STUDIES ON THE VACCINATION OF CATTLE AS A MEASURE AGAINST INFECTION WITH TUBERCULOSIS WITH THE LIVING VOLE ACID-FAST BACILLUS

By JAMES A. YOUNG and J. STUART PATERSON

From the Institute of Animal Pathology, University of Cambridge

(With 4 Figures in the Text)

CONTENTS

I. INTRODUCTION

The use of the vole acid-fast bacillus (Wells, 1937) for vaccination against bovine tuberculosis was suggested by Griffith & Dalling (1940), who, in reporting encouraging results from experiments carried out on a small number of calves, advised an extension of the investigation and we took up the work where they had left it.

In our first experiment successive groups of vaccinated and control calves were subjected to resistance tests with virulent strains of bovine type at intervals of from three months to three years from the date of vaccination. As the earlier groups were slaughtered for examination, ample confirmation was forthcoming for the claim that vaccination with the vole bacillus could enhance the resistance of an animal to tuberculosis to a remarkable degree, but as the later groups were examined in turn, it became evident that there was a time limit to the period of enhanced resistance. Our main task has been therefore to determine, by means of adequately controlled experiments carried out mainly under laboratory conditions, whether there is a possibility of making practical use of a method of vaccination which induces an enhanced resistance over a limited period of time.

The criterion of any vaccine is its efficacy under field conditions. For this reason, in addition to carrying out experiments under laboratory conditions to test the efficacy and safety of the vaccine, we seized an early opportunity of trying it out in the field, and we feel that with the conclusion of this long-term field experiment the time has come to assess progress.

The laboratory experiments varied considerably in purport, some being designed with several objects in view and few being complete in themselves. A limited amount of accommodation for the quarantining of animals before and after infection necessitated a certain amount of dovetailing of the experiments according to their size and duration rather than their purport, and the numbers allotted to the experiments in our records would be likely to prove confusing if we attempted to use them in this communication. We have, therefore, regrouped the experiments under letters in place of numerals.

II. TECHNIQUE

Except where specifically mentioned in the text, the techniques detailed in this section have been used throughout all the experiments.

(*a*) Vaccination and resistance tests

All cultures, whether of vole strain for vaccine or bovine strain for resistance test, were 7-day-old on unglycerinated Dorset's egg medium and the bacterial emulsions were prepared by Griffith's method of grinding weighed amounts of moist growth between ground glass plates with the gradual addition of normal saline and pipetting off of the emulsion until all the growth formed an even suspension in the requisite amount of saline.

Vaccination. The greater part of our work was done with strain LV 285, a strain isolated by Griffith from material from a vole forwarded to him by Wells in 1937 and used by Dalling and himself in their investigations.

During the course of tests to check the virulence of strain LV 285 in 1942, it was apparent that the strain had fallen off in virulence for small animals. Full virulence was restored by repeated passage through Orkney voles without intervening culture, and this passage strain is now being maintained by vole to vole transfer, cultures being made at the time of each transfer so that a recently isolated strain is always available. This passage strain has been used for our later experiments.

In one experiment we used a fresh strain, G 564, selected from a number of recently isolated strains kindly forwarded to us by Dr Wells.

Intravenous vaccination was performed by inoculation into the jugular vein. In the case of the few animals in which vaccination by the oral route was tried, the vaccine was placed on the back of the tongue by means of a glass pipette.

Resistance tests. The resistance tests have consisted of the oral administration of 7·5 mg. of virulent bovine bacilli. It appears to us to be a very debatable point whether a test dose should be of blunderbuss character and prepared from a number of strains or whether it should consist of one strain only. We have used both methods and latterly have preferred to use a single strain. We feel that it is unjustifiable to conclude that, in the event of some difference existing between various strains used collectively for a test dose, there will be uniformity in the degree with which each strain will exercise its pathogenic powers in each individual animal. When a single strain is used, it is certain that all lesions have been caused by the same virus and that any difference between the lesions in the control and vaccinated animals is the result of vaccination. The degree to which the control animals are infected provides evidence as to the suitability of the strain for the purpose for which it was selected. All the strains which we have used for resistance tests were recently isolated from cattle with severe natural infection.

In the case of young calves we found little difficulty in delivering the test dose to the back of the tongue by means of a glass pipette. With older animals, however, this is a somewhat hazardous proceeding, and we had recourse to the use of a rubber catheter fitted to a syringe. The animals were cast and gagged,

but even then it was not always easy to pass the end of the catheter over the tongue. Latterly, we used a silver catheter into which had been soldered the butt end of a wide-bore needle to fit a Record syringe. The curve of the catheter was widened so that the tip of the instrument could slide back readily between the tongue and the roof of the mouth while its rigidity enabled the operator to gauge the exact point of delivery of the inoculum even when this was not visible as a result of arching of the tongue.

(*b*) POST-MORTEM EXAMINATIONS

When the animals were due for examination, they were slaughtered either in the Institute's post-mortem room or, as was found more convenient in the case of animals returned from field tests on farms, at a local slaughterhouse under our supervision.

The organs were examined by palpation and multiple slicing of their substance but, with the exception of the lungs, involvement of the organs with tuberculous infection was a rare occurrence, and little more was necessary than an occasional histological examination of some lesion of dubious origin. In the case of cattle examined at the slaughterhouse it was not always possible to examine the liver and kidneys minutely, but it was our practice to bring back the lungs and any other organs which appeared macroscopically abnormal.

In all cases the lymphatic glands were subjected to an exacting search, group by group. In some cases the head glands, consisting of submaxillary, parotid and post-pharyngeals, were dealt with separately for guinea-pig inoculation tests. In other cases they were included with the other glands draining the alimentary tract, the coeliac, hepatic, mesenteric and colonic glands. The bronchial and mediastinal glands formed the thoracic group, while the carcass group was composed of prescapular, precrural, popliteal, iliac and renal glands.

The glands were stripped clean from fat and other connective tissue and examined by inspection and palpation. If any lesions were detected, material from these lesions was removed for cultural and biological examination. If no lesions were detectable, material was removed by random sampling, numerous specimens being taken and pooled from each group of glands for biological tests. The glands were then finely sliced in a continuation of the search for lesions. By using a Heiffor microtome knife it is possible to make from sixteen to twenty slices to the inch, a procedure which, except in the case of extremely minute lesions, permits the securing for bacteriological examination of uncontaminated material from any lesions which come to light. Fresh sterilized knives and cutting blocks were used for each group of glands, so that in the event of lesions being found that were too small to permit of sterilization of the surface by searing, the risk of contamination from outside sources was small.

(*c*) BACTERIOLOGICAL EXAMINATION

Material from definite lesions was emulsified in Griffith's tubes and examined microscopically for the presence of acid-fast bacilli, and cultures were made on unglycerinated and glycerinated egg media from a portion of the emulsion after treatment with 5% potassium hydroxide. A further portion was retained in cold storage as reserve material, and the remainder was inoculated subcutaneously into two guinea-pigs.

Material taken by random sampling from groups of glands was emulsified in mortars with the minimum amount of saline which would yield an emulsion of a consistency suitable for use in a syringe. Two or three cubic centimetres were inoculated into two guinea-pigs, the remainder being kept in cold storage in reserve. In the event of animals dying from infection with secondary organisms, the reserve material was treated with 6% sulphuric acid, neutralized with potassium hydroxide and inoculated into a further pair of guinea-pigs. Lesions found only after material had been taken for random sampling were treated separately for cultural and biological tests.

The guinea-pigs were killed for examination after periods varying from 3 weeks to 3 months according to the presence or absence of signs of infection. Cultures were made from the guinea-pigs in which tuberculous lesions were found, and latterly we have made it a practice to culture from the regional glands of all guinea-pigs inoculated with material from vaccinated calves or cows after we had made the chance discovery that it was possible for viable organisms of the vole strain to reach and lie dormant in these glands without causing any visible local lesion or enlargement of the glands.

(*d*) HISTOLOGICAL EXAMINATION

On several occasions we relied on histological examination to confirm the tuberculous nature of small lesions, but for reasons outside our control we were unable to make a systematic histological study of the lesions in the vaccinated animals.

In those experiments, however, in which we attempted to follow up the effects of intravenous inoculation of the vole bacillus in the calf, an extensive series of sections was prepared from all the tissues which seemed likely to be affected, and our description of the changes seen in those tissues is based on sections stained with haematoxylin and eosin and with Ziehl-Neelsen's stain.

III. PROTECTION EXPERIMENTS

(*a*) Introduction

In our experiments we have sought, not only to confirm the findings of Griffith & Dalling that the vaccination of cattle with the vole bacillus can bring about an enhancement of their resistance to infection with the bovine tubercle bacillus, but also to determine the duration of the period of enhanced resistance. In most of our work we have used the intravenous route for inoculation, but we have also given a short trial to the method of oral administration. The unsightly abscess formation which follows subcutaneous injection and the resultant injury to the hide rule out this method of vaccination for general use in the field, and the same objections would hold in the case of intradermal inoculation. We have, however, given a trial to the method of multipuncture skin inoculation.

In order to present our results in the most concise manner we have excluded from the summaries of the port-mortem findings any mention of intercurrent disease such as actinobacillosis or corynebacterial infection. The term 'lesion' refers only to a lesion of undoubted tuberculous origin, and it can be accepted that when it is used, the bovine bacillus was recovered from the animal in question or, in a few cases where a full bacteriological examination appeared unnecessary, the diagnosis was made by the demonstration of acid-fast bacilli in films and by histological section. It can also be accepted that where it is stated that there was no evidence of tuberculous infection, a full biological test was made with negative results.

In the reports on some of the experiments we have inserted figures in which the experiments are set out in schematic fashion, the relative degree of infection in each animal being indicated by plus signs. In the case of the laboratory experiments the number of plus signs shown against each animal represents our opinion as to the severity of the infection present. In the long-term field experiment, however, in which the animals were exposed to natural infection, we adopted a different method of assessing the number of plus signs to be allotted to each animal, attempting to judge each animal according to its potential danger to the rest of the herd rather than according to the amount of disease present.

(*b*) Single dose of vaccine intravenously, followed by exposure to infection *per os* in the laboratory

(1) Experiment A. *Vaccination with 5 mg. of strain LV 285*

In this experiment we followed the suggestion of Griffith & Dalling that a further test should be made of the immunizing power of the vole bacillus administered intravenously in a dose of 5 mg. We did not confine ourselves, however, to testing the resistance merely 6 months after vaccination, and sufficient calves were used to permit of observations being made after different intervals of time.

We commenced the experiment with twenty-four calves, all of which had been tuberculin tested and classed as negative reactors. When approximately 3 months old, fourteen of these animals were vaccinated intravenously with 5 mg. of the vole strain LV 285, the remaining ten animals being left to serve as controls.

Groups of two vaccinated and two control animals were subjected to a resistance test consisting of the oral administration of 7·5 mg. of virulent bovine bacilli at intervals of 3, 6 and 9 months after vaccination. Under the original plan four vaccinated and two control animals were to be tested at 12 and 18 months, but when the results in the earlier groups appeared distinctly promising, it was decided to extend the duration of the experiment up to 3 years. Four more tuberculin-negative animals of approximately the same age were added to the controls, and groups of two vaccinated and two control animals were tested at 12 and 18 months and at 2 and 3 years.

Throughout the experiment all animals were tuberculin tested at regular intervals, and the animals were slaughtered for examination approximately 6 months after their respective resistance tests.

The experiment is set out schematically in Fig. 1, and the results of the post-mortem examinations are given below in summarized fashion, the number of days between resistance test and slaughter being shown in each instance.

Summary of post-mortem examinations

Group A (vaccinated 3 months)

Controls

Calf RC 146 (188 days). Multiple lesions of infiltrative type in post-pharyngeal, coeliac, mesenteric and colonic glands.

Calf RC 149 (185 days). Numerous lesions of infiltrative type in mesenteric glands and a single lesion in one hepatic gland.

Vaccinated

Calf RC 127 (198 days). One encapsulated lesion of considerable severity in one post-pharyngeal gland and a smaller lesion of similar nature in one mesenteric gland.

Calf RC 140 (195 days). One small encapsulated lesion in one mesenteric gland.

Group B (vaccinated 6 months)

Controls

Calf RC 145 (171 days). Moderately numerous lesions of infiltrative type in mesenteric glands.

Calf RC 148 (177 days). Numerous lesions of infiltrative type in mesenteric glands.

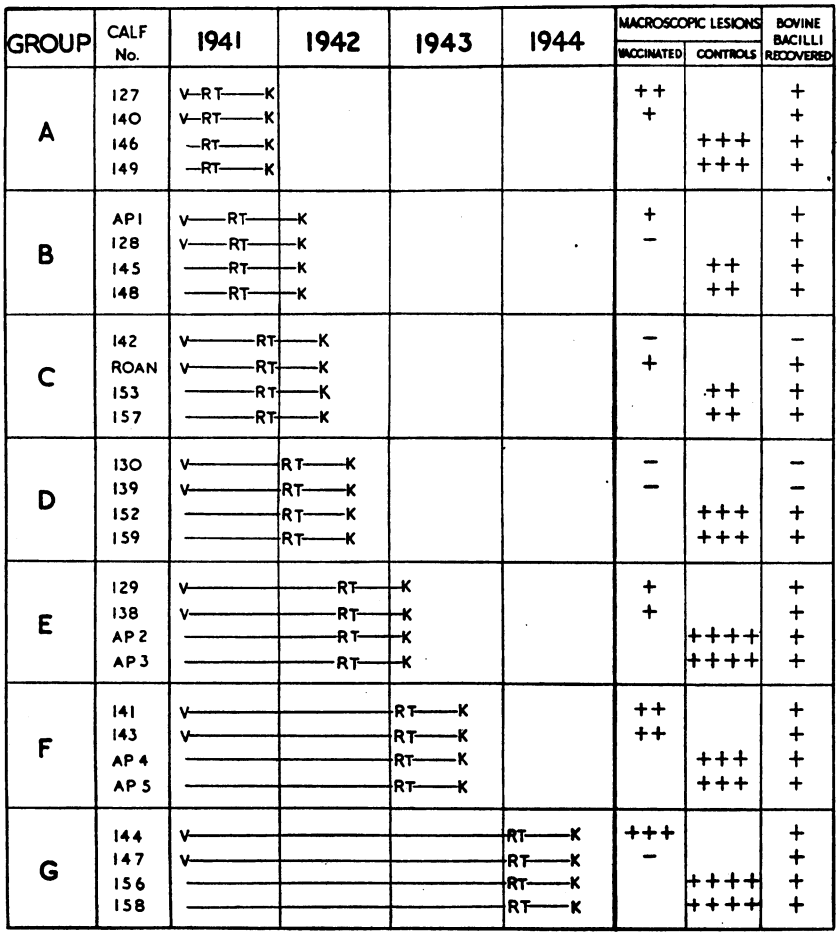

Fig. 1. Experiment A. Single vaccination with strain LV 285. Vaccinations simultaneous. Infections staggered. V, vaccinated; RT, resistance test; K, killed. Biological tests: positive +, negative –.

Vaccinated

Calf AP 1 (175 days). Two small encapsulated lesions in mesenteric glands.

Calf RC 128 (181 days). No macroscopic lesions seen, but strain of bovine type recovered by biological test from mesenteric glands.

GROUP C (vaccinated 9 months)

Controls

Calf RC 153 (175 days). Lesions of infiltrative type in both post-pharyngeal glands and a moderate number of similar lesions in mesenteric glands.

Calf RC 157 (168 days). Lesions confined to mesenteric glands and only in moderate number. Mostly of infiltrative type, but several showing signs of encapsulation.

Vaccinated

Calf RC 142 (171 days). No lesions of tuberculous nature found and biological tests negative.

Calf 'Roan' (177 days). Several tiny encapsulated lesions in mesenteric glands. Strain of bovine type recovered from one lesion, and another lesion examined histologically shown to be tuberculous, yet random sampling of mesenteric glands proved negative in biological tests.

GROUP D (vaccinated 12 months)

Controls

Calf RC 152 (173 days). Numerous lesions of infiltrative type in coeliac and mesenteric glands.

Calf RC 159 (180 days). Moderately numerous lesions of infiltrative type in coeliac and mesenteric glands and one lesion in a hepatic gland.

Vaccinated

Calf RC 130 (176 days). No evidence of tuberculous infection.

Calf RC 139 (183 days). No evidence of tuberculous infection.

GROUP E (vaccinated 18 months)

Controls

Calf AP 2 (174 days). Very numerous lesions in post-pharyngeal, coeliac, mesenteric, colonic and hepatic glands. Although some of infiltrative type, majority beginning to show signs of commencing resistance on part of host.

Calf AP 3 (177 days). Very numerous lesions in sub-maxillary, bronchial, mediastinal, coeliac, mesenteric and colonic glands. Majority still of infiltrative type, but some showed signs of encapsulation.

Vaccinated

Calf RC 129 (175 days). Several tiny lesions in mesenteric glands. Section of one examined histologically showed tuberculous changes, and biological test of pooled alimentary glands yielded culture of bovine type.

Calf RC 138 (178 days). One tiny lesion found in one submaxillary gland but biological test negative. Biological test, however, of pooled head glands yielded culture of bovine type.

GROUP F (vaccinated 2 years)

Controls

Calf AP 4 (181 days). Numerous lesions of infiltrative type in mesenteric and colonic glands.

Calf AP 5 (183 days). Several small lesions in one post-pharyngeal gland and a moderate number in the mesenteric glands. Lesions caseo-calcareous and tending to be circumscribed.

Vaccinated

Calf RC 141 (182 days). Numerous small lesions in coeliac, mesenteric and colonic glands mainly tending towards encapsulated type but some of more infiltrative character.

Calf RC 143 (184 days). Lesions of encapsulated type in two coeliac and one mesenteric gland.

GROUP G (vaccinated 3 years)

Controls

Calf RC 156 (176 days). Very numerous lesions in post-pharyngeal, bronchial, mediastinal and mesenteric glands. Some lesions of caseo-calcareous type, others of infiltrative granulomatous type with calcareous granules and spicules.

Calf RC 158 (176 days). Numerous lesions in sub-maxillary, parotid, post-pharyngeal and mesenteric glands. Lesions varied in size from pin-head to hazel nut, and were mainly soft caseous in character with a tendency to encapsulation.

Vaccinated

Calf RC 144 (181 days). Fairly numerous lesions in post-pharyngeal and mesenteric glands.

Calf RC 147 (184 days). No macroscopic lesions found, but biological test positive for a bovine strain from pooled alimentary glands.

(2) EXPERIMENT B. *Vaccination with 5 mg. of strain* G 564

In this experiment groups of calves of different ages were vaccinated at various intervals before all were exposed to a resistant test at the same time. This was done with a twofold purpose: first, to ensure that each animal was exposed to a similar risk of infection, and secondly, to determine if age might be a factor influencing results. In addition, by reducing the tuberculin testing after vaccination to a minimum in a number of the animals we sought to gain information regarding the effect of repeated tuberculin testing on (or withholding the same from) vaccinated animals.

It was decided that for this experiment we should use a freshly isolated strain of the vole bacillus, and Dr A. Q. Wells kindly supplied a number of young primary cultures. Subcultures were made from these strains and one, G 564, was selected as that least likely to require many subcultures before yielding sufficient growth for our purpose. The dose and route, namely, 5 mg. intravenously, were the same as those used in Exp. A.

Fourteen calves of approximately equal age were selected. Of these, four were earmarked for immediate vaccination, four for vaccination 9 months later, four for vaccination 10½ months later and two to serve as unvaccinated controls.

Of the first group of four vaccinated, one calf died 4 weeks after vaccination, death being attributable to a severe reaction to the vole strain. The fact that a recently isolated strain was to be used had precluded any preliminary investigation of its pathogenicity by tests on the smaller laboratory animals, and, as it was important to decide at once if it was safe to continue to use strain G 564, a second calf was sacrificed for immediate examination. Although a few tiny foci were found in the lungs, the lesions were not of sufficient severity to give cause for abandoning the experiment.

Four more calves were obtained and vaccinated. Again one calf succumbed to the effects of the vaccination, but as the remaining three continued to thrive they were added to the two survivors of the first group to form group A, a group of five instead of four as originally planned.

Groups B and C, each containing four animals, were vaccinated 9 and 10½ months later. None of these animals showed any ill effects following vaccination.

To complete the programme six young calves approximately 9 months younger than the other fifteen animals were included in the experiment, two (group D) being vaccinated at the same time as the animals in group B and two (group E) along with the animals in group C, while the remaining two served

as controls. None of the young calves showed any severe reactions following vaccination.

As in Exp. A the resistance test consisted of the oral administration of 7·5 mg. of virulent bovine bacilli. Group A had been vaccinated 12 or 13 months, groups B and D 3 months and groups C and E 6 weeks when the resistance test was made, and all animals were slaughtered for examination approximately 6 months after the test.

The experiment is shown in schematic fashion in Fig. 2 and the post-mortem findings are summarized below, the number of days between resistance test and slaughter being shown in each instance.

Summary of post-mortem examinations

GROUPS A, B AND C

Controls

Calf RC 292 (174 days). Numerous lesions mainly of infiltrative type in both post-pharyngeal glands. Lesions in mesenteric glands confined to four large glands, but very numerous in these glands and of infiltrative nature.

Calf AP 31 (177 days). Numerous small lesions in one post-pharyngeal gland, in the coeliac glands and in the larger mesenteric glands. One calcium granule found in one mediastinal gland. Lesions mainly calcareous granules or spicules in granulomatous tissue without much evidence of caseation.

Group A (vaccinated 12 months)

Calf RC 312 (198 days). Minimal tuberculin testing. Several tiny lesions in one post-pharyngeal gland from which the bovine strain was recovered. The biological test showed that bovine bacilli were present also in other head glands but failed to detect similar infection of mesenteric, thoracic or carcass glands. The vole strain, however, was recovered from the regional glands of the guinea-pigs inoculated with material from the carcass glands. The vaccinating strain had survived in the calf for 565 days.

Calf RC 314 (177 days). Repeated tuberculin testing. No evidence of infection with bovine strain, but vole strain recovered through guinea-pig from thoracic glands. Survival period in calf 544 days.

Calf RC 316 (199 days). Repeated tuberculin testing. No evidence of infection.

Group A (vaccinated 13 months)

Calf RC 300 (181 days). Minimal tuberculin testing. No lesions found, but bovine strain recovered by means of biological test from pooled alimentary glands.

Calf RC 301 (174 days). Repeated tuberculin testing. No lesions found and biological tests negative.

Group B (vaccinated 3 months)

Calf RC 304 (203 days). Minimal tuberculin testing. No evidence of infection with bovine strain, but vole strain recovered by biological test from carcass glands.

Calf RC 305 (188 days). Minimal tuberculin testing. No evidence of infection.

Calf RC 306 (178 days). Repeated tuberculin testing. No evidence of infection with bovine strain, but vole strain recovered by biological test from carcass glands.

Calf RC 307 (189 days). Repeated tuberculin testing. One small focus found in one post-pharyngeal gland, section of which showed it to be of caseo-calcareous nature with scanty giant cells present. No acid-fast bacilli seen and biological tests negative.

Group C (vaccinated 6 weeks)

Calf RC 299 (196 days). Minimal tuberculin testing. No evidence of infection with bovine strain, but vole strain recovered by biological test from thoracic glands.

Calf RC 308 (203 days). Minimal tuberculin testing. No evidence of infection.

Calf RC 311 (195 days). Repeated tuberculin testing. One small lesion in one post-pharyngeal gland from which a strain of bovine type was recovered. Histological examination of this lesion showed that there was little evidence of encapsulation, and that invasion had extended further into the tissues than had been apparent on macroscopic examination.

Calf RC 315 (182 days). Repeated tuberculin testing. A few tiny lesions in one submaxillary gland, both post-pharyngeal glands severely affected and converted into large loculated caseous abscesses and one encapsulated lesion in a mesenteric gland.

GROUPS D AND E

Controls

Calf RC 359 (175 days). Heavily infected with numerous lesions of infiltrative type in post-pharyngeal, left bronchial, coeliac and mesenteric glands.

Calf RC 358 (175 days). Heavily infected with numerous lesions of infiltrative type in post-pharyngeal, mediastinal, coeliac and mesenteric glands.

Group D (vaccinated 3 months)

Calf RC 363 (184 days). Minimal tuberculin testing. No macroscopic evidence of infection, but biological test yielded a bovine strain from the alimentary glands and a vole strain from the thoracic glands.

Calf RD 351 (185 days). Repeated tuberculin testing. No evidence of infection with a bovine strain, but non-viable acid-fast bacilli found in local lesion and regional gland of one guinea-pig inoculated with pooled thoracic glands. Type of organism unidentifiable by morphology.

Group E (vaccinated 6 weeks)

Calf RC 353 (191 days). Minimal tuberculin testing. No evidence of infection.

Calf RC 360 (192 days). Repeated tuberculin testing. No evidence of infection.

(3) EXPERIMENT C. *Vaccination with 0·005–5 mg. of strain LV 285 (vole-passaged)*

While awaiting the outcome of our longer-termed experiments, we carried out investigations to determine the fate of the vole bacillus in the calf, and as a result of the histological examination of certain organs misgivings arose regarding the possibility that

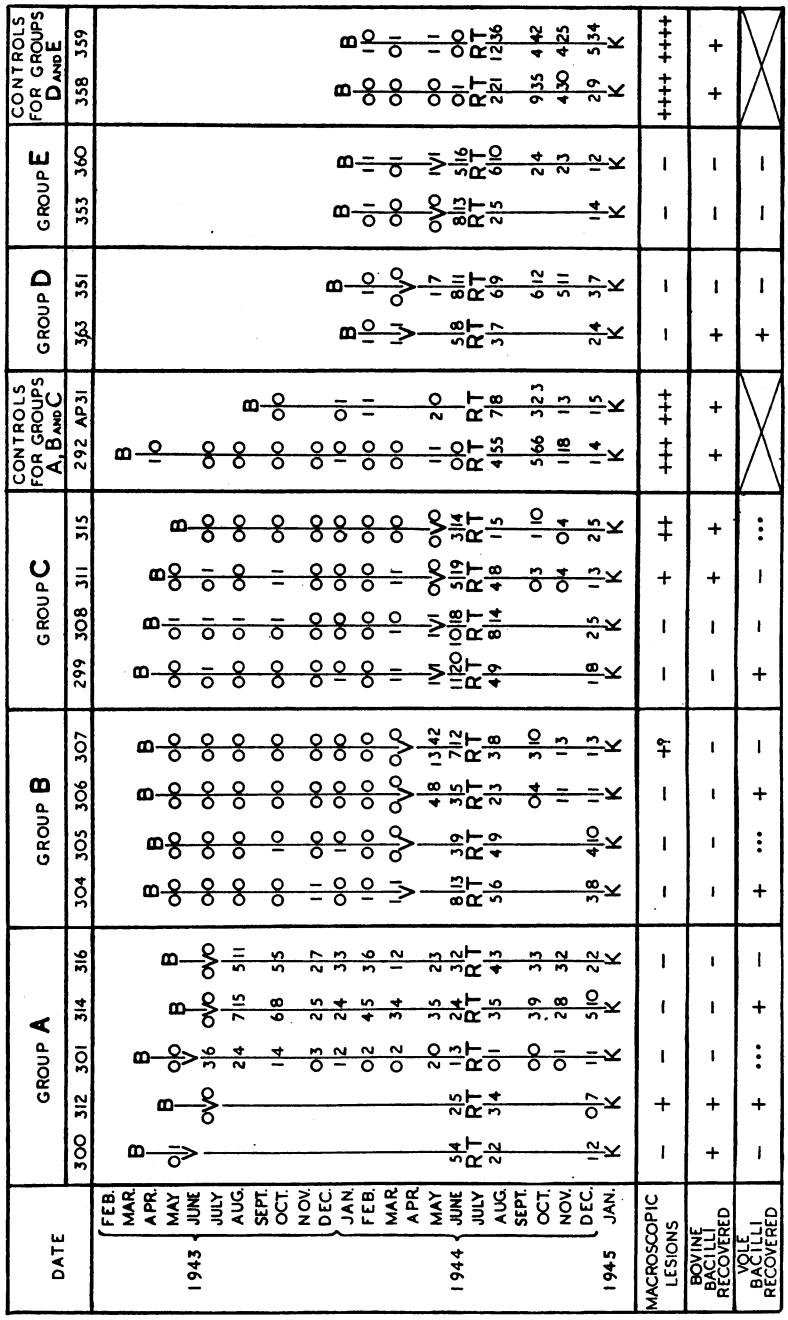

Fig. 2. Experiment B. Laboratory test of single vaccination in two lots of calves of different ages—vaccinations being staggered and resistance tests simultaneous. B, born; V, vaccinated; RT, resistance test; K, killed. Tuberculin tests: left-hand figure, avian; right-hand figure, mammalian; increases in mm. at 72 hr. Biological tests: positive +, negative −, not done ....

permanent damage might be caused, especially in young calves, by the intravenous inoculation of a dose of 5 mg. It seemed desirable that smaller doses of the vaccine should be tried and that the results should be assessed, not only as regards the efficacy of smaller doses of the vaccine in bringing about an enhanced resistance to subsequent infection, but also as regards any effects on the tissues arising as a result of the intravenous inoculation of the vole bacillus. An experiment was designed with this twofold purpose, some of the vaccinated animals being earmarked for protection tests (Exp. C) and others for histological examination (Exp. O).

For reasons explained elsewhere in this report we were led to believe that our stock laboratory strain, LV 285, was beginning to show signs of becoming unsuitable for general use as a vaccine, and it seemed that it might be advisable to replace it with its 'passage' modification. As the virulence of the passage strain, judged by tests on small laboratory animals, was much greater than that of the stock laboratory strain, Exps. C and O seemed good opportunities of testing the safety of the passage strain as a vaccine in cattle, since varying doses were to be used. It was decided therefore to use the passage strain at four levels of dosage commencing with our customary dose of 5 mg. intravenously and reducing this dose by decimal dilutions. Four groups of six calves (groups A, B, C and D) were inoculated intravenously with doses of 5, 0·5, 0·05 and 0·005 mg. respectively, two further calves being set aside as controls for the resistance tests. Two calves from each group were earmarked for histological examination, and further work on this will be described in a later section under Exp. O.

The vaccine was prepared from 7-day-old cultures of the strain in its seventh generation 5 months after its re-isolation from the tenth Orkney vole in the passage series.

As in Exp. B, some calves were tuberculin tested at regular intervals between the dates of vaccination and resistance test, while others were tested only immediately before the resistance test.

The resistance test was carried out 6 months after vaccination. One calf had died of corynebacterial pneumonia shortly after vaccination, but the remaining fifteen calves together with the two control calves were tested by the mouth with 7·5 mg. of virulent bovine bacilli and killed approximately 6 months later.

The experiment is set out in Fig. 3 and details of the post-mortem results are given below. In this experiment cultures were made from every guinea-pig in the biological tests which proved negative for a bovine infection in an attempt to determine the length of time over which the vole strain could survive in the calf. We failed to recover it from any of the calves 1 year after inoculation.

Summary of post-mortem examinations

CONTROLS

Calf 490 (163 days). Multiple lesions of infiltrative granulomatous type in head, coeliac, mesenteric and colonic glands.

Calf 503 (163 days). Large fibro-calcareous lesions in post-pharyngeal glands. Moderately numerous pin-head lesions in thoracic glands and numerous lesions in coeliac, mesenteric and colonic glands, mostly in small groups of greyish yellow foci containing heavy blocks of calcium.

VACCINATED CALVES

Group A (5 mg.)

Repeated tuberculin testing

Calf 510 (170 days). No evidence of infection.

Calf 498 (177 days). No evidence of infection.

Minimal tuberculin testing

Calf 523 (183 days). No macroscopic lesions found, but guinea-pigs inoculated with pooled head glands infected and bovine strain isolated.

Calf 486 (189 days). No evidence of infection.

Group B (0·5 mg.)

Repeated tuberculin testing

Calf 509 (169 days). No macroscopic lesions found, but guinea-pigs inoculated with pooled alimentary glands infected and bovine strain isolated.

Calf 500 (176 days). No macroscopic lesions found, but both guinea-pigs inoculated with pooled head glands and one of two inoculated with pooled alimentary glands infected and bovine strain isolated.

Minimal tuberculin testing

Calf 512 (182 days). One extremely small lesion found in mesenteric gland, but no acid-fast bacilli (a.f.b.) seen and biological test negative. Guinea-pigs inoculated with pooled alimentary glands infected and bovine strain isolated.

Calf 487 (188 days). Two pin-head lesions in mesenteric glands containing chalky material. A.f.b. numerous and bovine strain isolated. Only one guinea-pig infected out of two inoculated with pooled alimentary glands. Bovine strain isolated.

Group C (0·05 mg.)

Repeated tuberculin testing

Calf 504 (168 days). Both post-pharyngeal glands heavily infected with multiple lesions pin-head to small pea in size. Not markedly encapsulated and contents gritty and chalky. One very tiny lesion found in mesenteric gland. Bovine strain isolated from head glands, pooled alimentary glands and single mesenteric lesion.

Calf 502 (175 days). Two or three dozen pin-point to pin-head lesions scattered through mesenteric chain of glands. A.f.b. present and strain of bovine type isolated. Guinea-pigs inoculated with pooled head glands infected and bovine strain isolated.

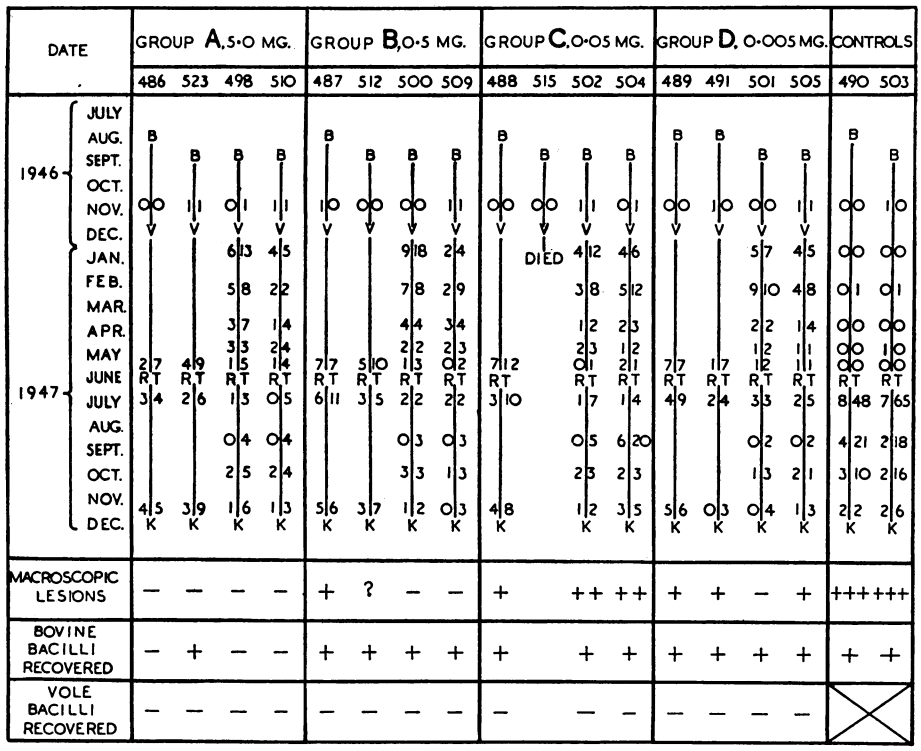

Fig. 3. Experiment C. Laboratory test of single vaccination with the passage form of strain LV 285 at four different levels of dosage. B, born; V, vaccinated; RT, resistance test; K, killed. Tuberculin tests: left-hand figure, avian; right-hand figure, mammalian; increases in mm. at 72 hr. Biological tests: positive +; negative −.

Minimal tuberculin testing

Calf 488 (185 days). Except for one tiny speck in one post-pharyngeal gland no macroscopic lesions found. Suspected lesion inoculated into one guinea-pig and animal infected. Bovine strain recovered. Guinea-pigs inoculated with pooled head glands not infected, but guinea-pigs inoculated with pooled alimentary glands infected and bovine strain isolated.

Group D (0·005 mg.)

Repeated tuberculin testing

Calf 505 (167 days). Two small lesions consisting of agglomerations of two or three small pin-head nodules with tiny greyish calcium foci in the mesenteric glands. Guinea-pigs inoculated from lesions infected and bovine strain isolated, yet in spite of one lesion just being missed in random sampling the pooled alimentary glands were negative to the biological test. Guinea-pigs inoculated with pooled head glands infected and bovine strain isolated.

Calf 501 (171 days). No macroscopic lesions seen in any gland, but guinea-pigs inoculated with pooled head and pooled alimentary glands respectively infected and bovine strains isolated.

Minimal tuberculin testing

Calf 491 (181 days). Two or three tiny yellowish foci in a small hempseed nodule in a mesenteric gland. A.f.b.+. Elsewhere in same gland three or four scattered pin-point yellow flecks. A.f.b.+. Guinea-pigs inoculated from lesions infected and bovine strain recovered.

Calf 489 (184 days). Two small hempseed fibrous nodules containing soft gritty calcium in mesenteric glands. A.f.b.+. Guinea-pigs inoculated from mesenteric lesions infected and bovine strain isolated. Guinea-pigs inoculated with pooled head glands infected and bovine strain isolated.

(4) Experiment D. *The observation over a prolonged period after exposure to infection of calves vaccinated intravenously with 5 mg. of strain LV 285.*

In the majority of our laboratory experiments we adhered to a period of 6 months as the standard time between exposure of the animals to infection and their slaughter for examination. By keeping to a standard time for examination after the administration of a standard infecting dose, the observer

becomes familiar with the type and severity of the lesions which he can expect to find in a control animal after that length of time, and the period of 6 months is ample for the purpose of demonstrating the presence of resistance in a vaccinated animal.

There was one question, however, which could not be answered in 6 months. Although in the majority of such of the vaccinated animals as were not completely protected, the lesions were small and circumscribed as compared with the lesions in the control animals, there was nothing to indicate what development these lesions might have undergone had the animals been allowed to live. We determined, therefore, to vaccinate and infect a number of animals and then kill these animals for examination at varying periods of time after infection.

Up to the time that Exp. D was initiated, no animals exposed to infection 3 months after vaccination had proved entirely resistant. It seemed likely that if we vaccinated animals and then infected them after this short period, we would be in a position to observe lesions at different stages of development.

Eight calves were vaccinated in the usual manner with strain LV 285 and subjected to a resistance test 3 months later. They were divided into four groups of two without any attempt at selection, and these groups, A, B, C and D, were earmarked for slaughter at 3 months, 6 months, 1 year and 2 years respectively. Two control animals were included, one to be killed with group A and the other with group B.

It cannot be claimed that this experiment was an outstanding success. For some reason the animals were particularly fractious on the day fixed for the resistance test, and in two instances disastrous results followed infection by inhalation of a portion of the test dose. In the remaining animals it was obvious that there had not been the initial equality in intensity of infection for which we had hoped. Group D was the only group which yielded valuable information.

Summary of post-mortem examinations

GROUP A (3 months)

Control

Calf AP 25. Head and mesenteric glands heavily infected. A few lesions in one mediastinal gland and one found in liver. Lesions different from those usually seen at 6 months, in that there was a thin semi-translucent capsule enclosing soft plastic tenacious material which could be shelled out to leave a clean-walled cavity. Calcareous spicules could not be detected macroscopically, but small granules were found microscopically.

Vaccinated

Calf AP 21. Two or three tiny semi-translucent foci with central yellow opacities in post-pharyngeal glands. Histological examination showed these to be of tuberculous nature.

Calf RC 293. One pin-head soft caseous mass surrounded by thin translucent zone of encapsulation. Histological examination showed this to be of tuberculous nature.

GROUP B (6 months)

Control

Calf RC 291. Numerous lesions in head and mesenteric glands, one in a parotid and a few in the thoracic glands. There was more caseation and less calcium than usual in a control animal at 6 months after infection.

Vaccinated

Calf RC 294. In one post-pharyngeal gland there was an area which appeared macroscopically to be of granulomatous nature. Histological examination showed granulomatous changes without any definite proof of the presence of tuberculosis, and the biological tests were negative.

Calf AP 22. Caseous cavities in right lung with spreading broncho-pneumonia. Several small lesions in thoracic glands. Biological test revealed presence of tubercle bacilli in head and mesenteric glands.

GROUP C (1 year)

Vaccinated

Calf RC 296. This animal, which had been earmarked for examination 1 year after infection, had to be destroyed 199 days after the resistance test owing to the onset of meningitis. Tuberculous lesions were found in lungs, thoracic glands, renal glands, brain and eyes.

Calf AP 24. No evidence of infection.

GROUP D (2 years)

Vaccinated

Calf RC 295. One single pea-sized lesion in one mesenteric gland consisting of a shaggy soft granular fibrous mass easily shelled out from surrounding tissue and containing gritty calcareous and caseous matter. Bovine bacilli of full virulence for rabbit recovered in culture.

Calf AP 23. No macroscopic lesions found, but one of two guinea-pigs inoculated with pooled material from the mesenteric glands infected and bovine strain recovered.

(5) EXPERIMENT E. *The development of immunity following intravenous vaccination with 5 mg. of strain LV 285*

With the object of demonstrating how soon evidence of resistance can be found in an animal after vaccination, we vaccinated six calves with 5 mg. of strain LV 285 at predetermined intervals before all were simultaneously exposed, together with two unvaccinated control calves, to a resistance test of 7·5 mg. virulent bovine bacilli administered by the mouth. The intervals between vaccination and infection were 42, 28, 14, 10 and 7 days respectively for five of the calves, while the sixth calf was vaccinated a few minutes before being infected.

The nature of the experiment did not call for a full bacteriological examination, attention being directed

rather to the character and extent of the tuberculous lesions when the animals were killed 6 months after infection. We could scarcely hope that there would be a steady gradation in the number and character of the lesions as between animal and animal according to their position in the series, for there is always a certain element of chance in the severity with which any particular group of glands becomes infected following infection by mouth, and there was in fact some considerable inequality between animal and animal. Nevertheless, some indication of the course of events could be elicited, as will be seen from the following summary of the post-mortem findings.

Controls

Both calves were severely affected with lesions of actively infiltrative character with much granulomatous tissue containing shining calcium spicules, the head glands of one animal being more severely affected than those of the other.

Vaccinated

In the 0-day calf there was little to distinguish this animal from the controls except for one comparatively large lesion in a post-pharyngeal gland in which there was a considerable amount of the moist chalky type of calcification which we have noted constantly in vaccinated animals.

In the 7-day calf there was no apparent reduction in the number of lesions, but changes of a granulomatous nature were reduced. Calcium deposition was of unusual type consisting of very large solid granules and nodules.

In the 10-day calf lesions were numerous in the head glands but considerably reduced in numbers in the mesenteric glands. There was an absence of macroscopic evidence of granulomatous changes, and calcium deposits consisted partly of hard granules and partly of soft chalky material.

In the 14-day calf lesions were still numerous in head and mesenteric glands, but there was evidence of commencing encapsulation, the encapsulated lesions containing calcium of moist French chalk type.

In the 28-day calf the post-pharyngeal glands were heavily infected with multiple lesions of varying size. The glands were granulomatous, but the calcification was of a soft chalky type. The lesions in the mesenteric glands were much fewer in number than in the earlier calves and were pin-point to pin-head in size with soft chalky caseous centres.

No lesions were noted in the 42-day calf.

Comments on Experiments A, B, C, D and E

Although these experiments varied in character, they were very largely complementary to one another and can be considered together.

In Exp. A the seven groups of animals were subjected to a resistance test at intervals after vaccination ranging from 3 months to 3 years. From Fig. 1 it will be seen that the evidence for an enhancement of resistance was progressively greater in each successive group up to 1 year (group D), at which time both the vaccinated animals (nos. 130 and 139) were negative to the biological test. After 1 year the resistance steadily declined, although in one of the vaccinated animals (no. 147) in the last group to be tested no visible lesions were found at the post-mortem examination, and latent infection with the bovine strain was detected only by the biological test.

It was not surprising that, having reached a peak, resistance should wane with the passage of time, but its apparent steady improvement over the first year after vaccination was unexpected. In seeking an explanation for this phenomenon several possible factors had to be considered. Although all animals were inoculated on the same day with the same batch of vaccine, the resistance tests were carried out at intervals, and in consequence there might have been inequality between the test doses. Secondly, the calves were in an active state of growth, and each successive group was 3 months older when it was tested. Thus the calves in group A were only 6 months old, whereas the calves in group D were 15 months old, when they were subjected to the resistance test. Thirdly, we felt that the repeated tuberculin testing to which all the calves were subjected between vaccination and infection might have had some influence on the resistance induced by vaccination.

In Exp. B, therefore, in addition to testing the relative merits of a recently isolated strain as a vaccinating agent, we tried to obtain information on some of the points which had emerged from Exp. A. We vaccinated calves at different ages, we staggered the dates of vaccination so that all animals could receive the same infective dose of the bovine strain, and we reduced the tuberculin testing of some of the animals to a minimum. The results of Exp. B indicate that age did not appear to be an important factor so far as vaccination was concerned, for equally good results were obtained with calves of different ages although, as might be expected, infection was somewhat more severe in the younger than in the older control animals. With regard to the possibility that resistance might be influenced by repeated tuberculin testing, the findings in the five calves in group A are interesting. When killed, three were found to be completely free from infection, and these had all been repeatedly tuberculin tested, whereas bovine bacilli were recovered from the two animals which were tested once only. This might be due to chance, but, if so, it was a 1 in 10 chance.

The results in Exp. B were better than those in Exp. A for twelve out of seventeen vaccinated animals were apparently unaffected by an infective

dose that set up severe infection in four control animals. From this it was clear that the recently isolated strain G 564 was more potent as a vaccinating agent than the more established laboratory strain LV 285, but it was equally clear that strain G 564 was too dangerous for use in the field. Not only did we lose two calves early in the experiment but also we recovered the vole bacillus from six of the vaccinated animals at the post-mortem examinations, in two instances after a survival period in the calves of over 18 months.

In Exp. C in which we tested strain LV 285 after its virulence for its native host had been restored, we obtained some further evidence regarding the part which virulence in the vaccinating strain may play in bringing about an enhanced resistance. It must be admitted that the evidence is slight, for there were only four animals, namely, those in group A, which received 5 mg., but the results in this group were better than any we had obtained previously by using the same dose of the strain in its less virulent form.

As in our other experiments, we had more than one object in view in designing Exp. C, one of which was to find the smallest dose that could set up a marked degree of resistance. Although the animals in group D vaccinated with 0·005 mg. developed considerably less disease than the controls, the fact that bovine bacilli could be recovered from every animal in groups B, C and D suggested that nothing under 5 mg. was likely to prove successful as a single vaccinating dose for use in the field.

Another point which we studied was the effect of repeated tuberculin testing on the vaccinated animals, and the manner in which Exp. C was designed enabled us to form a definite opinion on the question. We are now certain that even the small amount of P.P.D. used for intradermal tests can bring about a partial desensitization, for at the test immediately prior to the resistance test those animals which had been repeatedly tested after vaccination were much less allergic than those which were being tested for the first time since vaccination. Further, in three out of the four groups those animals which had been repeatedly tested and which were less allergic at the time of the resistance test were less severely affected by the bovine bacillus.

Exp. D, in which we attempted to follow up the ultimate fate of small lesions such as had been found in vaccinated animals six months after infection, did not run its course according to plan, but the two animals in group D killed 2 years after infection yielded important information. In one of these there was one small lesion from which the bovine strain was recovered. No macroscopic lesions could be found in the other animal, yet the bovine strain was recovered from the mesenteric glands by the biological test just as the infecting strain was recovered from eight animals in Exps. A, B and C, in which no lesions could be found 6 months after infection.

Exp. E brought out some important points relative to the nature of the resistance set up by vaccination, namely, the early reduction in the degree to which granulomatous changes take place and the early tendency to encapsulation of lesions together with an alteration in the character of calcium deposition. Another point to which reference will be made later was the exaggerated response in the post-pharyngeal glands of the 28-day calf. The freedom of the 42-day calf from macroscopic lesions was in keeping with some of the results in Exp. B, but as no bacteriological examination was made we cannot claim that the animal was entirely resistant and free from infection.

(c) Two spaced doses of vaccine intravenously, followed by exposure to infection *per os* in the laboratory

Experiment F. *Revaccination with 5–50 mg. following primary vaccination with 5 mg. intravenously*

The imperfect degree of protection attained by the technique of single vaccination led us to test the efficacy of revaccination. In the report of Griffith & Dalling (1940) it is recorded that a calf which was inoculated intravenously with 0·01 mg. followed by 2 mg. 116 days later, died 8½ days after the second injection. Death was considered to be the result of an allergic reaction which had caused consolidation of the lungs. Although other calves, in which a larger initial dose had been used, had been revaccinated without ill effects, the possibility of losing some animals through allergic reactions was not overlooked, and our experiment was designed so that animals were revaccinated after different intervals of time and with doses of different sizes. By this we hoped to determine not only the safety of the practice of revaccination but also the optimal dose and time for revaccination.

Twenty calves were vaccinated intravenously with 5 mg. of strain LV 285. Four of these animals were not reinoculated—to serve as controls on the efficacy of double vaccination as opposed to single vaccination. The remaining sixteen animals were revaccinated intravenously with varying doses and at varying times as follows. Seven were revaccinated with 5 mg. of vole bacilli after intervals of 7 days, 10 days, 14 days, 4 weeks, 8 weeks, 16 weeks and 6 months respectively. A further three animals were reinoculated with doses of 10, 25 and 50 mg. respectively at 7 days, and similar doses were used for three more animals at 4 weeks and again at 6 months.

Resistance tests consisting as usual of the oral administration of 7·5 mg. of virulent bovine bacilli were made 6 months after the second vaccination in

Table 1. *Experiment F. The immunizing effect of single and double vaccination with LV 285*

Group	No.	Revaccinating dose (mg.)	Interval between vaccinations	Period from last vaccination till R.T.	Lesions	Culture
Single vaccination	RC 203	—	—	4 months	−	+
	RC 235	—	—	4 months	+	+
Controls	AP 8	—	—	—	+ + + +	+
	AP 9	—	—	—	+ + + +	+
Single vaccination	RC 249	—	—	1 year	−	−
	RC 253	—	—	1 year	+	+
Control (Exp. D)	RC 291	—	—	—	+ + +	+
A	55127	5	7 days	6 months	+ +	+
	RC 244	10	7 days	6 months	+ + +	+
	RC 195	25	7 days	6 months	+ +	+
	RC 247	50	7 days	6 months	+ +	+
B	RC 241	5	10 days	6 months	+ + +	+
C	RC 237	5	14 days	6 months	+ +	+
Control for A, B and C	AP 17	—	—	—	+ + + +	+
D	RC 246	5	4 weeks	6 months	−	−
	RC 188	10	4 weeks	6 months	−	−
	RC 250	25	4 weeks	6 months	−	−
	RC 202	50	4 weeks	6 months	−	−
Control D	RC 180	—	—	—	+ +	+
E	RC 251	5	8 weeks	6 months	−	−
Control E	RC 200	—	—	—	+ + +	+
F	RC 255	5	16 weeks	6 months	+	+
Control F	RC 216	—	—	—	+ + + +	+
G	AP 10	5	6 months	6 months	−	−
	AP 11	10	6 months	6 months	−	−
	AP 12	25	6 months	6 months	−	−
	AP 14	50	6 months	6 months	−	−
Controls G (Exp. A)	RC 156	—	—	—	+ + + +	+
	RC 158	—	—	—	+ + + +	+

the case of the reinoculated animals. The single vaccination controls were tested in pairs after periods of 4 months and 1 year. For unvaccinated controls we had six calves earmarked for the purpose from the beginning of the experiment, and in addition we were able to make use of some of the control animals for other experiments in which resistance tests were being carried out contemporaneously. All animals were slaughtered for examination approximately 6 months after the resistance test.

The design of the experiment is shown in Table 1.

Two facts must be mentioned which might have some bearing on the results, the first being that it was only after the experiment had started that we realized that we had omitted to make provision for single vaccination controls. The four calves originally earmarked for revaccination at 6 months appeared to be the most suitable and they were accordingly diverted for this purpose. Four more calves were introduced into the experiment as replacements for group G, and in consequence these were vaccinated at a later date than the other animals with a later batch of vaccine. The second fact is that at one stage of the experiment, for reasons which need not be discussed here, we experienced difficulty for several months in obtaining satisfactory egg media, and an adequate amount of growth could be won only with difficulty even by using numerous tubes. This was especially the case when the growth was being gathered for the resistance test on group D, and it may be that the test dose was unsatisfactory for, as will be seen from Table 1, infection in the control animal was less pronounced than was desirable. The apparently excellent results of revaccination may be discounted to a certain extent so far as this one group is concerned.

Summary of port-mortem examinations

Single vaccination (4 months)

Controls

Calf AP 8 (183 days). Very numerous lesions in post-pharyngeal, coeliac and mesenteric glands. Lesions rather more circumscribed than usual in a control animal with little macroscopic evidence of invasive granulomatous tissue. Only slight caseation and calcification more chalky than spicular.

Calf AP 9 (188 days). Scattered lesions in submaxillary and post-pharyngeal glands. Numerous lesions in thoracic and very numerous lesions in mesenteric glands. Lesions of invasive type especially in mesenteric glands.

Vaccinated

Calf RC 203 (184 days). No macroscopic lesions, but strain of bovine type recovered from mesenteric glands by biological test.

Calf RC 235 (187 days). About seven or eight tiny lesions in mesenteric and colonic glands consisting of encapsulated sandy-like granules. Strain of bovine type recovered by biological test from mesenteric glands.

SINGLE VACCINATION (1 year)

Control

Calf RC 291 (181 days). (Also for Exp. D.) Numerous lesions in parotid, post-pharyngeal, thoracic and mesenteric glands of infiltrative type.

Vaccinated

Calf RC 249 (182 days). No evidence of infection.

Calf RC 253 (184 days). Single lesion of actively infiltrative type in mesenteric gland. Strains of bovine type recovered by biological tests from head glands, thoracic glands and mesenteric glands.

GROUP A (revaccinated after 7 days)

Control

Calf AP 17 (185 days). Very numerous lesions of infiltrative type in post-pharyngeal, thoracic, coeliac and mesenteric glands.

Vaccinated

Calf 55127 (189 days). Revaccinated with 5 mg. One tiny lesion in submaxillary and several pin-head lesions in post-pharyngeal glands. Moderate number of small lesions in mesenteric glands. Lesions all of encapsulated type with little evidence of calcification.

Calf RC 244 (188 days). Revaccinated with 10 mg. Rather heavily infected with encapsulated lesions in submaxillary and post-pharyngeal glands tending to break down with abscess formation. Numerous small lesions in mesenteric glands, in two of which infection was of infiltrative granulomatous nature.

Calf RC 195 (185 days). Revaccinated with 25 mg. Moderate number of small encapsulated lesions in mesenteric glands.

Calf RC 247 (181 days). Revaccinated with 50 mg. Two lesions, one large, in post-pharyngeal glands. A few pin-head lesions in mesenteric glands. Lesions of encapsulated soft caseous type.

GROUP B (revaccinated after 10 days)

Control as for Group A

Calf RC 241 (189 days). Revaccinated with 5 mg. Heavily infected with grapes on the pleurae. Marked visceral and parietal peritonitis. Severe granulomatous infiltration of mediastinal gland and numerous tiny lesions in mesenteric glands evidenced by tiny calcium granules.

GROUP C (revaccinated after 14 days)

Control as for Group A

Calf RC 237 (188 days). Revaccinated with 5 mg. The amount of infection with bovine bacilli was difficult to assess in this animal since Johne's disease was present as well, and much of the granulomatous tissue seen in the mesenteric glands was probably due to this cause. One small millet seed lesion was present in a submaxillary gland and one lesion in a mesenteric gland, which had the appearance of a bovine lesion, yielded a culture of bovine type.

GROUP D (revaccinated after 4 weeks)

Control

Calf RC 180 (190 days). A few caseo-purulent abscesses with thick fibrous walls in submaxillary and post-pharyngeal glands, and only one definitely calcareous granule in one mesenteric gland.

Vaccinated

Calf RC 246 (191 days). Revaccinated with 5 mg. No evidence of infection.

Calf RC 188 (192 days). Revaccinated with 10 mg. No evidence of infection.

Calf RC 250 (189 days). Revaccinated with 25 mg. No evidence of infection.

Calf RC 202 (188 days). Revaccinated with 50 mg. No evidence of infection.

GROUP E (revaccinated after 8 weeks)

Control

Calf RC 200 (181 days). Numerous lesions in submaxillary, parotid, post-pharyngeal and mesenteric glands. Lesions chiefly small calcareous spicules with little macroscopic evidence of surrounding reaction. In some lesions calcium present as soft chalky material and caseation commencing in other lesions.

Vaccinated

Calf RC 251 (180 days). Revaccinated with 5 mg. No evidence of infection.

GROUP F (revaccinated after 16 weeks)

Control

Calf RC 216 (183 days). Multiple lesions of invasive type in submaxillary, post-pharyngeal and mesenteric glands.

Vaccinated

Calf RC 255 (184 days). Revaccinated with 5 mg. One tiny lesion seen in one mesenteric gland. Lesion itself lost in attempt to remove for culture, but strain of bovine type recovered by biological test from alimentary glands.

GROUP G (revaccinated after 6 months)

Control

Calf RC 156 (176 days). (Also for Exp. A, group G.) Very numerous lesions in post-pharyngeal, bronchial, mediastinal and mesenteric glands. Some lesions of caseo-calcareous type, others of infiltrative granulomatous type with calcareous granules and spicules.

Calf RC 158 (176 days). (Also for Exp. A, group G.) Numerous lesions in submaxillary, parotid, post-pharyngeal and mesenteric glands. Lesions varied in size from pin-head to hazel nut and were mainly soft caseous in character with a tendency to encapsulation.

Vaccinated

Calf AP 10 (178 days). Revaccinated with 5 mg. No evidence of infection.
Calf AP 11 (177 days). Revaccinated with 10 mg. No evidence of infection.
Calf AP 12 (183 days). Revaccinated with 25 mg. No evidence of infection.
Calf AP 14 (185 days). Revaccinated with 50 mg. No evidence of infection.

Comments on Experiment F

This experiment demonstrated that revaccination even with relatively large doses could be carried out with safety, for no untoward sequellae followed the second inoculation and, in fact, the animals were less inconvenienced by the second inoculation than by the first.

We failed, however, to determine if there was any real advantage in increasing the dose for the second injection, for, with the exception of calf 255 in group E, every animal revaccinated after 4 or more weeks from the date of the primary vaccination proved resistant to the test dose of bovine bacilli. For the same reason we also failed to determine the best time for revaccination, but we did establish one very important point. The results in groups A, B and C, in which animals were revaccinated after 7, 10 and 14 days, were worse than those found in any of the single vaccination animals. Here we would refer to one calf mentioned by Buxton & Griffith (1931) in their first report on vaccination of calves with B.C.G. This calf appears to have been the only animal revaccinated inside 3 weeks, and when subjected to a resistance test it was even less resistant than the unvaccinated control animals. In Buxton & Griffith's case the resistance test was applied within 2 months of the last vaccination. In our experiment there was an interval of 6 months between the last vaccination and the resistance test, a period sufficiently long to dispose of any suggestion of 'negative phase'.

Our results suggest that revaccination enhances still further the resistance set up by primary vaccination provided that revaccination is delayed for at least 4 weeks, the period during which, as we shall show in a later section, the cellular activity which follows vaccination steadily progresses to a maximum and then begins to wane. It is also during this period that sensitivity to tuberculin is rapidly developing.

(*d*) Single dose of vaccine *per os* followed by exposure to infection *per os* in the laboratory

Experiment G. *Vaccination with* 5, 50 and 100 *mg. of strain LV* 285

This was a short pilot test carried out to determine if skin sensitivity could be set up in calves fed with the vole bacillus, but as the animals were subjected later to a resistance test, the experiment may conveniently be considered here.

At the start one calf, WR, was fed with 100 mg. of strain LV 285, and when tuberculin tested with avian and human P.P.D. 5 weeks later, it showed a sharp reaction to mammalian P.P.D. inoculated intradermally. Sensitivity waned, however, remarkably quickly.

Two more calves, RC 155 and RC 166, were then fed with 50 and 10 mg. respectively. Calf RC 166, fed with the smaller dose, failed to show any reaction until tested 100 days after dosing, when a slight positive response was obtained to the mammalian P.P.D. Calf RC 155 showed a moderate response to the mammalian P.P.D. when tested 6 weeks after the test oral dose, but sensitivity had waned completely by the end of 6 months.

The three dosed calves were housed separately but each had an uninoculated calf as a companion. The companion to calf RC 155 died shortly after the experiment had started, but calf WRA, in contact with calf WR, and calf RC 167, in contact with calf RC 166, did not react at any of the frequent tuberculin tests.

All animals were finally exposed to a resistance test administered orally in the usual manner and then slaughtered approximately 6 months later. Calves WR and RC 166 with their companions were tested along with group B in Exp. A. Calf RC 155 was left till a later date to permit of further observations on the fluctuations in skin sensitivity and was finally tested along with group D in Exp. A. Summaries of the post-mortem examinations are given below.

Summary of post-mortem examinations

Controls

Calf WRA (169 days). Numerous large lesions of infiltrative type in coeliac, mesenteric and colonic glands.
Calf RC 167 (177 days). Multiple lesions of infiltrative type in one coeliac and several mesenteric glands.

Vaccinated

Calf WR (181 days). Fed 100 g. Resistance test 6 months later. Five small pin-head lesions in one coeliac gland.
Calf RC 166 (185 days). Fed 10 mg. Resistance test 4 months later. Numerous lesions in mesenteric gland, but most of these small and of less infiltrative character than lesions in control animals.
Calf RC 155 (168 days). Fed 50 mg. Resistance test 10½ months later. No macroscopic lesions found, but strain of bovine type recovered by biological test from pooled alimentary glands.

Comments on Experiment G

The degree of protection conferred by oral vaccination was surprisingly good in view of the evanescent nature of the skin sensitivity, but it did not appear

to be superior to the resistance induced by intravenous inoculation.

(e) SINGLE DOSE OF VACCINE FOLLOWED BY EXPOSURE TO INFECTION UNDER FIELD CONDITIONS

(1) EXPERIMENT H. *Vaccination with 5 mg. of strain LV 285 intravenously*

During the summer of 1942 one of us (J.S.P.) was concluding the first part of an experiment on the vaccination of cattle against brucellosis and the freshly calved heifers were available for other work. This appeared to be a valuable opportunity of commencing observations on the efficacy of vole vaccination against the risk of infection with tuberculosis in the field, and arrangements were made to loan these heifers for a period of up to 5 years to farmers whose dairy herds were heavily infected with tuberculosis. Half the animals sent to each farm were to be vaccinated and the other half left unvaccinated, and if for any reason the farmer wished to dispose of an animal from one group, he must agree to surrender another for comparison from the other group within a reasonable time.

Thirteen heifers were available, six of which were vaccinated and seven left as controls. The vaccinating dose was 5 mg. intravenously of strain LV 285. Nine animals, four vaccinated and five controls, were sent to farm A and four animals, two vaccinated and two controls, were sent to farm B. We would have preferred to have kept the animals free from exposure to infection for a few weeks after vaccination, but circumstances compelled otherwise. The animals were lactating, and as insufficient staff was available for milking we had no alternative but to send the animals to the farms immediately. For this reason the experiment may have been weighted against the vaccine.

Some 6 months later the owner of farm B decided to clear out his stock and establish a tubercle-free herd. The four animals were returned to the Institute, and whilst awaiting transfer to a third farm, farm C, one vaccinated animal aborted. This, together with a control animal, was slaughtered for examination. The other two were at a later date transferred to farm C, but meanwhile were exposed to constant risk of infection through contact with large numbers of infected animals passing through the Institute in connexion with tuberculin 'test and slaughter' experiments.

In 1943 a further ten heifers were available on release from the brucella experiment, and these were sent to farm A, five being vaccinated and five left as controls.

At irregular intervals animals were returned for one reason or another, the choice of the second animal in each pair being left to the farmer. In practice this plan worked quite well, for it suited our purpose as well as the farmer's own interests that the animals in best condition should be left till the last. At the expiry of the 5 years period four animals still remained on farm A, and these were called in so that we could complete our observations. Whilst on the farm the cattle were tuberculin-tested at 6-monthly intervals, and all animals were slaughtered and examined as they were returned from the farm.

At the start of the experiment we had intended to limit the post-mortem examinations to a macroscopic examination of organs and glands, unless any animal presented some exceptional feature which made it desirable that a full biological test should be made. Thus a full examination was not made in the case of heifers nos. 10 and 11, killed early in the series, or in the case of no. 17, a non-reactor to the tuberculin test in which no lesions could be found. We had planned to carry out a full test on heifer no. 16, a vaccinated animal which had given a positive reaction to the tuberculin test, but unfortunately through a mistake at the slaughter house this animal was killed under the impression that it was no. 17, and as no lesions were found the mistake was not discovered until the following morning, when the second of the pair was due for examination. In the case of all other animals a full examination was made.

The results of the post-mortem examinations are given briefly below, their order being arranged according to the length of exposure of the animals to field infection. In Fig. 4 the experiment is set out schematically to show the length of exposure of each animal, the increases in skin thickness at the 72nd hour of the 6-monthly comparative tuberculin tests, together with the results of the post-mortem examinations. The following forms the basis on which we determined the number of plus signs to be allotted to each animal.

+	Slight macroscopic tuberculosis in one group of glands.
+ +	Slight macroscopic tuberculosis in more than one group of glands or severe tuberculosis in one group of glands.
+ + +	Severe tuberculosis in more than one group of glands or tuberculosis in one organ and its associated lymph glands.
+ + + +	Tuberculosis in one organ and marked lesions in more than one group of glands.
+ + + + +	Generalized tuberculosis.

Summary of post-mortem examinations

No. 23. Control (8 months' exposure on farm)

Scattered pin-head calcareous lesions in mesenteric glands.

No. 20. Vaccinated (11 months)

No evidence of tuberculous infection.

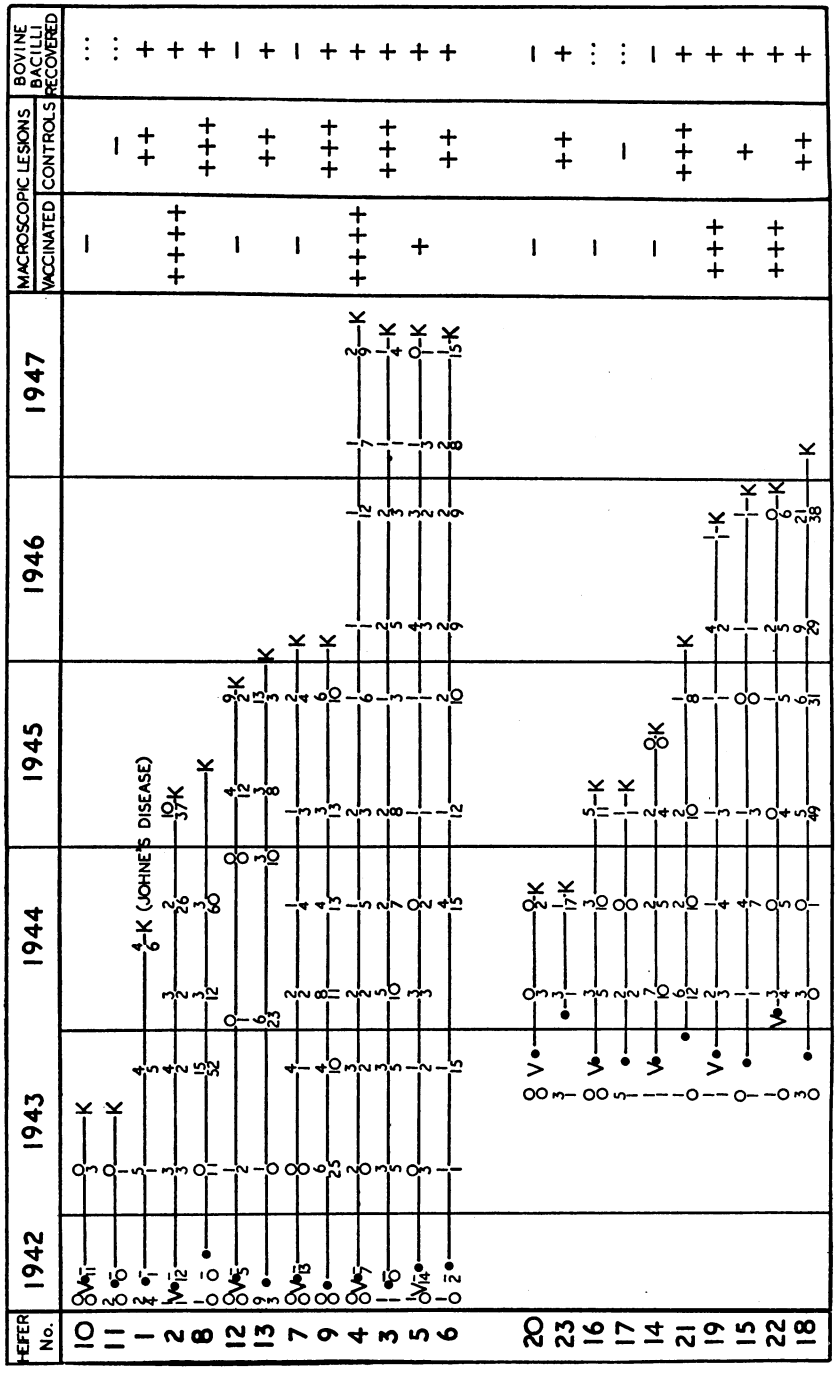

Fig. 4. Experiment H. Exposure of freshly calved heifers to field infection. V, vaccinated at laboratory; ●, moved to infected farm; K, killed. Tuberculin tests: upper figure, avian; lower figure, mammalian; increases in mm. at 72 hr. Biological tests: positive +, negative −, not done ....

No. 10. *Vaccinated* (11 *months*)

No macroscopic evidence of tuberculous infection.

No. 11. *Control* (11 *months*)

No macroscopic evidence of tuberculous infection.

No. 17. *Control* (1 *year and* 6 *months*)

No macroscopic evidence of tuberculous infection.

No. 16. *Vaccinated* (1 *year and* 7 *months*)

No macroscopic evidence of tuberculous infection.

No. 14. *Vaccinated* (1 *year and* 9 *months*)

No evidence of tuberculous infection.

No. 1. *Control* (1 *year and* 11 *months*)

Scattered lesions in bronchial and mediastinal glands, some of infiltrative type. Animal also suffering from Johne's disease.

No. 21. *Control* (2 *years and* 2 *months*)

Scattered fibrous walled and caseous centred lesions in lung, thoracic and post-pharyngeal glands.

No. 2. *Vaccinated* (2 *years and* 8 *months*)

Numerous caseous lesions in post-pharyngeal and mesenteric glands. Multiple small caseous abscesses in diaphragmatic lobe of right lung and severe infection of thoracic glands. One hepatic gland infected.

No. 8. *Control* (2 *years and* 8 *months*)

Numerous caseous abscesses in apical lobe of right lung and numerous caseous lesions in thoracic glands. Several small caseous lesions in one mesenteric gland.

No. 22. *Vaccinated* (2 *years and* 10 *months*)

Numerous caseo-calcareous lesions in diaphragmatic lobe of right lung, and in thoracic glands. Lesions present in post-pharyngeal and mesenteric glands.

No. 19. *Vaccinated* (2 *years and* 11 *months*)

Severe infection of post-pharyngeal glands with large abscess formation. Scattered caseous abscesses in thoracic and mesenteric glands.

No. 15. *Control* (3 *years and* 2 *months*)

A few hempseed lesions of chronic fibrocalcareous type in mediastinal glands.

No. 12. *Vaccinated* (3 *years and* 3 *months*)

No evidence of tuberculous infection.

No. 13. *Control* (3 *years and* 4 *months*)

Numerous fibrocalcareous lesions in thoracic glands.

No. 18. *Control* (3 *years and* 4 *months*)

Lesions confined to post-pharyngeal glands, both of which were much enlarged and contained multiple caseo-calcareous lesions.

No. 9. *Control* (3 *years and* 6 *months*)

Numerous pea-sized fibrocaseous lesions in both lungs and numerous hempseed lesions in thoracic glands.

No. 7. *Vaccinated* (3 *years and* 6 *months*)

No evidence of tuberculous infection.

No. 6. *Control* (5 *years*)

Scattered pin-head lesions in mediastinal glands. A number of pin-head lesions in one coeliac gland and a few lesions in a long mesenteric gland, one of which was walnut-sized and of granulomatous nature.

No. 5. *Vaccinated* (5 *years*)

One post-pharyngeal gland slightly enlarged and containing a caseous abscess about the size of a hazel-nut kernel. Smaller lesion at one pole.

No. 3. *Control* (5 *years and* 1 *month*)

Two small fibrocaseous lesions in right diaphragmatic lobe near root. Scattered small fibrocaseous lesions in mediastinal glands. Several lesions in coeliac and mesenteric glands, one of which appeared very old with grey coral-like calcium.

No. 4. *Vaccinated* (5 *years and* 2 *months*)

Several groups of lesions in diaphragmatic lobe of left lung, the lesions consisting of small fibrous walled abscesses containing bright yellow caseous material with a few calcium granules. A few fibrocaseous nodules in long posterior mediastinal gland. Fibrocaseous nodule in one post-pharyngeal gland and multiple lesions throughout mesenteric chain, some consisting of granulomatous areas from 1 to 1½ in. in length.

(2) EXPERIMENT J. *Vaccination with* 100 mg. *of strain LV* 285 *per os*

In this experiment we exposed six calves, which had been vaccinated 6 months previously with 100 mg. of strain LV 285 by the mouth, to the risk of natural infection on a farm where the incidence of tuberculosis of both avian and bovine types was high. With these vaccinated calves we placed five unvaccinated tuberculin-negative calves as controls. During the 6 months between vaccination and exposure to infection these calves had been used in a study of the type of tuberculin sensitivity induced by oral vaccination. This is discussed later in §VII, but here it may be stated that the vaccinated calves developed a sensitivity to mammalian P.P.D., but when tested just before being sent to the farm they were non-allergic.

Whilst on the farm the animals were subjected to the single intradermal comparative test at 7, 10 and 14 months. One of the vaccinated animals accidentally killed itself after it had been on the farm for 9 months. This animal had been a non-reactor at the 7-month test, and no lesions of a tuberculous nature were seen at the post-mortem

examination. The experiment was concluded when the calves had been just under 15 months on the farm, and they were slaughtered at a local slaughter-house under our supervision. Pressure of other work prevented the carrying out of a full bacteriological examination with biological tests, but macroscopic examination was checked by means of stained films and by histological examination in the case of animals in which lesions were found.

The results of the tuberculin tests while the animals were on the farm are important, and the 72nd hour readings are set out in Table 2.

Table 2. *Tuberculin reactions of orally vaccinated calves after exposure to natural infection (single intradermal comparative test) together with results of post-mortem examinations*

	Period of exposure on farm			Macroscopic lesions at post-mortem
	7 months	10 months	14 months	
	Controls			
RC 165	4/15	5/11	3/14	Present
RC 189	3/23	4/27	3/27	Present
RC 192	0/0	5/0	0/6	Absent
RC 193	0/0	6/1	7/18	Absent
RC 194	3/21	4/18	3/12	Present
	Vaccinated			
RC 161	1/0	2/0	8/18	Present
RC 161	0/0	0/0	1/8	Present
RC 164	1/0	9/0	7/3	Present
RC 169	0/0	0/0	0/5	Present
RC 170	0/0	0/0	0/7	Present

The left-hand figure represents the increase in mm. to the avian P.P.D. and the right-hand figure the increase to the mammalian P.P.D. at the 72nd hour.

The results of the post-mortem examinations are given in summarized form below.

Summary of post-mortem examinations

Controls

Calf RC 165. Mammalian reactor at 7 months. Three large lesions in mediastinal gland of partly encapsulated caseo-calcareous type but still some infiltration.

Calf RC 189. Mammalian reactor at 7 months. Numerous lesions of infiltrative type in thoracic glands.

Calf RC 192. Avian reactor at 10 months; mammalian at 14 months. No macroscopic evidence of tuberculosis.

Calf RC 193. Avian reactor at 10 months; mammalian at 14 months. No macroscopic evidence of tuberculosis.

Calf RC 194. Mammalian reactor at 7 months. Numerous lesions of infiltrative type in thoracic glands.

Vaccinated

Calf RC 161. Slight avian reactor at 10 months; strong mammalian at 14 months. One post-pharyngeal gland much enlarged containing large granulomatous lesion with burrowing tracks of stringy creamy pus.

Calf RC 162. Mammalian reactor at 14 months. Group of small encapsulated lesions in left bronchial gland.

Calf RC 164. Avian reactor at 10 months; avian and slight mammalian at 14 months. Group of small lesions of unusual type in mediastinal gland. Lesions semi-translucent fibrous nodules containing yellow streaks. Strain of bovine type isolated by direct culture.

Calf RC 169. Mammalian reactor at 14 months. Small consolidated area in right lung and two small encapsulated lesions in bronchial gland.

Calf RC 170. Mammalian reactor at 14 months. Small group of tiny encapsulated lesions in mediastinal gland.

Comments on Experiments H and J

These experiments were very severe tests for any vaccine. As already explained there was no interval of any practical significance in Exp. H between vaccination and exposure to the risk of infection, and the dairy herds to which the animals were admitted were heavily infected with bovine tuberculosis. Farm A which received the majority of the animals had a bad record under the Tuberculosis Order, no less than five animals being seized in 1942 with a further three being taken during the course of the experiment. In the second batch of animals sent to this farm the immediate risk was particularly great, for the animals entered the dairy herd when this was housed for the winter.

Reference to Figure 4 will show that the vaccinated animals possessed a marked resistance to infection as compared with the unvaccinated control animals. No infection was found in six out of eleven vaccinated animals, whereas ten out of twelve controls were infected. Two vaccinated animals were free from infection when examined 3 years and 3 months and 3 years and 6 months respectively after first exposure to field infection, whereas no control was free from infection after 18 months on the farm.

Nevertheless, one cannot but feel a certain uneasiness regarding those vaccinated animals which did become infected, for in four out of the five the lesions were severe, even more severe on average than the lesions in the control animals. Further experience of work under field conditions might suggest that this was merely due to chance, but the possibility cannot be overlooked that intravenous vaccination with a relatively avirulent strain may not result in a balanced state of resistance in which there has been an equal stimulation of the various factors which combine to form the defence mechanism. There may even be an initial overstimulation of certain tissues or cells with a subsequent loss of functional efficiency which would become apparent only in long-term experiments embracing large numbers of animals.

The results of Exp. J did not suggest that oral vaccination was likely to prove of much practical value. All the vaccinated animals developed a

sensitivity to mammalian P.P.D.'s following dosing with 100 mg. by mouth, but the sensitivity was not maintained as long as that induced by 5 mg. intravenously, possibly because the number of bacilli that found their way into the tissues was small. The same reason may account for the resistance set up being unsatisfactory. From Table 2 it will be seen that, judged by the tuberculin reactions, three of the five control animals were infected within 10 months of exposure to risk, at which time all five vaccinated animals were apparently free from infection. By 14 months, however, all the vaccinated animals had become mammalian reactors and all were found to be infected, although the majority of the lesions appeared to be of less active type than those found in the control animals. In one instance, however, there was a massive enlargement of one post-pharyngeal gland with a central burrowing caseous abscess.

(*f*) MULTIPLE-PUNCTURE SKIN VACCINATION FOLLOWED BY EXPOSURE TO INFECTION *PER OS* IN THE LABORATORY

EXPERIMENT K. *To test the efficacy of multiple-puncture skin vaccination when applied to cattle*

In view of the objections to intradermal or subcutaneous vaccination, it was suggested that we might try the multiple-puncture method of skin vaccination described by Rosenthal (1939) and practised by Birkhaug, and Dr A. Q. Wells very kindly offered us the use of an instrument designed for the purpose similar to that used by Birkhaug. As this was intended primarily for use on the human skin, there was some doubt as to whether or not it would be suitable for cattle, but a trial test on a calf with Indian ink and a culture of vole bacilli showed that penetration was adequate. Sections prepared from the inoculated areas of skin revealed that both the carbon particles and the vole bacilli were carried into the vascular layers of the skin, and there was evidence of some dispersal of the bacilli from the point of entry even at 2 hr. from the time of inoculation.

Seven young calves were assembled and five of these were vaccinated. The trial test had shown that the most suitable area was over the rump, and an area of skin was shaved sufficiently large to allow the plate of the instrument to be applied closely to the skin. The strength of the vaccine was 10 mg./c.c. The remaining two calves were left as unvaccinated controls for the resistance test.

The inoculation sites were examined at weekly intervals for the first month and thereafter at less frequent intervals up to 3 months, the sites being shaved where necessary to facilitate observation. Nothing definite was noted until the examination carried out 6 weeks after inoculation, when for the first time it was possible to make out something of the pattern made by the needles, which was apparent as a series of small pale reddish brown macules. In no case was the pattern more than two-thirds complete. Subsequent examinations again did not show anything very definite.

The vaccinated calves were tuberculin tested at intervals and the results are shown in Table 3.

Table 3. *Tuberculin reactions of calves vaccinated with vole bacilli by means of multiple skin puncture*

Calf no.	Weeks post-vaccination 3	4	13	25	Prior to slaughter
68	1/7	—	6/13	3/5	4/8
69	—	1/1	1/0	0/1	1/9
72	0/2	—	0/6	0/2	0/30
73	—	7/7	2/4	2/3	2/10
74	—	4/4	2/5	1/0	9/22

Left-hand figure: increase in mm. to avian P.P.D. Right-hand figure: increase in mm. to human P.P.D. Dose: 0·1 mg. Readings at 72nd hour.

Six months after vaccination the five vaccinated calves, together with the two control calves, were subjected to a resistance test consisting of 7·5 mg. of virulent bovine bacilli administered by the mouth. Six months later the animals were slaughtered and a full post-mortem examination was carried out on each animal. The bovine strain was recovered in culture from one or more sites in each case, and the post-mortem findings are summarized below.

Controls

Calf AP 71 (181 days). Severely infected with multiple lesions in post-pharyngeal, mediastinal, coeliac, mesenteric and colonic glands and one lesion in one hepatic gland. Lesions pin-head to filbert in size and caseo-calcareous in type with a tendency towards encapsulation.

Calf AP 76 (185 days). Small aggregate of spicular pin-head lesions in one post-pharyngeal gland. Main brunt of infection borne by mesenteric and colonic glands, practically every one of which contained lesions and some being riddled throughout their substance. Lesions showed little evidence of surrounding granulation tissue and consisted mainly of calcium spicules lying singly and in aggregates inside thin sheaths of translucent fibrous tissue.

Vaccinated

Calf AP 68 (188 days). Infection confined to mesenteric and colonic glands, a considerable number of glands being free from infection, but lesions fairly numerous in some of the larger glands. Lesions pin-head to large pea in size and of unusual type. The smaller lesions contained hard calcium granules, but the larger lesions consisted of yellow shaggy fibrous nodules containing a soft somewhat glutinous paste. Another unusual feature was the frequency with which lesions could be found projecting through the capsule in a partly pedunculated manner.

Calf AP 69 (189 days). Lesions much less numerous than in either of the control animals but still much more numerous than usually found in a vaccinated animal. One pin-head lesion in one post-pharyngeal gland. Majority of mesenteric and colonic glands free from macroscopic infection but moderately numerous lesions in such glands as were affected, pin-head to hempseed in size and all of one type. Small hard calcium crystals or spicules lying singly or in groups with a translucent coating of fibrous tissue and little or no reaction in the surrounding tissues. In a few instances lesions could be detected by reason of a slight thickening and puckering of the overlying capsule, but the majority were found only when the slicing knife struck calcium.

Calf AP 72 (184 days). Multiple lesions in submaxillary, post-pharyngeal, mediastinal, hepatic coeliac, mesenteric and colonic glands, varying in size from pin-head to hazel nut. Calcium deposition mainly of hard spicular type, but in some lesions there was also a considerable degree of soft caseation producing the 'over-frozen ice cream' type of lesion seen frequently in unvaccinated animals. The majority of the lesions showed signs of encapsulating fibrosis.

Calf AP 73 (183 days). Three pin-point lesions in one post-pharyngeal gland and only about half a dozen mesenteric glands infected, although lesions numerous in these. Mostly irregular aggregates of glistening calcium spicules lying just under the capsule and arranged in a plane parallel to the capsule. Lesions up to $\frac{1}{4}$ in. in diameter and detectable by thickening and puckering of capsule, but apart from capsular change no apparent surrounding tissue changes.

Calf AP 74 (182 days) Several lesions in one post-pharyngeal gland and in one coeliac gland. All the larger mesenteric and several colonic glands contained multiple lesions consisting of small very hard yellow calcium crystals embedded in thin translucent fibrous coating. Larger lesions made up of groups of crystals.

Comments on Experiment K

The tuberculin reactions exhibited by the vaccinated animals were extremely irregular and in the main did not attain the magnitude that has been noted in animals inoculated intravenously even with small doses. The test applied immediately before slaughter presented little difficulty in interpretation, since the mammalian reactions were large and diffuse, indicating the presence of bovine infection.

In calf AP 72 the infection differed little in character or extent from that seen in the controls. In the other vaccinated animals, vaccination, although affording a very imperfect degree of protection, had modified the course of infection in an interesting manner. Normally in vaccinated animals we have found not only a reduction in the number of lesions but also a very marked alteration in character. In this experiment, however, in spite of an obvious reduction in the number of lesions in four of the vaccinated animals, although there was an absence of granulomatous infiltration, the calcium deposits resembled those commonly found in non-vaccinated animals and there was little evidence of encapsulation.

The method of vaccinating cattle with the vole strain by multiple puncture of the skin does not appear to be capable of setting up an adequate degree of resistance in the vaccinated animal.

(*g*) Notes on the history and the virulence of the vole strains used in these experiments

As noted earlier in this report, the bulk of our work has been done with strain LV 285, one of the strains used by Griffith & Dalling in their investigations. This strain, when we first used it in 1940, had already been maintained on artificial media for over 3 years. It grew readily on unglycerinated egg and the growth was easily emulsifiable, but bacterial suspensions had a tendency to precipitate somewhat rapidly and also to form a slight scum on the surface of the fluid which tended to cling to the sides of the containers.

This strain has now been in culture for over 11 years. It is still readily emulsifiable and the tendency to sediment is not noticeably greater than it was 8 years ago, but the tendency towards scum formation has steadily increased, and this is undesirable in a strain intended for vaccine purposes.

In 1942 the virulence of strain LV 285 was tested on field voles and Orkney voles, and the limited amount of infection set up in these animals made it evident that there had been a loss of virulence since the strain was first tested by Griffith (1939). Macroscopically there was little evidence of generalized spread from the area of the local lesion and regional glands, but the bacillus could still be recovered in culture from the internal organs, and it was decided to carry on the strain by animal to animal passage in the Orkney vole, since this animal appeared to be no less susceptible than the field vole, and is a much more convenient animal for use in the laboratory. Virulence was rapidly regained, but as the inocula, usually consisting of emulsions of spleen, were not standardized no comparisons could be drawn between the survival periods of the animals. In fact, a number of the animals in the series were killed before showing signs of advanced infection.

After 3 years in passage without intervening culture, the strain was re-isolated and tested for virulence with measured doses, in parallel with the stock laboratory strain, on both field voles and Orkney voles. The virulence of the laboratory strain was low, and only a very localized infection was set up in either field or Orkney voles, but the passage strain produced a severe generalized infection with all doses in both species. The maintenance of the strain has been continued in passage, and we are still uncertain that the maximum possible virulence has been reached, for while there has been no appreciable alteration in the survival times, we have noticed a tendency for the strain to attack bone in the last few animals in the series. Abscesses have been seen

in the region of the knees, ribs and the vertebrae, and some of the animals have had to be killed owing to the onset of paralysis in the hind legs following the onset of caries of the spinal column.

The cultures recovered from Orkney voles are very smooth and they grow rapidly in comparison with strains freshly isolated from naturally infected voles. Emulsifiability is easy and suspensions show no signs of scum formation, although they do show a tendency to precipitate on standing. The precipitate, however, is easily resuspended, and if further experience shows that the passage strain is no more dangerous than the old laboratory strain as a living vaccine for cattle, it should prove the better strain for the work.

The one other strain with which we have worked is strain G 564 used in Exp. B shortly after its primary isolation. Culturally this strain differs in some respects from strain LV 285, and Wells (1946), from whom we received it, has noted how the 'G' strains differ from other vole strains. Strain G 564 adapted itself rapidly to growth on artificial media and yielded, after one or two subcultures, a profuse growth that was very smooth and easily emulsifiable. Its virulence, however, has been shown to be too great for use as a living vaccine, and although the results obtained in Exp. B were distinctly good so far as resistance against fresh infection was concerned, we have hesitated to make use of the strain again.

In our notes on Exp. B we have reported the loss of two calves shortly after vaccination. In the first calf which died 4 weeks after inoculation, there were numerous small millet-seed semitranslucent nodules in the lungs chiefly near the surface close to the pleura but also in smaller numbers in the depths of the lungs. A larger, firm, caseous-like lesion, measuring about 1 in. in its longest diameter, was found close to a bronchus. The thoracic glands were enlarged, firm and granular in texture. Apart from a few tiny white specks in or immediately under the capsule of the liver, no other macroscopic lesions were found, but the vole bacillus was recovered from lung, liver, spleen, kidneys and thoracic glands. In the second calf which was killed in a moribund condition 8 weeks after inoculation, there were multiple lobular areas of hepatization in the lungs and a few pin-point nodules in the peribronchial tissue. There was a general adenitis of all the lymph glands of the thorax and abdomen with many of the smaller glands showing up which normally escape notice. Numerous tiny greyish spots were seen in the cortex of the kidneys below the capsule, and the pelves and calyces were lined with a firm muco-purulent substance which gave them a glazed appearance. The vole strain was recovered from lungs, spleen and thoracic and mesenteric glands.

Histological examination of tissues from both of these calves revealed widespread changes of somewhat indefinite tuberculous nature in various organs and lymphatic glands. In a number of instances, however, one would be justified in designating the lesions as tubercles.

Although Exp. B was completed without any further mishap, we recovered the vole strain from six of the calves at post-mortem examination, in two instances after survival periods of 565 and 544 days, and a subsequent study of the virulence of the strain for the smaller laboratory animals confirmed our opinion that it was too dangerous for use as a vaccine, first on account of the progressive lesions set up in some of the animals, and secondly because of its powers for survival in the tissues even in the apparent absence of progressive infection.

The fact that vaccination with strains of vole bacilli had already been commenced in human beings led us to issue a warning in a paper published in the *Lancet* (1947), in which the more important of our results in small animals were set out in detail. The maximum survival period of strain G 564 has not yet been ascertained, for since the paper referred to above was published, the strain has been recovered from a rabbit 3 years and 45 days and from a guinea-pig 3 years and 364 days after inoculation.

IV. A COMPARISON OF THE TYPES OF TUBERCULOUS LESIONS FOUND IN VACCINATED AND UNVACCINATED CATTLE, TOGETHER WITH A NOTE ON THE SUPERIMPOSITION OF INFECTION WITH VOLE BACILLI ON CATTLE ALREADY INFECTED WITH BOVINE TUBERCULOSIS (EXPERIMENT L)

In the preceding section we did not attempt to give post-mortem reports in full detail on all the animals mentioned in the various experiments, partly because doing so would have meant excessive demands on space and partly because a sharper contrast could be drawn by means of summaries which would throw into greater relief the extent of infection in vaccinated and unvaccinated animals respectively. In addition, however, to the extent of the disease there were also differences in the types of lesions, and although we attempted in the abridged reports to indicate these differences by the use of the terms 'infiltrative' and 'encapsulated', a fuller description of the varying types of lesions is necessary in considering the processes at work which go to build up the resistant state.

In our laboratory experiments the majority of the animals were killed 6 months after exposure to a single dose of bovine bacilli. At this stage of infection a very sharp contrast was usually observable macroscopically between the type of lesion found in the

unvaccinated animals and that of such lesions as were present in the vaccinated animals.

Control cattle

In the unvaccinated animals the lesions were by no means always of uniform type. Differences in age or in degrees of natural resistance may have accounted partially for this fact, but, in addition, it has to be remembered that with infection by the oral route there must be an element of chance in the degree to which the glands draining the several parts of the head and alimentary tract become infected, and therefore varying intensity of infection at the different sites may have had a considerable effect on the character of the lesions seen in different groups of glands. Occasionally lesions of frankly infiltrative nature might be found in one group while circumscribed lesions were seen in another.

In animals with the least resistance, such as young calves, the lesions tended to consist of uncircumscribed areas of pinkish granulomatous tissue, scattered through which lay yellow crystalline-like spicules of calcium. Except for a thin moist coating to the calcium, there was little evidence to the naked eye of caseation or necrosis. In other animals the lesions seemed to be more advanced. There were areas of granulomatous tissue, but central necrotic changes had taken place resulting in abscess formation, sometimes of considerable size, the abscesses being filled with a thick creamy caseous pus containing many brittle calcium spicules and resembling over-frozen ice cream. In a third type of lesion the granulomatous changes were much less marked. The typical picture was one of several small yellow fibrous-walled nodules containing caseo-calcareous material adjacent to one another and tending to fuse into larger lesions. In our opinion the presence of this type of lesion is an indication of some degree of developing resistance, and not infrequently we have found such lesions in one group of glands whilst at another site the lesions have been of frankly infiltrative character.

Vaccinated cattle

The most common type of lesion found in the vaccinated animals consisted of small sharply demarcated and circumscribed abscesses lying in what appeared to the naked eye to be unaltered tissue. The contents of the abscesses resembled a paste of moist French chalk sometimes containing a few granules of hard calcium, and a striking feature was the ease with which they could be evacuated leaving behind a clean-walled cavity. Although to the naked eye these lesions appeared encapsulated, histological examination showed that fibrotic changes were not marked. Acid-fast bacilli, although not present in large numbers, could usually be found without much difficulty, but on several occasions they were not so strongly acid-fast as usual and morphologically they were inclined to be longer and thicker than normal. There was, however, no loss of virulence for guinea-pigs and rabbits, and strains recovered from these animals were similar in their cultural characteristics to the parent strains used for the resistance tests.

Still smaller lesions have been found recognizable only as tiny yellow specks or rods and often lying singly. In a number of instances bovine bacilli have been recovered from these extremely small lesions when random samples which included portions of the glands in which the lesions lay failed to yield a positive result in the biological test.

It was not an invariable rule, however, that the lesions in vaccinated animals were of a less severe type than those in the controls, for in a small number of cases we found lesions which must be described as severe. These were found chiefly in the post-pharyngeal glands which were converted into large granulomatous tumours with considerable areas breaking down to form ragged-walled abscesses containing a thin, sometimes bloodstained, caseo-purulent fluid in which lay flakes of necrotic tissue and irregular granules of calcium. Lesions of this type were found in animals exposed to infection at relatively short intervals after vaccination, such as calf no. 127 in group A of Exp. A, calf no. 315 in group C of Exp. B and the 28-day calf in Exp. E. They were also marked in another experiment which we have not yet described and which it would be convenient to report here.

EXPERIMENT L. *Effects of vaccination on the development of tuberculosis in calves already infected at the time of vaccination*

Our primary object in carrying out this experiment was to determine what risk might lie in vaccinating animals already infected with bovine tuberculosis but in which infection was so recent that it was not detectable by the tuberculin, but we extended our series of animals so as to cover the full range of time over which sensitivity normally reaches its peak and then wanes.

Simultaneously with the application of the resistance test to group E of Exp. A, six tuberculin negative calves were similarly fed with 7·5 mg. of the same bovine culture. Thus the group E controls served to demonstrate the virulence of the strain.

After 7 days one of the calves was inoculated intravenously with 5 mg. of vole strain LV 285. The other calves were similarly inoculated after intervals of 10, 14, 28, 56 and 112 days respectively. No tuberculin tests were made between the time of infecting the calves with the bovine strain and the time of inoculation with the vole bacilli.

There was a marked rise in temperature following

the inoculation of the vole bacilli lasting for 10–14 days. In the case of the animal inoculated after an interval of 10 days, weakness suddenly appeared in the hind legs 3 weeks after the inoculation of the vole bacilli, the animal having to be lifted on to its feet for 4 or 5 days. During this period the temperature was elevated (104–105° F.). The weakness then disappeared as suddenly as it had appeared.

The animals were slaughtered for examination approximately 6 months from the date of first infection. The nature of the experiment did not call for a full bacteriological examination.

Post-mortem examination

It is difficult to give by way of summary more than a very general description of the trend of events following the inoculation of the vole bacilli, for in both the control and vole-inoculated animals the lesions were somewhat more widespread than usual, and there was a considerable degree of irregularity in the severity with which the various sites were affected which was obviously unrelated to the effects of inoculation with the vole strain.

The control animals were heavily infected but the lesions were not predominantly of infiltrative type, there being some tendency towards encapsulation.

In the vole-inoculated animals the lesions were not noticeably altered in numbers but they were considerably altered in character, an important point being the very slight amount of caseation present in the animals inoculated with the vole strain after a period of 28 days from the date of infection with the bovine strain.

In such of the head glands as were infected the effect of super-imposing the effects of the vole strain on those of the bovine strain appeared to be an exaggerated formation of granulomatous tissue and an acceleration of encapsulation, together with a tendency towards rapid abscess formation. This abscess formation appeared to be more of the nature of a necrotic liquefaction than the result of massive caseation. These changes were least marked in the calf inoculated 7 days after infection. Excessive formation of granulomatous tissue was greatest in the 28-day calf.

In the mesenteric glands of the calves inoculated 7, 10 and 14 days after infection the lesions were larger, yet of a more discrete type, than those seen in the control animals. In the 28-day calf granulomatous changes were very marked, but in contrast the lesions in the 56- and 112-day calves, although very numerous, were detectable only as small calcium spicules and granules lying in tissue which appeared little altered to the naked eye.

A very unusual finding was the presence of pleural 'grapes' in three of the vole-inoculated animals, viz. the 10-, 28- and 112-day calves. The only other animal in our whole series of experiments in which we found grapes was calf RC 241, revaccinated after 10 days in Exp. F, and in this animal both the pleura and peritoneum were severely affected.

Comments

We have enlarged on the macroscopic differences between the lesions seen in vaccinated and unvaccinated animals because we feel that a consideration of these, along with the histological changes which follow vaccination and with the curve for developing tuberculin sensitivity, both of which subjects will be discussed in later sections of this communication, may help towards an understanding of the processes at work which collectively create a state of enhanced resistance.

In the vaccinated animal the changes of a granulomatous nature which are set up by subsequent infection with a bovine strain may be intensified or they may be reduced. If the interval between vaccination and infection is short, the granulomatous changes are intensified. On the other hand, lesions of infiltrative type are rarely seen when infection is delayed beyond 3 months after vaccination. The same course of events appears to be followed when the vole bacillus is inoculated into an animal already infected with bovine tuberculosis, and it would seem that when a second infection is superimposed on a pre-existing infection while the tissue cells of the host are still in the primary stages of reaction to the first infection, the tissue changes characteristic of these primary stages are intensified, whereas if superinfection is delayed there is a reduction in what for want of a better term at this stage we may call the state of tissue cell irritability.

The absence of necrotic changes in the circumscribing walls of the small lesions in the vaccinated animals suggests two possibilities. The tissue cells may have been rendered insusceptible to the cytotoxins of the tubercle bacillus, or cytotoxins are not being liberated from the bacilli owing to limitation of their growth by an unfavourable environment. The morphological appearance and poor staining reactions of some of the bacilli seen in films prepared from the lesions is in keeping with the second hypothesis, whilst the striking difference between the calcium deposits in the vaccinated and unvaccinated animals strongly suggests a difference between the physico-chemical conditions present in the two types of lesion.

V. BACTERIOLOGICAL AND HISTOLOGICAL EXAMINATION OF THE TISSUES OF CALVES INOCULATED INTRAVENOUSLY WITH VOLE BACILLI

A series of experiments was carried out in an attempt to determine the length of time the vole bacillus might survive in the tissues of calves and also to observe the nature and extent of the tissue changes

caused by the intravenous inoculation of vole bacilli. It was hoped that the latter observations would help to elicit any risks which might be attached to the use of the vole bacillus as a vaccine and at the same time provide information on the processes at work which bring about an enhancement of resistance to tuberculosis. In the experiments to be described below some calves were inoculated with the stock laboratory strain, some with the 'passage' strain, some with killed vole bacilli, while one group was inoculated for comparative purposes with B.C.G. When due for examination the animals were killed by cutting the large vessels of the throat following mechanical stunning. (In some cases the stunning was carried out electrically, but generally the captive bolt pistol was used.)

(*a*) Experiment M. *Intravenous inoculation with 5 mg. of strain LV 285*

Heifer calves were used in this experiment in order to observe whether there was any danger of the vole bacilli colonizing in the developing mammary glands. In an experiment not here recorded it was noted that the bacilli could persist for prolonged periods after inoculation into the ducts of a lactating udder. Five calves aged 3 months were inoculated intravenously with 5 mg. of strain LV 285. At intervals of 7, 14, 28, 56 and 84 days respectively these animals were killed by bleeding after electrical stunning, and bacteriological and histological examinations were made of the lungs, liver, spleen, kidneys, meninges, udder and thoracic and other lymphatic glands. Other tissues examined histologically were the ovaries, heart valves, bone marrow, pancreas and suprarenals, and in one case the synovial membrane of a swollen joint.

Macroscopic post-mortem examination

The macroscopic changes were not severe and some of these may have been due to, or intensified by, the method of killing. The changes which were observed were confined largely to the lungs and the thoracic lymph glands. Numerous petechiae, scattered over the surface and throughout the substance of the lungs, were noted in the majority of the calves, but these may have been due to the electrical stunning. In the calves killed at 7, 14, 28 and 56 days there was an increase in the size of the smaller lymphatic glands of the thorax, this being most marked in the 28-day calf. In the calves killed at 14 and 56 days the medullary portions of the thoracic glands were stained a dusky carmine hue with the colour fading off gradually into the cortex. The calf killed at 28 days had exhibited signs of lameness in the fourth week after vaccination; both its hock joints were swollen, and the synovial membrane covering the non-articulating surfaces was yellow and oedematous while around the margins of the articulating surfaces it was haemorrhagic. All quarters of the udder of this calf were noticeably larger than those of the other calves in the series. In the calf killed at 84 days there were a few small flat hepatized areas present at the margins of the anterior lobes of the lungs, whilst in one kidney there was a small lesion resembling an old-standing infarct and in the other a large cyst-like swelling involved the whole of one lobe. These abnormalities were not considered to be due to the inoculation of vole bacilli.

Recovery of the vole bacillus

In the bacteriological examination we employed both direct and indirect methods of cultivation, using for the latter the Orkney vole in preference to the guinea-pig which has a considerable degree of resistance to the vole bacillus and in which it seemed possible that small numbers of viable vole bacilli might fail to establish themselves. We did, however, try the guinea-pig in a number of cases with more success than we had anticipated, for in some animals there was macroscopic evidence of infection both at the site of inoculation and in the regional glands, while in other cases we recovered the vole bacillus in cultures from regional glands which were free from macroscopic lesions. The Orkney vole, however, proved the more reliable animal, for in several instances the strain was recovered through the vole when cultures from the guinea-pig proved negative. The results of the bacteriological examination are shown in Table 4.

Table 4. *Recovery of vole bacilli from calves*

Site	RC 391 7 days	RC 392 14 days	AP 36 28 days	AP 37 56 days	AP 38 84 days
Lungs	+	+	+	o	+
Liver	+	+	o	o	o
Spleen	+	+	+	+	o
Kidneys	o	+	o	+	o
Meninges	o	o	o	o	o
Udder	o	o	o	o	o
Thoracic glands	+	+	+	+	+
Other glands	o	+	+	+	o

+ = vole bacillus recovered. o = vole bacillus not recovered.

Histological examination

The histological changes which can be observed for the first few weeks after intravenous inoculation of the vole bacillus appear to fall into two distinct categories. On the one hand there are the specific changes which are characteristic of tuberculous infection, and on the other hand there is a stimulation into activity of the cells of the reticulo-endothelial system which, although it may be regarded as being of less specific nature, is nevertheless part of the

reaction of the inoculated animal to the inoculum. The vole bacillus is only feebly pathogenic to cattle, and tissue changes pathognomonic of tuberculous infection are slight. This may account for the throwing into greater relief of the changes associated with the reticulo-endothelial system.

Lungs. The maximum degree of reaction in the lungs appeared to be attained between the 14th and 28th days. At the 14th day there was a generalized and pronounced swelling of the septal cells, and there was capillary congestion with some extravasation within the septa and occasionally into the alveoli. Although there were numerous aggregations of epithelioid cells and lymphocytes associated with easily found bacilli lying free among the cells there were no giant cells or other definite evidence of tubercle formation. In the 7-day calf the changes were similar but not so advanced or generalized. In the 28-day calf the septal swelling was more uniform but less intense. The aggregations of cells were less marked and acid-fast bacilli could not be found. By the 56th day the septal swelling had largely subsided, but it was still present in a few isolated areas. Cellular aggregates were fewer and acid-fast bacilli were not seen. At the 84th day, apart from a few small areas where the septal thickenings still persisted, the lung was substantially normal.

Thoracic glands. The most noticeable feature was the development of an intense sinus reticulosis which reached its maximum somewhere between the 14th and 28th days and receded before the 84th day, leaving the gland architecture somewhat distorted by fibrosis. Giant cells of Langhans type were found in the 28-day calf but not in the other calves. Acid-fast bacilli were not seen after the 14th day, although macrophages filled with acid-fast material were numerous.

Liver. Nothing diagnostic of tuberculous infection was seen in any of the sections from the livers, although a few scattered minute cell aggregations could be found in the 14-day animal. No acid-fast bacilli were seen. The reaction of the Kuppfer cells and of the finer elements of Glisson's capsule was a more prominent feature than any suggestion of tubercle formation. At 7 days the nuclei of the sinusoidal cells were very deeply stained, and by 14 days these cells had increased both in size and number. There was no further increase in the reaction of these cells, but the histiocytes of Glisson's capsule continued to proliferate up to at least the 28th day. By the 56th day the reaction had passed its peak and the 84-day liver was normal except for the presence of numbers of macrophages filled with acid-fast material. Similar macrophages had been noted in all sections from the different livers, particularly under the capsule and in the adjacent trabeculae.

Spleen. None of the sections showed any changes which could justify a diagnosis of tuberculous infection. No acid-fast bacilli were found, although there were large numbers of macrophages filled with acid-fast material.

Kidneys. No changes of a tuberculous nature were seen in the kidney sections. In the calves examined after 7, 14 and 28 days there appeared to be a progressive increase in the activity of the interstitial histiocytes both in size and numbers. This had subsided by the 56th day. Some degree of congestion of the boundary zones and pyramids was noted in all calves, but it was most marked in the 7- and 28-day calves. Macrophages containing acid-fast material were seen in small numbers in the perivascular tissues of the 14-, 28- and 56-day calves.

Mesenteric glands. Varying degrees of sinus catarrh were seen, but changes of true tuberculous nature were absent. No acid-fast bacilli were found, but macrophages filled with acid-fast material were present in all sections.

Meninges. No changes of a tuberculous nature were observed, but macrophages packed with acid-fast material were found up to the 84th day.

Udder. The only lesions noted were in the 28-day calf. In one area there were a number of unmistakable tubercles. These lesions, which were not difficult to find, lay in loose reticular tissue and contained giant cells of Langhans type in addition to epithelioid cells and lymphocytes. No acid-fast bacilli were found despite prolonged searches of several sections. Numerous macrophages containing acid-fast material were seen in all calves.

In the 14- and 28-day calves there were signs of activity and proliferation of the epithelium of the ductules, this being particularly marked in the 28-day calf in which in some areas of the sections the cells were lying several cells deep with many mitotic figures present. Although in the 56- and 84-day calves ductules were more numerous the epithelium in these was of normal character. Specimens for section appear to have been overlooked in the case of the 7-day calf.

Suprarenals. In the earlier calves the interstitial cells of the glomerular and fascicular zones were very prominent, the nuclei being large and deeply stained and the cytoplasm more clearly definable than is normally the case. The glomerular cells showed signs of active multiplication.

Other sites. These showed the same activity of interstitial cells and the presence of macrophages containing acid-fast material noted elsewhere. We found the changes in the section from the tibio-tarsal joint difficult to interpret, but these have been studied by Dr D. V Davies, together with other sections from affected joints, and his report is given later in this section.

(*b*) EXPERIMENT N. *A comparison of the tissue reactions which follow the intravenous inoculation of various types of vole vaccine and of B.C.G.*

In this experiment we continued the study of the tissue changes which follow the intravenous inoculation of vole bacilli. In particular, we wished to discover any difference that might exist between the effects of living and killed vaccines, but we also included calves inoculated with the more virulent 'passage' form of strain LV 285 and we further included for comparison two calves inoculated with B.C.G. The killed vaccines were prepared from the stock laboratory strain LV 285, one consisting of bacilli killed by heating at 65° C. for 30 min. and the other of similarly killed bacilli after washing twice in normal saline to remove soluble metabolites which might be present. The dose for each of the vole vaccines was 5 mg., while that for B.C.G. was 50 mg., the dose used at present in field vaccination of calves.

From Exp. M it had appeared that the activity of the reticulo-endothelial system reached its peak before the 28th day, and there appeared to be little object in continuing observations beyond this point. Calves were therefore vaccinated in pairs, one calf from each pair being killed on the 14th day and the other on the 28th day.

Post-mortem examinations

As in the previous experiment the macroscopic changes were for the most part slight and not such as would arrest attention in the absence of any history. In the calves inoculated with the living bacilli there was a prominence of the small lymphatic glands of the thorax, and the lungs of all the calves inoculated with vole bacilli had a few small areas of hepatization. In the calves inoculated with B.C.G. areas of hepatization were more numerous, amounting in some places to a degree of lobular pneumonia. In the B.C.G. calf killed at 28 days the lung tissue appeared to be peppered with numerous tiny foci resembling tubercles.

Bacteriological examinations

Cultures were made by direct and indirect methods from lungs, liver, spleen, kidneys and thoracic, mesenteric and carcass glands of the six calves inoculated with living bacilli.

The strain was recovered both at 14 and 28 days from every site in the calves inoculated with the 'passage' strain, but in the case of the stock laboratory strain there were three failures from spleen, mesenteric glands and carcass glands respectively at 14 days and one failure from kidneys at 28 days. The B.C.G. strain was recovered from all sites at 14 days, but not from the spleen or carcass glands at 28 days.

Histological examination

Lungs. At 14 days in all calves there was a marked response to the inoculum on the part of the septal cells, the septal walls being congested and thickened. Changes of a tuberculous nature were most marked in the B.C.G. calf in which there were numerous tubercles with epithelioid cells, giant cells and moderately numerous acid-fast bacilli. A number of epithelioid cells were present in the calves inoculated with the living LV 285 strains, and there was a suggestion of attempts at tubercle formation somewhat more marked in the case of the 'passage' strain than in that of the old laboratory strain. Acid-fast bacilli were found in the former but not in the latter. In the calves inoculated with the killed bacilli few cells of epithelioid nature could be seen

At 28 days the septal response was greater than at 14 days in the calves inoculated with living but less in those inoculated with killed bacilli. In the B.C.G. calf, numbers of what had appeared at 14 days to be early tubercles had not progressed, and the more mature tubercles appeared to be resolving. The giant cells were breaking down and no acid-fast bacilli could be found. On the other hand, changes of a tuberculous nature were now more marked in the calves inoculated with the living bacilli consisting of loose aggregations of epithelioid cells, granulocytes, lymphocytes and imperfectly formed multinucleate cells. There was some suggestion of attempts at tubercle formation in the calf inoculated with the heat-killed bacilli but none in that inoculated with the heat-killed and washed bacilli. A striking feature in the latter was the presence of large numbers of granulocytes in the septal thickenings.

Liver. At 14 days in all animals there was swelling of the sinusoidal cells and perivascular histiocytic proliferation. In the B.C.G. calf the normal columnar arrangement of the liver cells was disrupted by the active growth of the sinusoidal cells. Numerous miliary tubercles were found in this animal, but nothing indicative of tuberculous infection could be seen in the others.

The activity of the sinusoidal cells was still greater at 28 days in all animals inoculated with vole bacilli and histiocytic proliferation had progressed along the branches of Glisson's capsule. A few aggregates of epithelioid cells and lymphocytes were noted in the calf inoculated with the living laboratory strain. In the B.C.G. calf infection appeared to be regressing.

Spleen. In all animals there was evidence of sinus reticulosis more marked at 28 days than at 14 days except in the case of the B.C.G. animals in which the condition was more marked at 14 days. Giant cell formation was a prominent feature, and although it was of foreign body type rather than Langhans type the fact that it was more marked at 28 days than at

14 days suggested that it was part of the reaction to the vaccination.

Kidneys. In all sections from the calves inoculated with the various vole vaccines the interstitial histiocytes were more prominent than they are normally to be seen. The most outstanding feature, however, was a congestion of the boundary zone which apparently was early in onset, for even in the 14-day calves inoculated with killed bacilli there was a considerable amount of blood pigment in the intertubular vessels. The same congestion was noted in the B.C.G. calves, but in these animals the interstitial histiocytes were less prominent at 28 days than at 14 days.

Thoracic lymph glands. At 14 days there was a marked degree of sinus reticulosis in the three calves vaccinated with living bacilli, more advanced in the B.C.G. and 'passage' strain calves than in that inoculated with the stock laboratory strain. The condition was only slight in the calves inoculated with the killed vaccines. Numerous giant cells were present in the B.C.G. calf and acid-fast bacilli were easily found. In the 'passage' strain calf there were numerous epithelioid cells fusing in some areas into small syncitial masses. Acid-fast bacilli were numerous. No acid-fast bacilli were seen in the stock laboratory strain calf, and changes of a tuberculous nature were slight.

At 28 days the sinus reticulosis appeared more active in the calves inoculated with living vole bacilli but less active in the B.C.G. calf and much less active in the calves inoculated with killed bacilli. Changes of a tuberculous nature were more advanced in the calves inoculated with living bacilli, epithelioid cells and giant cells being numerous. In the B.C.G. calf tubercle formation was slight and giant cells were less numerous.

Other lymphatic glands. Changes were not marked but were suggestive of a mild sinus reticulosis.

Suprarenal glands. Activity of the interstitial cells was marked in the glomerular and fascicular zones of all the calves but was particularly noticeable in the calves inoculated with killed bacilli and examined at the 14th day. At 28 days while the nuclei of these cells were still prominent, the cytoplasm was less apparent. At 14 days the cells of the glomerular zone still retained for the most part their normal arrangement but at 28 days they lay in uneven and confused groups in which cells at different stages of development could be seen. The stimulation into activity of the zone appeared to occur earlier in the B.C.G. calves, for it was marked at 14 days and less marked at 28 days.

(c) EXPERIMENT O. *The histological response to the intravenous inoculation of the 'passage' strain LV 285 in doses varying from 5 to 0·005 mg.*

In this experiment we sought to discover how far variation in the size of the dose of vole bacilli might affect the histological changes that occur in vaccinated calves. As Exps. M and N had indicated that the maximum response occurred nearer to the 28th day than to the 14th day, it was decided to inoculate calves in pairs and to kill the first of each pair on the 21st day and the second on the 28th day.

The calves for this experiment were inoculated at the same time as those for Exp. C, the 'passage' form of strain LV 285 being used in a dose of 5 mg. and in decimal dilutions of this dose down to 0·005 mg.

The experiment was started in December 1946, and the exceptionally severe weather which followed later in the month and lasted into January 1947 had an adverse effect on the well-being of the young calves, with the result that our observations were complicated by a spontaneous outbreak of corynebacterial pneumonia which started the week after vaccination and affected almost every calf to a greater or less degree during the following 4 weeks. In the circumstances we felt that too much reliance could not be placed on the histological findings in these animals, and we therefore repeated the histological examination with calves inoculated with 5, 0·5 and 0·05 mg. Apart from the lung lesions attributable directly to corynebacterial infection in the first group of calves, the macroscopic appearances of the organs at post-mortem examination and the histological picture of both groups of calves were so similar that they can be considered together.

Post-mortem examination

As in Exps. M and N the macroscopic appearances attributable to vaccination were not marked. No cultural examination appeared necessary, so it was possible to examine four animals in one day and therefore to compare the organs side by side.

Lungs. There were slight areas of hepatization in all lungs, these being most marked in the lungs of the calves which had been inoculated with 5 mg.

Thoracic lymph glands. There was a marked enlargement of the glands of the calf inoculated with 5 mg. and killed at 21 days, but nothing was obvious in the other calves. At 28 days the enlargement of the glands of the 5 mg. calf was not very marked.

Liver. Congestion was present varying in degree according to the size of the inoculating dose.

Spleen. There was nothing to distinguish between the spleens of the calves killed at 21 days, but at 28 days the spleen of the calf inoculated with 5 mg. was pale, the cut surface presenting a homogeneous appearance with inconspicuous trabeculae and diminution of red pulp. In the calf inoculated with 0·005 mg. the spleen would have passed as normal, while the other two spleens were graded between the above two extremes.

Kidneys. Of all the organs the kidneys showed the most definite differences from normal, even if they were not gross in character. The changes, which were associated with congestion, were more marked

in calves killed after 21 days than in those killed after 28 days. The degree of congestion was directly related to the size of the dose. Thus in the kidney of the calf inoculated with 5 mg. and killed after 21 days there was a purplish congestion of the boundary zone extending down the pyramids to the papillae, whereas in the animal which received the smallest dose the congestion was slight and confined to the boundary zone. At 28 days congestion was no longer evident in the pyramids and was much reduced in the boundary zones. It was still present in slight degree in the papillae of the animals inoculated with the two larger doses, while the kidney of the animal inoculated with the lowest dose appeared normal.

Mysenteric and other lymph glands. These were normal in appearance.

Histological examination

Lungs. There was a considerable degree of septal thickening in all lungs at 21 days, but aggregation of epithelioid cells into tubercle-like masses, although marked in some sections, was absent from the lungs of the calf which received the smallest dose. The septal response at 28 days was much less marked than it was at 21 days, but epithelioid cells were more numerous and in the case of the two larger doses there were occasional giant cells of Langhans type. Acid-fast bacilli were seen only in the lungs of the calf inoculated with 5 mg. and killed at 21 days.

Thoracic lymph glands. There was a marked degree of sinus reticulosis at 21 days in the two calves inoculated with the larger doses, and there were multiple sheets of epithelioid cells and many giant cells. Acid-fast bacilli were numerous in the section from the calf which had received 5 mg. but were not found in the sections from the other calves. Sinus reticulosis was much less marked in the calf inoculated with 0·05 mg., and was present to only a slight degree in the calf which received the smallest dose. Although acid-fast bacilli were not seen in any of the sections from the 28-day calves, the degree of sinus reticulosis was slightly more pronounced with each dose than it was at 21 days. Giant cells were prominent in all sections except those from the calf inoculated with the smallest dose.

Liver. There was no definite tubercle formation, although a few aggregations of cells were seen in some sections. In all sections there was swelling and proliferation of the sinusoidal cells and perivascular histiocytes. These changes were in proportion to the dose inoculated, and they were slightly more marked at 21 days than at 28 days.

Spleen. There was remarkably little difference to be seen between any of the sections taken from either the 21- or 28-day calves. Varying degrees of sinus reticulosis were seen, but giant cell formation was scanty.

Kidneys. In contrast to the kidney changes which were noted with the stock laboratory strain, the interstitial histiocytes were not severely affected, nor was perivascular infiltration marked in any of the kidneys. The most prominent feature was a marked congestion of the vessels in the boundary zone, extending down into the pyramids in the case of the larger doses but confined to the boundary zone in the case of the smaller doses. There appeared little difference between the 21- and 28-day calves.

Mesenteric and other lymph glands. Some slight sinus reticulosis was noted in the mesenteric glands from the calves inoculated with the two larger doses both at 21 and 28 days. The iliac glands from the same animals were similarly affected. Otherwise any other changes were slight.

Suprarenals. The changes in these glands were not so marked as in the previous experiments. The arrangement of the cells of the glomerular zone was confused, but the interstitial cells did not show the same degree of activity.

(*d*) *The histopathology of lesions occurring in joints of vaccinated animals*

The most disturbing clinical feature that followed the intravenous inoculation of vole bacilli was a transient but occasionally severe lameness that affected a proportion of the animals. Dr D. V. Davies, School of Anatomy, Cambridge University, kindly undertook to make an examination of the limb joints of the calves in Exps. C and O, as they were slaughtered, to find out the cause of this lameness. The following section summarizes his findings.

The animals may be divided for convenience into two groups: (i) those killed 3 and 4 weeks after vaccination (fourteen animals in Exp. O), and (ii) those killed 6 months after exposure to a test dose of tubercle bacilli, i.e. 12 months after vaccination (fifteen animals in Exp. C).

In all groups the search for joint lesions was concentrated on the metatarso-phalangeal joints (hind fetlocks), as these were known to be the most frequent seat of lameness following injection of the vole bacillus vaccine. Tissues from several regions within these joints were subjected to histological examination in every case, and the synovial fluid to histological examination when its quantity or macroscopic appearance suggested any abnormality. Search for the vole bacillus both within the tissues and in the synovial fluid was made by staining with the Ziehl-Neelsen technique and, in a few cases, by inoculation into voles or guinea-pigs. In all cases the following joints were also examined macroscopically: the metacarpo-phalangeal (fore fetlocks), the tibio-tarsal (hocks) and the femoro-tibial (stifles). Tissues were removed for histological examination in all cases showing lesions on naked eye inspection.

(i) *Animals slaughtered on the 21st or 28th day following injection of the vole bacillus vaccine*

The joint lesion in all these animals was similar, differing only in its severity from animal to animal. Only one animal (no. 516) showed any objective signs of a joint lesion during life. On the 23rd day following vaccination this animal developed a lameness of both hind fetlocks. The joints involved were hot, swollen and tender. The lameness became progressively worse until the 26th day, when the animal refused its food and was recumbent. Its temperature was 104·2° F. Thereafter, although the temperature dropped (102·8° F.) and the animal took some food, the lameness continued. The fetlocks were swollen, hot and tender at the time of slaughter on the 28th day.

The first and most significant feature in this series was the occurrence of pathological changes in the hind fetlocks of all animals and frequently in the other joints examined, despite the absence of any signs of a joint lesion during life in all except one (no. 516). The lesion varied in its severity from one animal to another, and its degree of development bore no relation to the massiveness of the dose of vaccine injected (5 down to 0·005 mg.).

In the mildest cases the lesion was confined to the blood vessels of the synovial membrane and subsynovial tissues; the endothelium of the arterioles, capillaries and venules became swollen, the vessels themselves dilated and engorged with blood; stasis occurred. In more severe cases there was exudation of fluid into the synovial and subsynovial tissues which became swollen and oedematous. The synovial lining cells now enlarged and multiplied, becoming several layers thick, whilst the subsynovial tissues became more cellular, the tissue cells increased in numbers and scattered lymphocytes appeared among them. A few polymorphonuclear leucocytes were also seen scattered throughout this tissue, whilst here and there small extravasatons of red blood cells occurred. Trauma could not be excluded as a causative factor in this extravasation.

With the progression of the lesion increasing numbers of nucleated cells, lymphocytes, macrophages and synovial cells appeared in the synovial fluid which became cloudy. In the most severe case (no. 516) a nucleated cell count of over 26,000 was recorded in the fluid, whilst in histological sections the detachment of the lining cells of the synovial membrane, to become free in the fluid, could be clearly seen. Even portions of villi became detached *en masse*. In the most severe cases fibrin or fibrinogen appeared in the fluid in quantity, clotting here to form fibrinous loose bodies similar to the melon-seed bodies of tuberculous joint lesions in man. In addition, the fibrin was deposited on the surface of the synovial membrane as a loose detachable membrane or pseudomembrane of yellow colour. No vole bacilli were detected within this nor, indeed, in the synovial tissues or fluid in any case; the animal inoculation tests also proved negative. In no case was there denudation of the surface of the synovial membrane and no ulceration occurred, whilst the articular cartilage in all cases appeared normal both on macroscopic and microscopic examination. In the milder cases the lesions in the synovial membrane were confined to the areolar areas, in the more severe cases spreading to the more fibrous areas, but always remaining less marked here. If, as would seem probable, the primary lesion were vascular in character, the delay in its development in the more fibrous regions of the synovial membrane would be due to mechanical factors preventing any marked dilatation of blood vessels or swelling of tissues.

No lesion even suggestive of tubercle formation could be identified in the joint tissues. Nor could any lesion analogous to the Aschoff body of rheumatic fever be found. Perivascular infiltrations of lymphocytes were not a feature of the pathology. The lesions, however, did resemble those which have been described in the joints in serum sickness, allergy and anaphylaxis. The lymphocytic reaction, however, though present, was not as intense as described by Klinge & Vaubel (1931) in rabbits following injections of horse serum intravascularly and into the periarticular tissues. The vascular reaction and that of the synovial lining cells were very similar. The time of development of the joint lesion in the present series lends support to an allergic basis for the lesions, whilst the rapidity with which the lame animals improve and lose their lameness is also suggestive.

(ii) *Animals slaughtered approximately 12 months following injection of the vole bacillus vaccine*

These animals all received their injections of the vole bacillus vaccine approximately 12 months prior to slaughter. None showed any outward signs of a joint lesion following the injection. The joint tissues were examined for any residual effects.

The same procedure was followed as in the previous group. The lesions were not of a gross nature, though definite, and were seen in both fore and hind fetlocks. No macroscopic changes were seen in the hocks and stifles excepting in one or two cases. The pathological lesions were best seen in the more areolar areas, and especially in the loose tissue lying between the sesamoid bones and the proximal end of the first phalanges. The synovial membrane was considerably more villous than normal whilst its blood vessels frequently remained filled with blood after slaughtering, giving it a pink appearance compared with the remainder of the joint lining. In histological section the synovial cells were larger than normal, and frequently two, three or even more layers deep.

In the subsynovial tissue small perivascular collections of lymphocytes occurred. In one case (no. 510) there was a large collection in one hind fetlock, almost amounting to a lymphoid nodule, close to the surface. No giant cell or necrotic areas could be seen in association with these lymphocytic infiltrations. They could not be considered as small tuberculous lesions.

In contrast to the earlier series, almost all animals here showed lesions of the articular cartilage in the fetlocks and in a few cases also in the hock. On the metacarpal and metatarsal bones these consisted of small pits, up to 2 mm. or more in diameter on the weight-bearing surfaces, generally adjacent to the ridges on the medial and lateral condyles. On the adjoining phalanges and also on the talus these lesions of the articular cartilage were less frequent and consisted of wedge-shaped areas, partially or wholly devoid of cartilage, extending inwards from the posterior margins of the articular surfaces. On the phalanges these wedge-shaped areas extended inwards opposite the central parts of the articular facets. Microscopically the defects displayed either a complete absence of articular cartilage and its replacement by fibrous tissue or of sunken areas of articular cartilage, i.e. indentations into the adjacent subchondral bone. Within these sunken areas the cartilage was more cellular than elsewhere. Its cells extended right to the joint surface where they persisted, presumably due to the absence of wear and tear. Furthermore, in these areas the capillary loops of the underlying bone and marrow had frequently extended into the calcified layer of the articular cartilage, whilst in defects filled by fibrous tissue this latter was vascularized up to the joint surface from the underlying bone capillaries.

The lesions on the cartilage here resembled some of those described by Bauer, Bennett, Marble & Claflin (1930) in cattle, but were not so marked. Their constant occurrence in all cases of this series, however, suggested a significant relation to the other lesions described. The findings in this series did not suggest any tuberculous affection of the joint tissues. The lesions resembled early degenerative joint disease of the rheumatoid type. The occurrence of this in these young animals as a constant feature suggested a causative relation to the previous joint lesion described in the 21–28-day group. The cartilage defects presumably followed some disturbance of its nutrition or growth at an earlier stage. In view of the constant occurrence of a joint lesion in the 21–28-day group it can reasonably be assumed that all animals in the present series suffered similar joint changes at the corresponding stage.

Comments on Experiments M, N and O

In the vole, the vole acid-fast bacillus produces a disease which is characterized by a massive histiocytic reaction with necrosis, caseation and other features also seen following infection of various species of animals with the appropriate type of virulent tubercle bacilli (Pagel, 1939; Robb-Smith, 1946).

In the calf, in our experience, the occurrence of typical tuberculous changes following vaccination with the vole bacillus is uncommon, even when cultures of high virulence are inoculated intravenously. Such tubercles as are produced are not of great cytological complexity, and they occur almost exclusively in the lungs and thoracic glands. They do not progress to a stage showing central necrosis, and the bacilli are difficult to find after 14 days, at which time one may find bacilli lying in or around loose aggregations of epithelioid cells which lack the classical features of tubercles, while later, at 28 days, one may find fully formed tubercles which lack the evidence of associated acid-fast bacilli. This picture differs from that seen in calves inoculated with B.C.G., for in these animals fully formed tubercles containing acid-fast bacilli can be found with ease at 14 days, whereas at 28 days the tubercles are markedly regressive. In spite of the fact that the lesions in vole-vaccinated animals are slower in forming than those in animals inoculated with B.C.G., they do not persist long, and in a matter of weeks they disappear.

In addition to lesions of specifically tuberculous character, there are other changes which might be classed as 'non-specific' although they are undoubtedly due to the presence of the vole bacillus or its metabolites in the body. There is an early stimulation into activity of the cells of the reticulo-endothelial system which steadily progresses to reach its peak in the 4th week and then begins to wane before the changes of a tuberculous nature have reached their maximum.

In the lungs there is swelling of the septal cells, only a small proportion of which undergo epithelioid change. In the liver there is a perivascular histiocytic proliferation and swelling and some proliferation of the sinusoidal cells, resulting sometimes in a loss of the normal columnar arrangement of the parenchymal cells. In the spleen and lymphatic glands it is the littoral cells of the sinuses which show the most change, but the fibrous cells of the trabeculae are oedematous and swollen and there are signs of fibroblastic activity. In the kidney there is some proliferation of the perivascular histiocytes and a certain amount of swelling of the interstitial cells. These non-specific changes occur whether the inoculum consists of living or of dead bacilli, and they are present in intensity in animals inoculated with small doses to a degree that is not proportionately less than that seen in animals inoculated with a dose 1000 times as great.

The joint lesions associated with lameness in the 4th week appear to be further manifestations of the

response of the reticulo-endothelial system rather than part of the tuberculous infection, for bacteriological examination has failed to detect the presence of acid-fast bacilli in the synovial membranes or fluid and no changes of a specific tuberculous nature can be seen. The histological examination suggests that the lesions are vascular in origin, and it is probable that the congestion and stasis noted in the boundary and pyramidal regions of the kidneys are of similar causation.

The proliferation of the epithelial cells of the mammary ductules and the activity of the glomerular zones of the suprarenal glands appear to be reactions associated with a different system of the body and may reflect the manner in which the general metabolism of the body is affected by tuberculous infection.

With regard to the survival of the vole bacillus in the calf, in spite of the fact that in Exp. B we recovered bacilli belonging to strain G 564 from two calves after a survival period of 18 months, we failed to recover strain LV 285 in its 'passage' form from any animal 12 months after inoculation. In Exp. N we recovered it from every site examined at 14 and 28 days, but we failed to recover the stock laboratory strain from several sites. As in Exp. M we had recovered the stock strain from the lungs and thoracic glands at 84 days, and from these sites only it would appear that strain LV 285 has no great powers for survival such as have been seen in the case of strain G 564.

VI. THE CLINICAL EFFECTS OF THE INOCULATION OF VOLE BACILLI INTO CATTLE

In the course of the experiments described in § III we used three different vaccines, the stock laboratory strain LV 285, its 'passage' form and a recently isolated strain G 564. The clinical response of cattle to each strain varied in certain respects.

(*a*) *The stock laboratory strain LV* 285

By the mouth. Doses of strain LV 285 ranging from 10 to 100 mg. have been given by the mouth (Exps. G and J) without any symptoms being noted.

Single dose intravenously. In the laboratory some fifty cattle have been given a single intravenous inoculation of 5 mg. of strain LV 285. These were mainly young castrated bull calves, but eleven freshly calved heifers (Exp. H) and five heifer calves (Exp. M) were also inoculated. In addition, a further 150 heifers and heifer calves have been similarly inoculated in the course of a small field trial which is still in progress on five farms. This field trial is not yet complete, but certain clinical observations made during its course are recorded below. We have observed both immediate and delayed effects in the vaccinated animals, the former consisting of a rise in temperature and a loss of appetite and the latter consisting of lameness occurring in the 4th week post-vaccination.

Temperature

There was a rise of from 3 to 4° to 104 or 105° F. within 24 hr. For the next 7 days the temperature fluctuated narrowly within the range 103–105° F., after which there was a gradual return to normal between the 14 and 20th days.

Appetite

Calves showed an almost complete inappetance 12–24 hr. after inoculation and there was a cessation of rumination. On the second and third days they took some food, the fourth day brought a great improvement, and by the seventh day appetite and rumination were normal. In yearlings and larger animals the inappetance was not so noticeable. In no case was there any obvious loss of condition in the period immediately following vaccination.

Delayed effects

During the course of Exp. L one calf suddenly developed an acute weakness of the hind legs 21 days after the intravenous inoculation of 5 mg. of vole bacilli. The animal was so severely affected that it had to be lifted to its feet for 4 or 5 days. The symptoms then disappeared as suddenly as they had arisen. As this calf had been deliberately infected with virulent bacilli by mouth 10 days before it was vaccinated, we did not at the time recognize the symptoms as sequelae to the inoculation of vole bacilli.

In Exp. F, however, during the fourth week post-vaccination fifteen out of sixteen yearling bullocks developed a lameness which was most marked in the hind limbs. The animals, some of which had to be lifted to their feet and persuaded to walk, had a depressed appetite and appeared very dull. The onset of the lameness was sudden, and the severe symptoms lasted 3–4 days. Recovery was gradual, but all animals were normal some 7–10 days after the onset. This batch of calves was housed in a large concrete paved yard and indulged in a lot of playful running about, and this may have had some effect in causing the symptoms observed. The one calf which did not exhibit any untoward symptoms was an undersized animal which did not take part in this running around.

Following this episode a more careful watch was kept for similar symptoms amongst vaccinated animals. As a result, many degrees of lameness, varying between a slight stiffness of one limb lasting for a few hours to severe lameness in all four limbs, have been observed. Symptoms are most commonly

noticed in yearlings and bulling heifers, rarely in young calves or calved heifers. The onset of symptoms has not been observed outside the fourth week after vaccination. Providing the animals are kept in strawed yards or at pasture, the lameness noted is slight, usually no more than a stiffness in one or two limbs lasting up to 24 hr., but anything up to 25 % of animals of a batch may be affected. These slight symptoms do not give rise to any anxiety and frequently pass unnoticed by the animals' attendant. However, where the animals are housed in concrete paved boxes, more serious lameness has been observed occasionally, and one alarming incident concerned a batch of six bulling heifers which, on the 21st day after vaccination, were driven about a quarter of a mile along a hard road and then placed in loose boxes. One hour later five of the six heifers were extremely lame and two could stand only with difficulty. When examined after a further hour the worst symptoms had passed, but the lameness was still very marked, particularly in the hind limbs. The joints of the lower limbs were swollen, hot and painful to the touch, and each leg was rested in turn. The animals were placed in a paddock with plenty of grazing. The lameness was still pronounced 48 hr. later, and some animals had to be made to rise. On the fourth day there was some improvement in the gait of four of the animals but all showed some loss of condition. On the eighth day, apart from one which was still slightly lame, the animals appeared normal and were feeding well. Subsequent history was uneventful. There were no remissions, all became pregnant and calved normally.

In two animals, one in the laboratory and one in the field trial, hard swellings developed in the skin and subcutaneous tissues over the point of intravenous inoculation. The swelling in the laboratory animal reached its maximum approximately 1 month after the date of vaccination. It slowly resolved over the next 2 months leaving no trace. The other animal had a hard lump—the size of a walnut—when examined 9 months after vaccination. There was no trace of the swelling after a further 8 months. On both animals exaggerated responses to the tuberculin tests occurred when these were sited close to the nodules, which were probably due to the leakage of a small number of vole bacilli into the subcutaneous tissues during vaccination.

Revaccination by the intravenous route

In Exp. F sixteen calves were revaccinated with doses varying from 5 to 50 mg. at intervals from 7 days to 6 months after primary vaccination with 5 mg. Eleven of these sixteen calves developed a severe lameness, of relatively short duration, after primary vaccination. Together with the four animals which acted as single vaccination controls in Exp. F, they make up the batch of fifteen animals already described in this section. The animals forming group G of the experiment were vaccinated and revaccinated at a later date and they did not develop lameness. The six animals comprising the revaccinated groups A, B and C were reinoculated before the lameness appeared, and group D (four animals) was revaccinated whilst the symptoms of lameness were just beginning to abate. Revaccination, irrespective of the dose employed, did not appear to have any effect on the course of the lameness, nor did revaccination itself appear to give rise to a similar condition. There was some diminution of the appetite and a slight rise in temperature following revaccination, but the animals returned to normal within 48 hr.

(*b*) *The 'passage' form of strain LV* 285

Thirty-two calves aged between 3 and 4 months have been inoculated intravenously in the laboratory, ten with 5 mg., eight with 0·5 mg., eight with 0·05 mg. and six with 0·005 mg. (Exps. C, N and O). As with the stock laboratory strain, both immediate and delayed effects have been noted. The diminution in the appetite, however, was much less marked, and the rises in temperature where observed did not exceed 1° F.

Symptoms of lameness were noted in one calf which had been inoculated with 0·5 mg. Symptoms appeared on the 23rd day after vaccination, the fetlocks of both hind limbs being swollen, hot and painful. For a further 72 hr. the condition progressed unfavourably, by the end of which time the animal was recumbent and had to be got to its feet. It refused food and its temperature was 104·4° F. Thereafter it began to improve, and the symptoms had somewhat subsided when the animal was slaughtered for examination 28 days after vaccination.

(*c*) *The recently isolated strain G* 564

Twenty-four calves were vaccinated intravenously with 5 mg. of this strain in the laboratory. No particular symptoms beyond some slight inappetance were noted following vaccination, but two calves did not continue to thrive. One of these died 1 month after vaccination, and the other was killed in a moribund condition 2 months after vaccination. The former went downhill almost from the time of inoculation. Its appetite was small and emaciation was progressive. The second calf was noted to be feeding poorly some 5 weeks after inoculation. In its case, also, emaciation was progressive over the next 3 weeks until it was killed. The post-mortem examinations of these animals have already been described in § III, but here it may be recalled that nothing was found which might indicate that the animals' deaths were due to any other cause than the effects of the inoculations.

Lameness was not observed in any of the calves inoculated with strain G 564, but as they were housed in a large well-strawed shed, it is possible that if slight lameness did occur it passed unnoticed.

VII. TUBERCULIN TESTING

We have carried out large numbers of tuberculin tests on vaccinated and control calves with a variety of P.P.D. preparations, and we have satisfied ourselves that the oral administration of single doses of vole bacilli of from 50 mg. upwards or the parenteral inoculation of doses as low as 0·005 mg. will provoke a skin sensitivity to bovine and human P.P.D., to a P.P.D. prepared from B.C.G. and to a lesser extent to avian P.P.D. We have not yet used a P.P.D. prepared from a strain of the vole bacillus because we have not yet succeeded in adapting the vole bacillus to grow on synthetic media.

Our main purpose has been to record the responses which result from the inoculation of the types of P.P.D.'s used in the 'official' test. During the period covered by our experiments there have been modifications in the method of applying the official test—the latest on 1 June 1947. We are unable, therefore, to state how vaccinated animals would react to this test.

In the earlier part of Exp. A and in Exps. G and J we used the double intradermal (D.I.D.) method of testing, making a second inoculation into the same sites after 48 hr., and the routine testing was carried out with human P.P.D. only. At first, this was 'Provisional Standard' P.P.D. supplied by Dr Hartley of the Medical Research Council at 0·75 mg./ml. and later we used a Burroughs Wellcome product at 0·67 mg./ml. From September 1942 onwards the single intradermal comparative (S.I.D.) method has been employed using P.P.D.'s prepared at the Ministry of Agriculture Veterinary Laboratory at Weybridge, the dosage being fixed at 0·025 mg. of avian and 0·1 mg. of human P.P.D. In Exp. C the dose of avian P.P.D. was raised to 0·1 mg. as, in our experience, this makes for an easier interpretation of the test.

In our laboratory experiments it has been our custom to house both vaccinated and control calves together in large open yards prior to the application of the resistance tests. Whilst thus living in contact with vaccinated calves all control calves have remained uniformly negative to human P.P.D., but some calves have exhibited slight avian reactions. Post-mortem examination subsequent to the resistance test did not show that the avian-sensitized animals were more resistant to the test dose than completely non-allergic animals.

Tuberculin reactions following oral administration

Fourteen days after feeding 10, 50 and 100 mg. of strain LV 285 to three calves respectively, the three animals were completely non-allergic. Six weeks after feeding, the two calves fed with 50 and 100 mg. were allergic, the skin increase at 48 hr. being 4 and 9 mm. respectively. A proportionately greater increase was noted following the second inoculation of tuberculin (0·075 mg. human P.P.D.). The reactions in these two animals after a further month were of a similar order, but thereafter the tests were negative. The calf fed with 10 mg. did not develop any really measurable allergy.

Six calves fed with 100 mg. were negative to a D.I.D. test (0·1 mg. human P.P.D.) 14 days after feeding. They had some allergy when tested 6 and 10 weeks after feeding, the skin increases being between 5 and 10 mm. at the 48th hour. The allergy faded rapidly, further tests being, in the main, negative. These calves were also simultaneously tested with bovine, B.C.G., avian, johnin and phlein P.P.D.'s. The increases to the bovine and B.C.G. P.P.D.'s were approximately equal and were about half those to the human P.P.D. (We have evidence from observations on tuberculous calves that this sample of bovine P.P.D. was of low potency.) The increases to the avian P.P.D. were of a low order. An occasional reaction was noted with the johnin P.P.D., but no reactions occurred with the sample of phlein P.P.D.

Tuberculin reactions following intravenous inoculation

In Exps. A, B and C we have been able to study the allergy at varying periods following the intravenous inoculation of 5 mg. of vole bacilli.

In Exp. A we were able to show that 14 days after the intravenous inoculation of 5 mg. of strain LV 285, the animals became very definitely allergic and in some cases the maximum response was observed at this test. The majority, however, had their biggest reactions when tested 6 weeks after vaccination. The maximum response of each animal to 0·075 mg. of human P.P.D. lay between 10 and 20 mm. at the 48th hour. There was usually some further slight increase at the 72nd hour following the second inoculation of tuberculin. Positive reactions continued to be noted for some time, but the animals which were available for test 9 months after vaccination were substantially negative, although slight responses of 2–3 mm. (D.I.D.) were noted for periods of up to 2 years. However, on the introduction of the single intradermal comparative test (groups F and G had still not been exposed to the resistance test at this time) it was apparent that these small reactions had been due in most cases to 'non-specific factors' as the responses to the 0·1 mg. of human P.P.D. did not exceed those to 0·025 mg. of avian P.P.D.

In Exp B, in which we inoculated calves with the newly isolated strain G 564, the maximum reactions were generally observed at the 4 weeks test (0·025 mg.

avian and 0·1 mg. human P.P.D.). After 12 months (group A) the reactions were slight, two animals having minimal avian excesses and three small mammalian excesses.

In Exp. C the animals inoculated with the smaller doses of the passage strain LV 285 developed their maximum sensitivity at a somewhat later date than those inoculated with the larger doses. This experiment clearly demonstrated that there may be some danger of jeopardizing the outcome of an experiment in trying to follow the course of the development of tuberculin sensitivity in animals which are subsequently to be subjected to a resistance test. A perusal of the 72nd hour increases in the test carried out on all animals immediately prior to the resistance test (see Fig. 3) shows that the calves which had not been tuberculin-tested since vaccination were markedly more allergic than those which had been repeatedly tested. We are unhappy about this desensitization and are of the opinion that in 'immunization' experiments the amount of tuberculin testing should be reduced to such tests only as are absolutely necessary for the detection of any extraneous infection.

Tuberculin reactions after multiple skin puncture

The responses in the vaccinated calves in Exp. K are shown in Table 3. They were not of a high order, the response to the mammalian P.P.D. exceeding 10 mm. in one instance only and, as in the other experiments, allergy had waned considerably by the 6th month. In contradistinction to what we found in the other experiments, the skin responses in the tests applied immediately before slaughter afforded an accurate ante-mortem forecast of the post-mortem findings. In Exps. A, B and C our post-mortem findings were not always in accordance with a forecast based on the ante-mortem tuberculin test.

Tuberculin reactions in vaccinated cattle exposed to natural infection

In Exp. H we had an opportunity of observing the tuberculin responses in vaccinated animals exposed to natural infection and of comparing them with those occurring in comparable unvaccinated animals. The 72nd hour increases at the half-yearly tests are set out in Fig. 4. The dose of avian P.P.D. was 0·025 mg. and that of human P.P.D. 0·1 mg.

If we can assume in the case of the vaccinated animals that the immediate reactions or any slight mammalian excesses under 4 mm. occurring during the 12 months after vaccination are due to the inoculation of the vole bacilli, and if we accept a mammalian excess of 3 mm. beyond this period as being indicative of bovine infection, it is possible to make the following points:

(1) That nine out of twelve control animals became bovine reactors and all were subsequently proved to be infected.

(2) Of three control animals which failed to react to tuberculin, two (nos. 11 and 17) were free from infection at post-mortem examination. The third animal was infected, but co-existing Johne's disease had undoubtedly made diagnosis by the test impossible.

(3) Seven vaccinated animals became bovine reactors, but only four were found to be infected at post-mortem examination. In three of these the tuberculin history suggested that resistance had been overcome, and this was proved to be the case. The fourth heifer (no. 19) was a bovine reactor 11 months after vaccination, but over the next 2 years was negative, although she was found at post-mortem examination to be badly affected. Nos. 7 and 12 were bovine reactors at one test only and subsequently they became negative. No tuberculosis was found in these two animals. In the case of heifer no. 16 we expected to find tuberculosis at the post-mortem examination, but no lesions were found.

(4) Four vaccinated heifers did not become bovine reactors (nos. 5, 11, 14 and 20). At post-mortem examination only one (no. 5) had tuberculosis, and this was localized and of very slight extent. This animal was on the farm for 60 months.

Although the number of animals in this field trial was small, it would seem justifiable to say that tuberculin testing would indicate the majority of animals whose resistance has been overcome but, on the other hand, there is a real danger in placing too much reliance on tuberculin as indicated by the low degree of sensitivity exhibited by the grossly infected animal (no. 19). The failure of animal no. 5 to react must also be noted.

Severe tuberculin reactions due to the vole bacillus

One last point calls for comment. In two animals extraordinarily large reactions were noted at the first tuberculin test after vaccination, the increase being 42 mm. in one animal 6 weeks after vaccination and 22 mm. after 9 months in the other. In each case there was visual evidence, in the form of a swelling at the point of intravenous inoculation, that some of the vaccine had lodged either in the skin or in the subcutaneous tissues. In neither animal was the swelling noticed at the time when the tuberculins were being inoculated, and by chance the sites chosen for the test were in close proximity to the swelling. It may be that this increased sensitivity was a local phenomenon. Subsequent tuberculin tests on these animals do not call for special comment.

VIII. DISCUSSION

There appears to be little need for us to recapitulate the evidence which we have given in §III regarding the capacity of vaccination with the vole bacillus to enhance resistance against subsequent infection with bovine tuberculosis. It is abundantly clear that the vole bacillus is entitled to be ranked alongside B.C.G. as a prophylactic agent, and that the question is, not if it can be used, but how it can be used to the best advantage. The results of our experiments suggest that just as has proved the case with B.C.G. there is little hope for the bestowal on an animal of a lifetime immunity, but any procedure which could lead to an enhanced resistance over a number of years might well find a place in a scheme for the eradication of tuberculosis from dairy herds and particularly from herds which have a high percentage of tuberculin reactors from which large numbers of reactors could not be cast at one time without seriously affecting milk production.

We suggest that, provided always there are facilities for the segregation of young stock, the entire milking herd of a self-contained farm could be replaced with fresh stock within a period of 4 years, the oldest animals being eliminated as fast as young vaccinated animals become available to replace them. Once the turn-over of the milking stock was completed no further vaccinations would be carried out, but in their turn during the following 4 years the vaccinated animals would be replaced with unvaccinated animals. In this manner the status of a non-reacting tubercle-free herd might be attained with a minimum of interference with farm management.

The question of farm management is one which must be uppermost in mind in drawing up a scheme for vaccination, for the success of the latter is dependent upon the willing co-operation of the farmer. Repeated vaccinations carried out at intervals over a number of years must necessarily entail periodic dislocations of routine (and sometimes an appreciable if temporary loss of milk production). This by itself would have been sufficient to make us reluctant to attempt to maintain resistance by repeated vaccinations but in fact we found evidence in Exp. L (§IV) that vaccination of an infected animal can modify the progress of the infection in a manner likely to mask its presence. We feel that once an animal has been exposed to the risk of infection nothing should be done which might delay recognition of the fact that it has become infected, and we are forced to the conclusion that our aim should be to give the animal as solid a resistance as possible at the time when it is admitted to the milking herd and provide for its removal from the herd before it can become a potential danger to its neighbours through the waning of resistance or earlier if a tuberculin test suggests that it has become infected.

How far does the vole bacillus meet the requirements for a vaccine likely to succeed when used for the above plan? It is clear that a single intravenous dose of 5 mg., although capable of inducing in some animals a very marked degree of resistance for periods up to 3 years, and possibly for a considerably longer space of time, is far from being capable of producing such a degree of resistance in every case. We must look for some way of intensifying the initial resistance, and we do not favour that of increasing the dose, for the vole bacillus is not the innocuous organism that it was at first considered to be. We believe from the evidence we have presented that the degree of resistance set up in calves is correlated with the virulence of the vaccinating strain for its native host, and any strain selected for use as a vaccine would have to be maintained at a high level of virulence. We have seen that at least one strain, G 564, can prove fatal for calves by causing a generalized adenitis, and although in the case of other strains a dose of 5 mg. can be borne without apparent ill effects of any lasting nature, we feel that this dose is the maximum that could be used with safety.

The alternative to increasing the dose for a single vaccination is revaccination, and by this we mean revaccination before the animal has been exposed to infection and not afterwards. In Exp. F we found that animals revaccinated after intervals of time varying from 4 weeks to 6 months after primary vaccination were much more resistant to infection than animals which had been vaccinated once only, but while the experiment showed that a greater initial resistance could be obtained by revaccination, it did not throw any light on the duration of that resistance for all animals were subjected to the resistance test six months after their last vaccination. It seems, however, reasonable to surmise that if revaccination can intensify resistance, it will also prolong it, and a further experiment has been planned to test the degree of residual resistance remaining in revaccinated animals after 3–4 years and to determine the optimal revaccinating dose and the optimal period between vaccinations.

Until this latter experiment has been completed, it will be impossible to make any final decision regarding the value of vaccination with the vole strain. Unless revaccination can result in resistance being prolonged well into the fourth year, we feel that it would be unwise to place too much faith in vaccination as a measure towards the control of tuberculosis in cattle, although it might be profitably employed in special circumstances, for example, where an owner is prepared to sacrifice milk production temporarily by eliminating his old stock more rapidly than he can replace it with young stock

and so shorten the period over which vaccinated animals would be at risk.

We have given priority to the question of the practical application of vole vaccination as a measure towards the eradication of bovine tuberculosis, for that has been the primary motive of our work, but in the course of our investigations we have collected a considerable amount of data bearing on the problems of vaccination against tuberculosis in other fields.

It has long been recognized that the greater the virulence of a primarily infecting strain, the greater is the resultant resistance to a second infection of similar nature, and vaccination with a living organism is to all intents and purposes the setting up of a mild infection. Some observers are of the opinion that B.C.G. is less effective now as an immunizing agent than it was at the time when its safety as a vaccine was still in question owing to the possibility of its regaining virulence. We ourselves have found that the degree of resistance set up by vaccination with a strain of the vole bacillus is related to the virulence of the strain for its native host, but we have also found that a more virulent strain causes less constitutional reaction than does a less virulent strain, and this suggests that in the case of the more virulent strain there is a slower rate of destruction of the bacilli and a slower liberation of bacterial products.

It may be that the fully virulent strain is more powerfully antigenic than the feebly virulent strain —it would be strange if this were not the case—but it would be unjustifiable to assume that any antibody stimulated in the calf by an antigen in the vole bacillus which is linked with the virulence of the vole bacillus for its native host will be effective against the bovine tubercle bacillus. The resistance set up by vaccination with the vole bacillus may be largely a cellular resistance due to the production of an allergic state in the tissue cells, and it may be that the rate at which the cells are exposed to the sensitizing proteins may determine their subsequent reactivity.

We have a considerable amount of evidence regarding the cellular changes which follow vaccination. In § V we have described the alteration in the cells of the reticulo-endothelial system in widely varying sites of the body which show evidence of an activity that is progressive for about 3 weeks and then subsides irrespective of the fact that processes of specifically tuberculous nature may still be progressive in those sites in which the vole bacilli persist longest.

We have not been in a position to study histologically the effects of revaccination on the cells of the reticulo-endothelial system, but we have evidence from Exp. F to show that revaccination can reduce or intensify resistance according to whether the revaccination is carried out within the period during which the cells of the reticulo-endothelial system are in an irritable state or delayed until the activity of the cells has subsided. It is obvious that there is room here for a further histological investigation which might lead to a closer understanding of the principles underlying immunization against tuberculosis.

It is noteworthy that in our laboratory experiments the majority of vaccinated animals in which serious lesions were found were animals that had been exposed to infection at relatively short intervals after vaccination, and it is probable that there is the same cellular basis for our findings in Exp. L in which we found that if an animal was inoculated with vole bacilli during the first few weeks of a bovine infection, granulomatous changes were intensified, but if the inoculation of vole bacilli was delayed for several weeks, changes of such nature were reduced.

How far revaccination may act by a process of desensitization is an open question. We have certainly considerable evidence that even the small amounts of P.P.D. used for the intradermal tuberculin tests can reduce sensitivity, and there is a strong suggestion that frequent tuberculin testing can affect the progress of infection. It is possible therefore that the further enhancement of resistance following revaccination is largely the result of desensitization.

Although we have stressed the role of cellular allergy, we are far from suggesting that it is entirely responsible for the enhanced resistance which follows vaccination with the vole bacillus. There are differences between the lesions of vaccinated and control animals which cannot be explained by the presence of allergy. There is, for example, the difference in character between the calcium deposits found in vaccinated and unvaccinated animals respectively which would seem to indicate some biochemical alteration in the tissue fluids that might well affect the capacity for survival and growth of the bovine bacillus. The altered morphology of a number of the bacilli seen in lesions from vaccinated animals suggests that the bacilli are existing in an environment which is not suitable for their active growth.

If there is some difference in the biochemical make-up of the vaccinated animal, might it not arise from an unnatural stimulation of the functional activity of the endocrine glands? One of us (J.A.Y.) has made prolonged observations on the suprarenal glands of guinea-pigs infected with tuberculosis and has noted a close correlation of the survival periods of infected animals with the functional activity of the cells of the cortical zone. It may be that natural resistance is related to the capacity of the cortex to fulfil its natural function, a possibility that would afford a ready explanation for variations in family, race or species resistance on physiological grounds.

For economic reasons this is a subject that is less easy to study in cattle, but the sections of the suprarenal glands of the animals studied in Exp. N leave little room for doubt regarding the activity of the cells of the cortical zone which follows intravenous vaccination. We suggest that the reactions of the endocrine glands to tuberculous infection in other parts of the body offer a rich field for study.

There is one further point for consideration—the ultimate fate of a vaccinated animal. So far as cattle are concerned, provided that vaccination can result in an enhanced resistance of sufficient duration to protect animals during the period of time necessary for the cleaning up of a herd, the possible ultimate fate of a vaccinated animal which did become infected would be a matter of little consequence, for all vaccinated animals would be regarded as 'suspect' and would be destroyed as rapidly as they could be replaced with clean unvaccinated animals. It is, however, argued in favour of B.C.G. vaccination of human beings that vaccination gives the body time to develop resistance against the virulent tubercle bacillus. This may hold good for human beings in whom there is unquestionably a much greater capacity for spontaneous recovery from tuberculous infection than exists in cattle, but in none of our experiments on cattle have we found any evidence that the mechanism of resistance is rendered in the long run a more effective defence against the virulent tubercle bacillus as a result of vaccination. Indeed, what evidence we have is to the contrary, for in Exp. H the vaccinated animals that did become infected were, on average, more severely infected than the controls. Our figures are small and by themselves would have no statistical significance, but our interpretation of them is supported by the work of Watson (1934), who found that although younger animals that had been vaccinated with B.C.G. held an advantage over control animals, this advantage was lost as the vaccinated animals grew older and the lesions in these animals tended to be more severe than those of similarly aged controls.

We are forced to the opinion that although vaccination with the vole bacillus can produce an enhanced resistance to tuberculosis in cattle, this resistance is of a temporary nature and is unbalanced in that only some parts of the defence mechanism are stimulated to activity. It may be that they are overstimulated, and while we have demonstrated that it is impossible to get an adequate degree of protection with doses of the vole bacillus vaccine smaller than that which we have adopted as our standard dose, it might be that smaller doses would prove effective if used in combination with a vaccine of dead virulent bovine bacilli or suitable extracts of these bacilli. In this way it might be possible to attain a state of resistance which would justify the use of the term 'acquired immunity', a state of resistance in which the cellular and humoral factors are more evenly balanced, a state of resistance in which there is less chance of exhaustion following overstimulation.

IX. SUMMARY AND CONCLUSIONS

1. A series of experiments is described in which the efficacy of vaccination with the vole acid-fast bacillus against tuberculosis in cattle has been studied. Various methods of vaccination have been tried and intravenous inoculation has proved to be the most satisfactory.

2. A single intravenous inoculation of 5 mg. of vole bacilli can raise the resistance of cattle to infection with the bovine bacillus to a high degree, this resistance being evidenced by a complete absence of infection or by a marked diminution in the severity of lesions following exposure to artificial or natural infection.

3. The resistance takes time to develop, reaches its maximum between 6 and 18 months after vaccination and then slowly wanes. The resistance induced by a single inoculation is of insufficient duration in many animals to warrant the practice of single vaccination being used in the field except in special circumstances.

4. Revaccination can intensify resistance provided that it is delayed until the end of the fourth week. A long-term experiment is being carried out to determine if revaccination, in addition to intensifying the initial resistance, can also lengthen its duration.

5. The degree of resistance set up in cattle is correlated with the virulence of the vaccinating strain of vole bacillus for its natural host. This fact will necessitate that any strain used for vaccine purposes will have to be maintained at a high level of virulence by repeated passage through its native host.

6. Care is necessary in the selection of a strain of the vole bacillus for vaccine purposes, for we have encountered one strain, G 564, which can set up a fatal infection in calves. Nothing has been noted which would contra-indicate the use of strain LV 285.

7. The histopathological changes pathognomic of tuberculous infection which follow the intravenous inoculation of vole bacilli are mainly confined to the lungs and thoracic glands. They reach their maximum about the 28th day and have undergone resolution by the 84th day.

8. The changes which occur in the cells of the reticulo-endothelial system following vaccination have been described. These changes must be taken into consideration in deciding the optimal time and dosage for revaccination and a further study of them is advisable.

Our thanks are due to Prof. T. Dalling for his unfailing interest in our work and for much helpful advice, and also to Dr A. Q. Wells for supplying cultures of vole bacilli and for the loan of his multiple-puncture instrument. We are indebted to Dr V. D. Davies for painstaking examination of the joint lesions and to Dr J. R. M. Innes, who examined many histological sections of vole-inoculated animals and drew up a short report of which we have made much use. Mr V. L. Mastin was associated with us during part of the work and we gratefully acknowledge the help he gave. We also owe a deep debt of gratitude to our technical assistants, particularly Mr W. Nicholson and Mr C. Spurgeon, much of the routine bacteriological work being done by the former whilst the latter prepared the bulk of the histological sections. The local slaughterhouse officials of the Ministry of Food, particularly Mr Bacchus, the slaughterhouse manager, and Mr C. Rose, the foreman slaughterman, have been most co-operative over the arrangements for the slaughter of many animals. Our thanks are also due to the farmers who allowed us to expose experimental animals to natural infection on their farms, and to these and other farmers who have placed their herds at our disposal for the purpose of field trials.

The cost of these investigations was met mainly by grants from the Agricultural Research Council, but partly from the funds of the Institute of Animal Pathology.

REFERENCES

BAUER, W., BENNETT, G. A., MARBLE, A. & CLAFLIN, D. (1930). *J. Exp. Med.* **52**, 835.

BIRKHAUG, K. (1944). *Acta med. scand.* **117**, 274.

BUXTON, J. B. & GRIFFITH, A. S. (1931). *Lancet*, **1**, 393.

GRIFFITH, A. S. (1939). *J. Hyg., Camb.*, **39**, 154.

GRIFFITH, A. S. & DALLING, T. (1940). *J. Hyg., Camb.*, **40**, 673.

KLINGE, F. & VAUBEL, E. (1931). *Virchows Arch.* **281**, 701.

PAGEL, W. (1939). *J. Hyg., Camb.*, **39**, 254.

ROBB-SMITH, A. H. T. (1946). *M.R.C. Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 259.

ROSENTHAL, S. R. (1939). *Amer. Rev. Tuberc.* **39**, 128.

WATSON, E. A. (1934). *Proc. 12th Int. Vet. Congr.* **2**, 2.

WELLS, A. Q. (1937). *Lancet*, **1**, 1221.

WELLS, A. Q. (1946). *M.R.C. Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 259.

YOUNG, J. A. & PATERSON, J. S. (1947). *Lancet*, **1**, 707.

(*MS. received for publication* 6. XII. 48.—Ed.)