27		53	Temperature reaction	44	
31				47	
32		64		49	
33		65	Temperature reaction	50	
51				55	
52		66	Rinderpest	56	
54		68	Temperature reaction	3657	
59				58	
63				60	
				61	
				62	
				67	
				69	
				70	
				71	
				72	

In Table III above are given the results of the test inoculations with virulent blood. It will be observed that out of 29 animals of type (1) (single injection of infective vaccine), 2 only, show slight temperature reactions; the charts are appended. Of 24 animals of type (2) (single injection of completely-inactivated vaccine), 11 reacted to the test inoculation, and of these 8 showed temperature reactions only, while 3 developed mild lesions of rinderpest; there were no fatalities. Of 37 animals of type (3) (injection of completely-inactivated vaccine followed by one injection of infective vaccine type 1), 1 only showed an extremely mild and transient temperature reaction on the 9th day. Attention might be drawn to the fact that while it was not possible to perform sub-inoculations of blood from

every animal showing a temperature reaction during the test, these have been carried out in the course of similar reactions on frequent occasions, and the first reactions in this test were confirmed as rinderpest by sub-inoculation. The tests in Table III took place from 12 to 15½ months after the vaccination.

Inspection of Table III is sufficient to convince one that the vaccines of types (1) and (3) are capable of provoking a very high-grade immunity which persists for a period in excess of fifteen months, and that vaccine type (2) does not afford as great a protection. Referring back now to the question of resistance to contact infection, it is noteworthy of the first five animals in each batch, four were submitted to three weeks' contact exposure and one to two weeks' exposure. The exposure was the most severe that it was possible to arrange at the laboratory, two test animals being placed in each box together with two animals passing through an acute attack of rinderpest, the infecting animals being replaced by fresh reacting animals whenever they died or recovered. Owing to the difficulty of providing stabling facilities and artificial feed, it was not possible to submit the remaining animals to such a contact exposure, although it was realized that such a procedure would have been of interest in view of the results obtained with the fifteen exposed animals. None of the fifteen exposed animals reacted in any way to contact infection, although three out of the five animals of type (2) reacted to the subsequent inoculation of virulent blood. It seems reasonable therefore to suppose that most, if not all, of the animals in Table III which gave only temperature reactions as a result of the inoculation of virus were nevertheless sufficiently immune to withstand infection by contact.

It is necessary in a field experiment of this kind to consider certain factors that are more or less beyond the control of the worker, and to ascertain how far such factors may influence or even invalidate the results obtained. In the course of the present experiment there are only two such factors that need be taken into consideration: (1) The question of susceptibility of the animals before vaccination, and (2) the possibility that the animals may have acquired a natural immunity as a result of an attack of rinderpest in the period elapsing between the vaccination and testing. In connection with this last criticism it has to be admitted that a small outbreak of rinderpest did occur on the farm in question some seven months after the vaccinations were completed; the outbreak, however, only affected unweaned calves and certain animals that had been immunized by the simultaneous serum and virus method a year previously, and the writer is informed by the manager that none of the vaccinated animals was ever within two miles of the infected area.

With reference to point (1), the original susceptibility of the vaccinated animals, which were an even lot comparable in every way, can be gauged quite fairly from the reactions that took place during the vaccinations with vaccine type (1). One hundred and eight animals reacted out of a total of 127 inoculated with this vaccine, and it is proposed to take this figure 108—127 as the index of susceptibility of the whole group, although the actual susceptibility was no doubt higher since the vaccine used on this lot was partially attenuated, and certainly did not represent virus in its most virulent form.

The possibility (2) that the immunity may have resulted from natural infection during the interim can best be examined by

statistical methods. The first question to be asked is whether the animals of type (2), Table III, i.e., those vaccinated with the weakest antigen, have any degree of immunity in excess of what might be expected to occur normally in a chance sample. We may gauge the initial susceptibility of these animals from the reactions observed in the batch of 127 animals inoculated with vaccine type (1) discussed above. The generally accepted method of treating this problem is shown in Table IV.

TABLE IV.

AFTER INOCULATION TYPE (2).

		Attacked.	*Immune.*	*Total.*
Observed	(m + x)	11.0 ...	13.0 ...	24.0
Expected	(m)	19.0 ...	5.0 ...	24.0
	x^2/m ...	3.31 ...	12.8 ...	

BEFORE INOCULATION TYPE (1).

		Attacked.	*Immune.*	*Total.*
Observed	(m + x)	108.0 ...	19.0 ...	127.0
Expected	(m)	100.0 ...	27.0 ...	127.0
	x^2/m ...	0.64 ...	2.33 ...	

$x^2 = 19.08$, $n = 1$.

Tables IV and V are in every way comparable to the illustrations from Greenwood and Yule, quoted by Fisher, page 83. If Pearson's tables are consulted for a value of 19.08, it will be found that the proportion in which such a distribution is liable to occur is .00001; the odds against the distribution shown are therefore 100,000 to 1, and in experimental work odds of 100 to 1, obtained in this manner, are universally accepted as evidence that a distribution has been significantly affected by the conditions of experiment, i.e., by the factor that necessitates separation of the different groups in the examination. Table IV then proves conclusively that the animals vaccinated with the weakest type of antigen—type (2)—had a degree of protection greater than normal 13 to 16 months after vaccination.

If it is denied that the immunity of the tested animals—Table III—is a direct result of the vaccination, then the only assumptions open to us are either that all the animals in the three batches shown in Table III were equally susceptible to natural infection seven months after vaccination when the outbreak of rinderpest occurred on the farm, or that their susceptibility varied according to the type of vaccine with which they had been treated. If the latter assumption is taken as correct, then the protection afforded by vaccination in these three groups may be supposed to have declined progressively up to the time of the natural outbreak, and at that time the animals of type (2) would be the most lightly protected. This group would consequently show a higher attack-rate during the outbreak of disease, and when the time came for testing would in consequence show a greater percentage of immunes. Inspection of Table III demonstrates that the very reverse was the actual case; the proportion attacked in type (2) during the test was more than seven times as great as that attacked in type (1) and more than eighteen times that of the attacked in type (3). This hypothesis must therefore be discarded.