The Pathology and Immunology of Some Parasitic Infestations in Domestic Animals.

by

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This thesis describes the development, from the early laboratory-test stage, of the first vaccine to be successfully used on a wide scale against a parasitic helminth. The process utilises X-irradiation to attenuate the infective larvae.

The work is presented in four parts, followed by technical, bibliographical and tabular appendices. The first part is a discussion on both parasitic immunity and the application of X-irradiation to helminths. The second part describes experiments designed to test a vaccine against Dictyocaulus viviparus. The third part is a quantitative and qualitative study of D. viviparus larvae under the respective effects of X-irradiation, antibody and diethyl-carbamazine. The fourth part concerns the testing of a vaccine against Haemonchus contortus.

Part I contains a general introduction to parasitic immunity, with a brief indication of the importance of parasitism in both animals and man. This is followed by a description of:

(a) previous attempts by other workers to vaccinate against helminth diseases;

(b) the background of work behind the present method of vaccination.

Part I ends with a review of various applications of X-irradiation to helminths.

Part II is introduced by a small experiment designed to test the longevity of both first and third stage larvae of D. viviparus under a wide range of laboratory conditions. A short description of the parasite is included. Then follow the four experiments which form the bulk of this part. These were designed to test the effectiveness of the D. viviparus vaccine in various combinations of dose against a challenge spectrum ranging from a large single dose of infective larvae to a natural farm-pasture challenge. Approximately 1,250 calves were involved in these experiments.

Part III is mainly concerned with histo-pathological and quantitative assessments on the distribution and fate of irradiated larvae in the normal host and of normal larvae in the immune host. This work was done to study the mode of action of, and the host-reaction to, the vaccine against D. viviparus in calves. It was known that a dose of irradiated larvae did not produce a population of adult worms in the bronchi, and this experiment showed where the larvae were ceasing to develop.

The pathological basis of the transient clinical signs sometimes seen after vaccination is described, as well as the correlation of lesions and clinical signs observed in the immune animal under heavy challenge.

The last experiment in this part is a histopathological study of the pulmonary lesions produced by treating calves, in the prepatent or patent stage of parasitic bronchitis, with diethylcarbamazine. This drug inactivates or kills the parasite, and the host-reaction to the drug-disabled worm is interestingly similar to the reactions of the normal-calf lung to irradiated larvae and of the immune-calf lung to normal larvae. The lesions in the calves treated at patency were correlated with the clinical signs sometimes seen following such treatment.

Part IV. The previous parts dealt with the successful application of the larval irradiation technique to a parasite with an extensive migratory cycle. It was thought that this method might be less effective in parasites which undergo only a limited histotrophic phase - such as some of the gastro-intestinal nematodes. The experiments in this part indicated that a servicable degree of immunity could be induced in sheep against Haemonchus contortus.

Duncan Banfield

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Thesis submitted for the degree of Doctor of Philosophy in the Faculty of Medicine, The University of Glasgow.

To Carol and Robert.

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PART II

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PREFACE.

This thesis describes the development, from the early laboratory-test stage to the point of wide scale application in the field, of the first vaccine to be used against a parasitic helminth. The parasite is Dictyocaulus viviparus, the cause of bovine parasitic bronchitis, and the vaccinating process involves the use of infective larvae, partially inactivated by exposure to X-irradiation.

The thesis also describes in detail the fate in the bovine of the attenuated larvae, and describes the host reaction to them. The sequence of pathological events in immune animals subjected to challenge with normal infective larvae is given. Due to the similarity of these reactions to those which occur when normal larvae of this parasite are arrested in the lungs by use of diethylcarbamazine, an experiment is included in which this drug was used in both the prepatent and patent stages of parasitic bronchitis.

This is followed by a description of experiments in which the same technique of immunisation was applied to Haemonchus contortus in sheep. This parasite, in contrast to D. viviparus, has a minimal migratory cycle in the host, but a potentially useful immunity was nevertheless elicited.

The work is presented in four parts, with technical, bibliographical and tabular appendices.

Part I contains a general introduction to parasitic immunity, preceeded by a brief indication of the global importance of parasitic disease in both animals and man.

This is followed by a more specific description of:-

(a) previous attempts by other workers to vaccinate against helminth diseases;
(b) the background of work behind the original idea of vaccination, including other methods of inducing active and passive immunity.

Part I ends with a review of the previous application of X-irradiation to helminths by other workers.

Part II is introduced by a small experiment designed to test the longevity of both first and third stage larvae of D. viviparus under a wide range of laboratory conditions. A short morphological description of the parasite is included. Then follow the four experiments which form the bulk of this section. These were designed to test the efficacy of the D. viviparus vaccine in various combinations of dose against a challenge spectrum ranging from an artificial and relatively large single dose of fully virulent larvae to a completely natural farm pasture challenge. Altogether, approximately 1,250 calves were involved in these experiments.

Part III is mainly concerned with histo-pathological and quantitative assessments as to the distribution and fate of irradiated larvae in the normal host and of normal larvae in the immune host. This work was done to study the mode of action of, and the host's reaction to, the vaccine against D. viviparus in calves. It was known that a dose of irradiated larvae did not produce a population of adult worms in the bronchi, and this experiment showed where the larvae were ceasing to develop.

The pathological basis of the transient clinical signs sometimes seen after vaccination is described, as well as the correlation of lesions and clinical signs observed in the immune animal under heavy challenge. The last experiment in this part is a histo-pathological study of the pulmonary lesions produced by treating calves, in the prepatent or patent stage of parasitic bronchitis, with diethylcarbamazine. This drug has the effect of killing or severely inactivating the parasite, and the host's reaction to the drug-disabled worm is interestingly similar to the reactions of the normal-calf lung to irradiated larvae and of the immune-calf lung to normal larvae. The lesions in the calves treated at patency were correlated with the clinical signs sometimes seen following such treatment.

Part IV. The previous sections dealt with the successful application of the larval irradiation technique to a parasite with an extensive systemic migratory cycle. It was thought that this method might be less effective in parasites which undergo only a limited histotrophic phase - such as some of the gastro-intestinal nematodes. The experiments in this section were performed to find if resistance of sheep to Haemonchus contortus could be induced.

The experiments described were done by one team of workers. I carried out the parasitological techniques involved, supervised the general running of the experiments and undertook the irradiation of larvae. I co-operated with Dr. Jarrett in the pathological aspects of the work.

PART I

GENERAL INTRODUCTION.

Before considering the general field of immunity to parasites it is worth stating some facts about parasitic disease as a whole, in order to outline the problem. It is now widely accepted that by A.D. 2,000 the world's human population will have doubled. It is also accepted that the greater proportion of both humans and animals in less developed countries, suffers from hunger and malnutrition. In particular, the human consumption of livestock and livestock products is extremely low, thus the tendency is for the food consumed to be qualitatively poor as well as lower in quantity.

In 1947, Stoll published a table listing the incidence of human infection of the 25 major human parasites in the eight main geographical divisions of the world.

He estimated that over 1,000 million people were infected with either (or both) Ascaris lumbricoides or hookworm spp., two of the most prevalent nematode parasites of man. More recently (1957) he considered that, with minor exceptions, parasitic worms are as numerous as ever.

The problem of parasitism in the human is one mainly of under-developed countries, but the initial steps towards a higher living standard in the form of irrigation projects

may lead to a worsening of the situation as did happen in the Aswan province of Egypt, the Belgian Congo and in Southern Rhodesia. In these areas the incidence of Bilharziasis rose to between 10 and 40 times the previous levels after the establishment or extension of such irrigation schemes; this was due to the vast increase in the numbers of water snails which are the relevant intermediate hosts. Wright (1951) and Vogel (1958) also give heavy emphasis to parasitism as a world problem.

Similarly, on the veterinary side, advances in agrarian techniques, leading to greatly increased concentrations of stock on pastures, have facilitated the spread of parasitism, thus reducing production of milk, meat and wool and causing serious financial loss; in America this has been estimated at $ 227 million per annum and although the present author has not seen a similar estimate for this country Spedding (1956) indicates that it is 'substantial'. He has shown (1957 a,b) that what are considered as sub-clinical helminth burdens in sheep may be responsible for up to 30% reduction in liveweight gain, with further deleterious effects present in the lambs of parasitised, as opposed to normal, ewes. One cannot but be impressed by the statement of account of parasitic helminth diseases in the Animal Health Yearbook of F.A.O. (1958). In almost all countries of the world gastro-intestinal nematodiasis, intestinal taeniasis, fascioliasis and parasitic bronchitis are stated to be 'wide-spread throughout the country'.

Medical Science is sometimes accused of being blinded by self motivation in that it constantly attempts to lower infant mortality and to increase longevity in under-developed areas, without considering the altered nutritional requirements of such communities. Human parasitic disease is perhaps unique in that 'efficient' parasites tend to have a relatively high morbidity but a low mortality rate; their hosts are far less capable of work although they require the same amount of food - or possibly more, due to selective requirements on the part of the parasite.

It is thus evident that elimination of human and animal parasitism would not merely shift a problem from one branch of science to another but would be a valid contribution to world health.

SPECIFIC INTRODUCTION

Parasitic Immunity

Helminth parasites, because of their visibility to the naked eye, were the earliest pathogenic agents of man and animals to be recognised. Yet little attention has been paid, until comparatively recently, and with the exception of some individual scientists, to the practical aspects of their elimination by immunological methods.

Partly, this has been due to the concentration of effort among parasitologists on morphology, taxonomy and life cycles with comparatively little emphasis on either the pathogenesis or immunology of the relatively few species of medical or veterinary importance.

Immunisation in protozoan parasitology apparently began in ancient times in the middle East. There, it was the practice to immunise children against Leishmania tropica by rubbing onto the scarified skin in an unexposed part of the body the exudate from an active sore of an infected person.

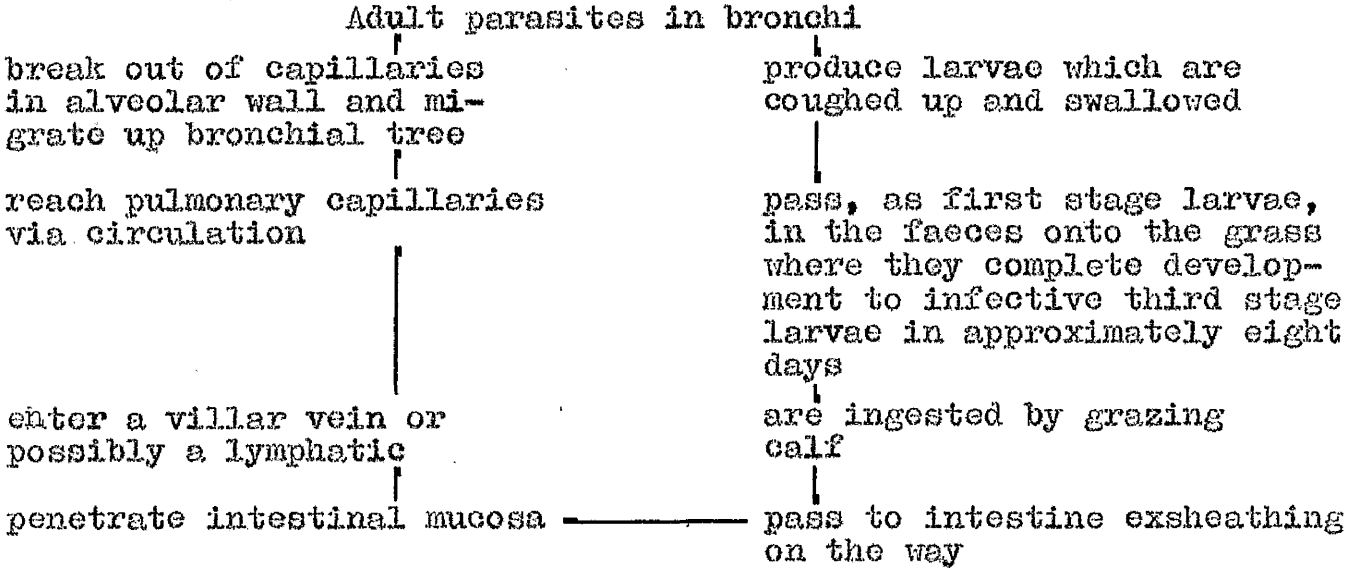
Natural immunity in the field of veterinary protozoology was observed in 1893 by Smith and Kelbourne, who found that cattle recovering from Texas Fever, caused by Babesia bigemina, were resistant to further attack.

The first recorded instance of experimentally acquired immunity to a helminth parasite is by Fujinama (1916).

He provided evidence, concluding with post mortems, that the horse could acquire immunity to Schistosoma japonicum.

That more and more workers are turning their attention to parasitic immunity is confirmed by the fact that seven reviews have appeared in the last 5 years (Stoll 1958; Soulsby 1957, 1958 1960, 1961; Stewart 1960; Urquhart, Jarrett & Mulligan 1962), whereas the previous 30 years produced only three (Taliaferro 1929, 1948; Culbertson 1941). These reviews give the general situation to date and are not summarised here.

D. viviparus is the parasite for which a vaccine utilising X-ray inactivated larvae was developed at Glasgow. Before describing the stages leading to that development it is worth describing the life cycle of the parasite and the pathogenesis of its associated disease, parasitic bronchitis, or 'husk'. The life c ycle of D. viviparus is comparatively simple in that no intermediate host is involved and it is best illustrated diagramatically.



The definitive site of the adult parasite is in the main and segmental bronchi and the pathogenic effects are confined to the lungs. These effects are complex and it is useful at this point to consider in moderate detail the disease process. This is relevant for two reasons:-

1) To provide a point of reference for the detailed description of the mode of action of, and host reaction to the vaccine, which follows in part III.

2) To illustrate the fact that once calves are showing clinical signs of parasitic bronchitis, it is usually too late to institute effective treatment. This underlines the obvious advantage that exists in preventing this frequently fatal disease.

As described before, (Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart 1960) the uncomplicated disease divides itself quite readily into four stages:-

1) Penetration by the larvae through the intestinal mucosa and their subsequent migration to the lungs. This phase is completed within days one to seven.
2) Prepatent stage. This occupies days seven to 25 and is the period during which the parasite is migrating within the lung to its definitive site.
3) Patent stage. This, by definition, is the time from the 25th to the 55th day, approximately, when the worms are mature and producing larvae.
4) Post-patent stage. The final period, from the 55th to the 70th day, at the end of the disease when there may be a flare-up of severe pulmonary symptoms.

1. The penetration phase is of comparatively little clinical or pathological importance in the fully susceptible host. The mechanism of penetration would no doubt be of considerable interest and the probable importance of this site in the immune animal is intriguing. However, when one considers the enormous mass of the intestinal tract of a calf compared to as many as a million larvae, one can appreciate the difficulties of finding even one lesion in hundreds of sections. The experimental cost/importance of result ratio is too high to give this aspect any priority although it would be satisfying to elucidate.

2. The Prepatent Phase is very important clinically. The main underlying lesion is blockage of small bronchi and bronchioles by the eosinophil exudate produced in response to the small developing larvae migrating up the bronchi. It is important to realise the difference between this lesion and the pneumonia produced later by adult worms. The blocked bronchi are not permanently damaged but the obstruction to air passing in, results in collapse of the alveoli distal to the block; as the air in these alveoli is absorbed into the blood and as no more takes its place, the alveoli collapse and are lost as functioning units. If the blockage is removed, these alveoli can return quickly to normal function.

The clinical picture depends on the size of the infecting dose as this determines the extent of bronchial involvement and the speed of development of the severely ill case. In heavy infections, there is often a dramatic change in the clinical signs towards the end of the second week; in the course of four or five days the respiratory rate doubles, reaching 70-80 per minute and coughing becomes marked. Towards the end of the third week, as the primary bronchial lesions regress, there is often a noticeable deceleration in the progress of the disease, although the animals are still ill and deterioration in condition continues.

In the third week, death may occur in heavily infected animals; this is usually associated with the development of acute complications. (see below - complications).

If an animal is autopsied in the prepatent phase, worms may not be seen; in the later phase they are visible, but microscopic examination by direct smear of bronchial mucus is necessary if they are to be found during the second and early third weeks. Many acute pneumonias are not diagnosed as husk because of this fact.

3. The Patent Phase is associated with the presence of adults in the bronchi, and while these cause much exudation into, and blocking of, air passages, a great deal of the clinical effect is caused by the primary parasitic pneumonia in which macrophages and giant cells engulfing aspirated eggs and newly hatched larvae, consolidate the lung lobules. In most cases it is this pneumonia and not secondary bacterial complications which produces the typical picture at autopsy.

The respiratory rate may become faster than the pulse rate, rising to over 100 per minute, coughing becomes more frequent, appetite is lost and growth ceases. On auscultation harsh bronchial breathing is evident and this may be augmented by emphysematous crackling or fluid sounds as complications supervene in serious cases. Dehydration may become very marked. During this period many animals die; in the survivors there is a slow regression of symptoms after the fifth week.

During this central stage of the disease the adult worms are laying eggs which hatch in the lungs or intestine and may be detected in faeces as larvae. Counts usually range from 100 to 500 per gram but higher levels are not infrequent. At autopsy the adult worms are easily seen in the trachea and bronchi and range in number from a few hundred to several thousand.

4. The Postpatent Phase. In this phase the majority of animals gradually recover; the respiratory rate steadily decreases, coughing lessens and weight increase is resumed. Complete recovery, however, is apparent only after a lapse of several months.

In approximately a quarter of those animals which were severely affected earlier the postpatent phase is different from the above and is characterised by a sudden exacerbation of dyspnoea, during the seventh or eighth weeks, which is often fatal.

This is caused by the spread of a lesion the aetiology of which is not known at present. During the patent phase, many alveoli show a swelling and proliferation of their epithelium so that instead of a thin membrane, easily permeable to gases, a thickened gland-like wall develops. In the postpatent phase, this alveolar epithelialisation spreads to involve whole lobes. This proliferative pneumonia has serious clinical consequences and by the time it has become widespread, all, or almost all, of the worms have been shed from the lungs, so the findings at autopsy are not those associated with patent parasitic bronchitis. At post mortem one finds large areas of red or pink rubbery lung which are not friable and consolidated, and which on palpation still show some crepitance.

REINFECTION PHENOMENA

A section of Part III is devoted to the experimental elucidation of the lung reaction to reinfection, but for completeness an anticipatory paragraph is given here.

Animals which have become immune to D. viviparus may show symptoms after reinfection; the magnitude of these varies with the numbers of larvae ingested. When these larvae penetrate into the body, a number may reach the lungs and be killed in small bronchioles; these dead larvae cause quite a marked reaction and if there are many of them, an obvious and sometimes even fatal clinical condition results.

The dead larvae cause a proliferation of epithelium and the development of fibrous and lymphoid nodules around the air passages. Usually, however, only mild coughing is caused, the lesion resolves quickly and the animal returns to normal in a short time. This process may be seen when vaccinated animals are first exposed to heavy infection. It is also frequently seen in outbreaks in adult cattle where, presumably, immunity has waned and a moderately large number of larvae reach the lungs before being killed. This may produce the sudden onset of severe and persistent coughing in a whole herd of adults with a number showing signs of severe pneumonia. Naturally larvae are not found in the faeces of these cases, although all of the other clinical signs of husk may be present.

COMPLICATIONS.

These may be classified as:-

a) occurring during the course of the disease:

(1) Pulmonary oedema.
(2) Emphysema.
(3) Secondary bacterial infection

b) occurring as sequelae:

(1) Bronchiectasis.
(2) Pulmonary fibrosis.

Pulmonary Oedema. This is one of the most serious complications and is the frequent cause of sudden clinical deterioration; the onset of heart failure in a dyspnoeic animal is the main cause.

The alveoli become filled with transuded fluid and this, together with the laying down of protein-containing 'hyaline membranes' on the alveolar walls, causes a serious diminution in the already reduced gas-exchanging area of the lungs. This augments the parasitic pneumonia and leads to further anoxia.

These changes are accompanied by a marked decrease in the volume or strength of the arterial pulse. Only occasionally is it possible to detect fluid râles on auscultation.

Emphysema. This frequent complication is usually of the interstitial type. The lobules of the bovine lung are separated by loose fibrous tissue and air may enter this from ruptured bronchioles. The air travels along the septa and may increase in volume to form large bullae. The lung is then incapable of proper contraction and respiratory distress becomes more marked.

The advent of emphysema is readily recognised clinically by the distinct and widespread crackling sounds heard on auscultation. Infrequently the emphysema may extend via the thoracic inlet to the subcutaneous tissues over the thorax and abdomen.

Secondary Bacterial Infection. This is less common than is generally supposed although it is frequent enough to justify the use of antibiotics in severe husk cases. The green pus often seen in the lungs of parasitic bronchitis cases is composed of eosinophil leukocytes exuded in response to the parasites and not to bacteria.

However, acute interstitial pneumonia may be superimposed and the occurrence of this complication may be suspected when moderately tachypnoeic cases suddenly become anorexic and very dull. Recumbency and death may ensue within 48 hours.

Bronchiectasis

After some husk infections, the bronchi of a lobe fail to clear themselves of exudates and these become the nidus of low grade chronic bacterial infections. Slowly the bronchi dilate and the lobules collapse until the lobe becomes transformed into several tubes of viscid pus. This is a common cause of the poorly thriving stunted animal with a persistent cough, and economic therapy is not possible.

Pulmonary fibrosis

This may remain after an infection in the intra- or peribronchial sites or in the septa, but while it may give rise to a mild chronic cough, it is not generally of much clinical significance.

From this brief account it is apparent that the disease processes initiated by D. viviparus are complex; furthermore in one group of calves the various phases of the disease may be encountered simultaneously in different animals. In general, however, the appearance and severity of clinical signs are determined by the numbers of larvae ingested and the immune status of the host.

The intake of larvae is very difficult to control effectively by grazing techniques and the solution to the problem of parasitic bronchitis depends on vaccination or specific therapy.

Between 1952 and 1954 a group of workers (Jarrett, Jennings, McIntyre, Mulligan and Urquhart) commenced an investigation into parasitic bronchitis. An early factor was the necessity of differentiating clinical parasitic bronchitis from another calf proliferative pneumonia characterised by an expanding peribronchial lymphoid hyperplasia and which was not caused by the lungworm, although it often complicated a husk pneumonia.

In 1954 the group published a number of basic facts concerning parasitic bronchitis, viz:

1) Age and seasonal incidence figures from both a knacker-yard and a farm survey;

2) Epidemiology including longevity of larvae on pasture, infection build-up and the course of the disease on a herd basis;

3) The correlation of symptoms and faecal larval counts;

4) A pathological description of some of the main pulmonary lesions such as alveolar epithelialisation and hyaline membrane formation following pulmonary oedema and leading to cardiac anoxia and heart failure.

This was followed by a more detailed investigation of the epidemiology with special reference to the over-wintering of larvae on pasture (Jarrett, McIntyre, Urquhart and Bell, 1955a) and a further more extensive knacker-yard survey also aimed at clarifying the method of survival of the parasite over the winter (Cunningham, Jarrett, McIntyre and Urquhart 1956). The descriptive pathological survey was later followed by a detailed study of the pathogenesis following experimental infection with moderate and high larval doses and serial killing of calves (Jarrett, McIntyre and Urquhart 1957). This work reproduced all the lesions seen in the naturally occurring disease and explained many hitherto puzzling features.

It was at this time becoming rapidly apparent to these workers that the traditional methods of attack on parasitic disease, viz; prevention, by removing access to infective larvae, and medicinal treatment of established infections were of little practical use; control of the disease on the farm by husbandry methods was either prohibitively expensive or impractical or both. In spite of the claims to efficacy of the many remedies then current, no drugs produced at this period had been proved to have any effect on the establishment of infection.

There was evidence that a strong immunity could be acquired under field conditions (Wetzel 1948; Taylor 1951; Jarrett, McIntyre and Urquhart, 1954) although little work

had been done on experimental animals to explore the nature of this resistance nor had it been quantitated in any way. Two experiments were set up in order to investigate this point (Jarrett, Jennings, McIntyre, Mulligan, Thomas & Urquhart 1959). In the first, 10 calves received a total of 20,000 infective larvae each, in three doses of increasing magnitude, at intervals of over five months. Although the calves were killed at a time which would otherwise have been the height of patency of the third infection, seven of them showed no lung-worms at autopsy. A second experiment on similar lines, although with a different dosing regime, confirmed that an infection with D. viviparus confers a high degree of immunity to subsequent reinfection.

A corollary of these findings was to attempt passive immunisation (Jarrett, Jennings, McIntyre, Mulligan & Urquhart, 1955). Serum was obtained from recovered field cases which had been subjected to experimental reinfection in order to boost the antibody titres. Seven of 14 parasite-free calves were given a total of 1,500 ml. of the immune globulin preparation intra-peritoneally and then challenged with 4,000 infective larvae. The maximum daily mean respiratory rate of the immunised group was 55 per minute as against 85 in the controls and the former had a group lungworm burden less than 5% of that of the controls - 37.4 as against 786.

The above findings suggested that it might be possible to protect animals against parasitic bronchitis by means of active immunisation other than natural or experimental infection. An experiment was performed (Jarrett, Jennings, McIntyre, Mulligan and Urquhart 1960a) in which a total of 62 calves received one or two doses of a freeze-dried preparation of D. viviparus worms collected from the bronchi of infected calves. This adjuvanted whole-worm antigen was injected intramuscularly. A further 50 non-immunised calves served as controls. Viewed as a vaccinating procedure the results were quantitatively disappointing although two vaccinated groups of 11 calves each showed a statistically significant 50% reduction in 'take'. Qualitatively, however, the pulmonary lesions in the immunised groups were both different and interesting; the series of lesions found showed an exagerated response to the parasites on the part of the host, associated with marked eosinophil infiltration and giant-cell phagocytosis. The main conclusions from the experiments with whole worm vaccine immunisation were that as a method of protection it seemed to be of little practical value. This was due partly to its comparatively low efficacy and partly to the difficulty of obtaining adequate numbers of adult lungworms to produce a vaccine on a wider scale.

It was realised that the problem of producing immunity would probably best be solved by getting actively metabolising but inactivated worms into macromolecular contact

with the immune apparatus of the calves. Ionising radiations from an X-ray source were chosen for the attempted inactivation and before detailing this aspect of the work it is pertinent to digress on to the general subject of parasites and ionising radiation.

As applied to the field of parasitology ionising radiation from an X-ray, cobalt-60, or radium source has been used in three main ways:-

1) To render potential butcher meat parasitically sterile for human consumption.

2) To study the biological effects resulting from irradiation.
   (a) whether radio-resistant strains could be produced,
   (b) whether recovery from irradiation effects could be induced,
   (c) whether stability of morphological characters could be assessed by studying type-frequency of mutations produced.
   (d) whether any constant morphological effect is produced.

3) To produce active immunity and to separate different factors in the immune response.

Each of these will now be considered briefly.

1) Sterilisation of Meat.

In 1916 Tyzzer and Honeij discovered that X-rays had a deleterious effect on Trichinella spiralis larvae and since then much work has been done on this aspect of trichinosis, although it was not until 1951 that the thread was picked

up by Alicata, who performed a series of experiments. On the subject of meat sterilisation he concluded that, although X-irradiation in sufficient quantity was effective and dependable in destroying trichinae in the meat, the procedure was of limited practical value due to the high doses (500,000 roentgens) required and to the difficulty of large scale irradiation. However, Gould, Van Dyke and Gomberg (1953), irradiating trichinous pork in slices less than two centimetres thick, found 25,000 roentgens to be effective and saw promise in commercial application of the method. Gould and Gomberg (1953) also noted that larvae encysted in meat were more resistant than those in vitro.

Gould, Gomberg and Bethell (1953), using chemical dosimeters embedded in trichinous pork, obtained accurate dose measurements and tested the efficacy of their various radiation levels by feeding 5,000 larvae from irradiated meat to rats compared with 5,000 normal larvae to the controls. 12,000 roentgens from a cobalt-60 source allowed some females still to develop but all were completely sterile. Magath and Thompson (1955), discussing the effects of T. spiralis irradiation on immunity from the public health aspect, considered that pork irradiation would reduce the acquisition of immunity in the consumers and increase the clinical manifestations if they contracted the disease elsewhere.

2) Biological Effects.

a) Radio-resistant strains. Gould, Gomberg, Bethell, Villella and Hertz (1955), in their series of papers on various aspects of T. spiralis, considered the chance of such strains arising, bearing in mind the possible mutagenic effects of ionising radiations. However, progeny produced from T.spiralis larvae irradiated at the sublethal and reproductive-permissive dose of 10,000 roentgens showed no evidence of resistance to irradiation. Alicata (1956) made similar observations in more detailed experiments on the same parasite by tabulating the percentage sterility among adult female T. spiralis in six generations following irradiation of the larval stage in each generation with 5,000 roentgens of X-rays. He concluded from his data that no resistant strain developed and that the ability or failure of individual egg cells to develop probably depends on the amount of radiation received per egg. Approaching radio-resistant from another aspect, Shikhobalova et al (1958) studied the variation in sensitivity to irradiation of the different stages of embryogenesis of three species of ascarids. A. lumbricoides, A. suum and A. galli were all X-irradiated at stages ranging from the blastomere to the infective larva. They found eggs to be more susceptible than larvae, especially at the blastula, morula and early gastrula phases; this assessment was made on the number of infective larvae produced and their ability, which was always reduced, to migrate within the host. That postembryonic development was affected by irradiation, was shown by increases in the female:male ratio.

Although we have not tried specifically to find radio-resistant strains, we have not seen any evidence of their existence. While, in the widespread use of an irradiated vaccine, this is a factor that has to be considered, a radio-resistant strain must produce progeny which are going to be themselves irradiated in order for such a mutation to 'benefit' the parasite. As there is, at the factory which produces the D. viviparus vaccine, no larval feedback from sources outside their own larval production unit, the problem does not arise there.

b) Possible recovery from irradiation.

The possibility that parasites might recover from the effects of ionising radiation, particularly if they are stored at temperatures approaching 0°C., was first suggested by Cook (1939). He reported that X-irradiated eggs of Parascaris equorum, kept at 5°C. for a long period, showed remarkable recovery from the radiation effects, as measured by later hatchability. However, Bachofer and Pahl (1955), having studied the influence of extended low temperature treatments on the recovery of X-irradiated A. lumbricoides var suis eggs, concluded that such irradiation causes the first cleavage to be delayed, which delay is not altered by keeping the eggs at 0°C. or 5°C. for periods of up to 35 weeks before incubating at 30°C., compared to controls.

Alicata (1956) irradiated T. spiralis larvae, then refrigerated them for one month after which they were fed to susceptible rats in a controlled experiment.

He found no recovery from irradiation effects to have taken place. Also, he found that performing the irradiation at 0°C. produced the same effects on T. spiralis larvae as irradiation at 'room temperature'.

Although performed in a different biological context, the work of Weiss (1960) on the effects of hypothermia and hypoxia on the sensitivity of HeLa cells to X-rays is interesting and possibly relevant. Using an in vitro cell system he was able to observe these two factors separately and he concluded that while hypoxia did produce a definite decrease in radiosensitivity, hypothermia led to no detectable change. Poynter (personal communication 1962), who has had considerable experience in working with X-irradiated D. viviparus larvae, does not consider that 'recovery' occurs. Each time that larvae of D. viviparus, H. contortus or T. colubriformis were irradiated for use in experiments recorded in this thesis it was considered important to dose the experimental animals as soon as possible after irradiation to avoid the variables introduced by time-lag factors. Irradiated D. viviparus larvae probably do not have a long enough 'shelf-life' for the possibility of radiation recovery to be a hazard.

c) Morphological stability.

Schiller (1957) introduced to parasitology a novel application of X-irradiation which he utilised to facilitate the study of morphological variations. Eggs of Hymenolepis nana were given a range from 5,000 to 40,000 roentgens and fed to mice.

The yield of mutations was directly proportional to the number of roentgens and Schiller considered this an important aid to taxonomists in evaluating the relative stability of given morphological characters.

d) Morphological effects.

Several workers have subjected parasites to arbitary doses of X-irradiation, have dosed the treated larvae to susceptible hosts and have examined the resulting worm population. Most workers including ourselves, have been impressed by the comparative radio-resistance of the female worm, which can survive for a period following doses of irradiation which eliminate 90% of the corresponding males. This will be commented on further in sections III and IV.

Alicata (1951), in an informative article on X-irradiation of T. spiralis, noted that the females, although surviving, show variously:- stunted growth, cuticular thickening, inability of eggs to cleave in utero and ovarian degeneration. Katz (1956) utilised X- and cobalt-60-gamma irradiation of sperm and/or ova to induce successive free living generations in Strongyloides papillosus in which this phenomenon does not appear to occur naturally. Although theoretically such gynogenetic and/or androgenetic induction ought to have been possible in view of the triploidy observed in the worm, the efforts were unsuccessful. However, differing male and female susceptibilities were noticed in that the dominant lethal effect on larval production was observed with males given 20,000 roentgens of gamma rays from cobalt-60 but not until 40,000 roentgens in the case of females.

Ciorda and Bizzell (1960) subjected T. axei to high intensity X-irradiation and observed that no males developed, in rabbits, above 60,000 roentgens whereas the dosage required to inhibit development of the females was 90,000 roentgens. Similarly, Riek and Keith (1960) found that the effect of 20,000 roentgens of X-rays on infective larvae of Oesophagostomum radiatum dosed to calves was to establish a predominantly female population.

3) Production and investigation of the immune response following dosing with irradiated larvae.

The work described under this heading has all been performed using T. spiralis, infections of which consist of a primary intestinal phase and a secondary muscle invasion by the progeny. Once a strong resistance to reinfection had been demonstrated (Ducas 1921; McCoy 1931; Levin and Evans 1942), the main problem was whether the host resistance to reinfection was elicited during the phase of intestinal mucosal penetration by adult trichinae, or as a result of dissemination of larvae into the skeletal musculature, or a combination of both.

Tyzzer and Honeij (1916), Schwartz (1921) and Semrad (1937) irradiated, with radium or X-rays, trichina larvae encysted in muscle and fed the treated material to mice or rats, but the first causal relation between irradiated parasitic larvae and the production of host resistance was demonstrated by Levin and Evans (1942), although in the previous year Waxler and Herrick had published a communication on immunisation against coccidiosis in poultry by the use of X-irradiated oocysts.

Working with reproducible infections of T. spiralis in rats, and finely quantitating the dose of X-irradiation used, Levin and Evans (1942) produced morphologically mature but reproductively sterile intestinal populations. These induced 90 to 99% protection to reinfection with normal larvae as estimated by the number of encysted larvae recovered from the muscle of dosed rats compared to appropriate controls. Since this high degree of protection was obtained by an uncomplicated intestinal infection it was concluded that the origin of the mechanism of host resistance to T. spiralis was 'in the intestine'.

Levin and Evans ended their discussion of the general application of irradiation in parasitic immunity by indicating that. "The suggestions..... are made in the hope that others will take up this work. The use of roentgen radiation as a tool for the study of host resistance to parasitic infections is urged".

Alicata (1951) studied a series of effects of X-irradiation on T. spiralis and established useful background data. Hendricks (1952) and Gould, Gomberg, Bethell, Villella and Hertz (1955a) reopened the question of active parasitic immunity. The latter group had been working on problems associated with X-irradiation and T. spiralis for several years and in 1955 published results of experiments confirming the observations of Levin and Evans that suitably irradiated larvae, dosed to rats, produced a sterile intestinal population and an immunity to reinfection.

The immune response was further calibrated by Zaiman, Stoney, Rubel and Headley (1955) using pairs of parabiotic rats. In particular they immunised one 'twin' with X-irradiated trichinae larvae and challenged the pair with normal larvae, together with control pairs of rats. The 'uninfected' twin showed higher resistance than the control pair in each case but not as high as the 'infected' twin. The duration of immunity in such vaccinated pairs was found to be five months (Zaiman, Stoney and Headley, 1955).

In order to define the critical irradiation limits for immunisation, Zaiman and co-workers (1961) X-irradiated T.spiralis larvae over a range of 8,000 to 20,000 roentgens and gave them in doses of 500 each to 265 rats in 11 groups. It is of interest that the degree of protection induced by larvae given 8,000 or 12,000 roentgens was greater than that resulting from the same number of normal larvae.

Sadun, Norman and Brooke (1956) extended the study on the location of immunity by investigating the antibody response in rabbits to both phases of T. spiralis; the uncomplicated intestinal phase they produced with irradiated larvae, the muscle phase alone with extra-intestinal larval innoculations. They wished to find whether either phase independently could elicit a detectable rise in complement-fixing or flocculating antibody. They observed that the muscle phase alone led to the production of antibody in relatively high titres.

Continuing along similar lines, Kim (1956) X-irradiated T. spiralis at close intervals of under 2,000 roentgens to eliminate one or more stages of the life-cycle which he divided into pre-adults, adults and 'larvae from the time of release to encystment'. The migrating and encysting larvae did not appear, by a process of elimination, to contribute to the total immunity, as measured by controlled challenge experiments and serology, whereas adults and, especially, pre-adults seemed to be responsible for the immune stimulus.

Finally, Larsh, Race and Goulson (1959), in a correlative histopathological study on irradiated T. spiralis larvae in mice, concluded that the pre-adult stage alone can produce a demonstrable immunity which is associated with the presence of serum antibody and a characteristic inflammatory response in the small intestine.

The use of ionising radiations by various workers has, therefore, proved a useful method of inducing active immunity and of analysing the host response to a complex life cycle, in the case of T. spiralis.

Following their limited success with the adjuvanted whole-worm vaccine (loc. cit.), Jarrett, Jennings, McIntyre, Mulligan and Urquhart considered that a more effective alternative might be to X-irradiate D. viviparus larvae to investigate whether these attenuated forms were capable of inducing immunity. A pilot experiment established that the radiation dosage for satisfactory attenuation lay between 20,000 and 60,000 roentgens.

An experiment was then performed (Jarrett, Jennings, McIntyre, Mulligan and Urquhart, 1960b) in which three groups of calves respectively received a single dose of 4,000 larvae irradiated at 20,000, 40,000 and 60,000 roentgens. Fifty days later the three groups, together with untreated controls, were challenged with 4,000 normal larvae and all were killed after a further 35 days. The groups receiving larvae irradiated at the two lower levels showed both a very reduced mean 'take' of approximately 15 worms and minimal pulmonary consolidation compared to the controls, whose lungs were markedly consolidated and contained over 1,000 worms. The 60,000 roentgen group was very similar to the controls in worm burden and pulmonary consolidation, indicating that at this level the larvae were over-irradiated and had failed to mature sufficiently to reach the immunising site or stage, or both.

In the same experiment a further three groups of calves had received, on day One, single doses of 4,000 larvae from the respectively irradiated batches, together with controls receiving 4,000 normal larvae. These calves were killed at the height of patency of the controls to assess the pathogenicity of the attenuated larvae. The three irradiation groups showed minimal or absent pulmonary consolidation and had mean worm counts of four, two and zero worms, respectively.

Finally, a group of calves given an immunising dose of 4,000 normal larvae had a mean of only 11 worms after the challenging infection of 4,000 larvae; quantitatively this protection was of a similar order to the lower two irradiation groups but

qualitatively the pulmonary consolidation from the immunising dose was severe.

Thus the use of X-irradiation appeared to be a promising method of attenuating D. viviparus so that it remained immunogenic without being pathogenic.

A series of experiments was then performed to elaborate on the vaccination procedure and to test the immunity under a variety of challenge conditions. These experiments are described in the next Part of this thesis.

PART II

PART II

Section I

Longevity experiments on Dictyocaulus viviparus

Before a large scale project involving the culture, harvesting, irradiation, counting, dosing and probable storage of the larvae of D. viviparus could be undertaken, it was important to carry out a preliminary investigation into some of the biochemical aspects, especially those relating to larval storage.

The provision for experimental purposes, of adequate numbers of larvae from what is a comparatively non-prolific parasite, involved three phases of activity.

a) culture calves - their larval dose and type of feeding

b) larvae-containing faeces - method of collection; culture and harvest

c) the separated third-stage larvae - their storage and laboratory handling

The procedures under categories a) and b) were already established at Glasgow and are briefly described later. It was, however, thought necessary to investigate methods of storing the larvae, and the main factors considered were:-

1. Whether larvae were best stored, unharvested, in faeces and if so at what combination of stage (first or third) and temperature (-20°C: 0 to 4°C.: 27°C.: 37°C.) ?

2. At what combination of one of eight different media (including

a tap water control) with one of three temperatures, (0 to 4°C.: 27°C.: 37°C.) did harvested third stage larvae survive longest?

Experiment 1. Longevity tests on first and third stage larvae stored unharvested in faeces under various conditions.

Larvae-containing faeces obtained from culture calves was bulked and thoroughly mixed. This was done on two occasions with seven days' interval between each so that, on the eighth day, there were two batches of faeces containing third and first stage larvae respectively.

Forty-eight 1 gm. samples were removed from each of the two batches and placed in 96 glass bottles of 30 ml. capacity. The 48 first stage samples were divided into three groups of 16, - A,B and C. Group A consisted of moist faeces only. Group B was similar to A with the addition of 0.2 ml. of 1:10,000 Merthiolate, which was stirred into each bottle to control fungal growth. Group C was also similar to A, with the addition of 100 units of penicillin and 100 mg. of streptomycin to each bottle to control bacterial growth.

The 48 third stage samples were similarly divided and treated. This resulted in altogether six groups of 16 samples; each 16 was subdivided into four groups of four each, for incubation at four different temperatures; -20°C.; 0 to 4°C.; 27°C. and 30°C.

Thus, the effect of the various conditions was tested on the two (first and third) larval stages, although as expected the first stage larvae were found to develop to the third stage after a week, in those samples wherein they remained alive. The samples were examined at approximately weekly intervals and the observations made are recorded in Tables I and II.

The main conclusion drawn from this part of the experiment was that the storage of larvae in the faeces was not a practical proposition.

TABLE I To investigate the feasibility of larval storage in faeces. All numbers expressed as percentage of larvae still alive.

		First stage larvae stored			
		-20°C.	0 to 4°C.	27°C.	37°C.
Examined at five days	A	0%	100%	50% (now 3rd)	0%
	B	0%	100%	0%	0%
	C	0%	100%	50% (now 3rd)	0%
Examined at 11 days. First stage were now third stage.	A	0%	10%	0%	0%
	B	0%	45%	0%	0%
	C	0%	45%	0%	0%
Examined at 21 days	A	0%	15%	0%	0% (decomposed)
	B	0%	33%	0%	0% (decomposed)
	C	0%	33%	0%	0% (decomposed)
Examined at 28 days	A	0%	25%	0%	0% (decomposed)
	B	0%	66%	0%	0% (decomposed)
	C	0%	66%	0%	0% (decomposed)

All bottles 'A' contained 1 gm. faeces with no added preservative.

All bottles 'B' contained 1 gm. faeces with 0.2 ml. of 1:10,000 Merthiolate.

All bottles 'C' contained 1 gm. faeces with 100 units penicillin + 100 mg. streptomycin.

TABLE II. To investigate the feasibility of larval storage in faeces.
All numbers expressed as percentages of larvae still alive.

		Third stage larvae stored			
		-20°C.	0 to 4°C.	27°C.	37°C.
Examined at five days	A	71%	90%	90%	40%
	B	66%	70%	66%	0%
	C	60%	100%	70%	0%
Examined at 11 days First stage were now third stage	A	100%(very sluggish)	60%	0%	0%(very decomposed
	B	50%	66%	0%(very decomposed)	0%(very decomposed
	C	100%	100%	0%	0%(very decomposed
Examined at 21 days	A	0%	66%	0%	0% (decomposed
	B	0%	20%	0%	0% (decomposed
	C	0%	50%	0%	0% (decomposed
Examined at 28 days	A	0%	15%	0%	0% (decomposed
	B	0%	20%	0%	0% (decomposed
	C	0%	40%	0%	0% (decomposed

All bottles 'A' contained 1 gm. faeces with no added preservative.

All bottles 'B' contained 1 gm. faeces with 0.2 ml. of 1: 10,000 Merthiolate.

All bottles 'C' contained 1 gm. faeces with 100 units Penicillin + 100 mg. streptomycin.

Experiment 2: The determination of the optimum medium for the storage of separated third stage larvae.

Larvae-containing faeces was collected from infected calves and cultured and harvested by the usual technique. The resulting larval suspension was diluted to contain 2,000 larvae per ml. and was then mixed with various media in the proportion of 0.25 ml. suspension to 4.75 ml. of medium. Strict sterile precautions were observed in the making up of all media, of which seven solutions were tested against a Glasgow tap water control at three temperature levels:
0 to 4°C., 27°C. and 30°C. Only third stage larvae were used in this experiment.

The media were as follows:-

1. Glasgow tap water as control.
2. Glasgow tap water + 100 units of penicillin per ml. + 100 gm. streptomycin per ml.
3. Phosphate buffer, pH 7. (Made up of 50 ml. of 0.2 m $KHPO_4$ + 29.63 ml. of 0.2 n NaOH used without further dilution).
4. Tyrode's solution.
5. Earle's solution. (treated with CO_2 before use until producing yellow colour with phenol red).
6. Earle's solution, containing 15% pre-coloestral serum).
7. Earle's solution, containing 0.6%lactalbumin hydrolysate.
8. Earle's solution, containing 0.6% lactalbumin hydrolysate + 0.6% yeast extract.

Twelve bottles were prepared, as detailed above, from each medium including the control and 0.25 ml. of the larval suspension was added to each. There was a total of 96 larval cultures, on which examinations were carried out on the fifth, 12th, 19th and 24th days after setting up the experiment.

Larvae which had been stored at 0 to 4°C. were allowed to stand on the bench, at approximately 17°C., for one hour before counting. The results are listed in Table III.

TABLE III. To determine the optimum medium for the storage of harvested third stage larvae.

		MEDIUM		
		Tap water	Tap water + Pen. & Strep.	Phosphate buffer
five days	0 to 4°C.	100%	90%	100%
	27°C.	100% 10%*	86% 10%*	100%
	37°C.	43% 19%*	52% 24%*	87% 19%*
12 days	0 to 4°C.	91%	93%	80%
	27°C.	10%	40% 10%*	10% 6%*
	37°C.	0%	0%	0%
19 days	0 to 4°C.	96%	85% 25%*	100% 45%*
	27°C.	29%	18%	0%
	37°C.			
24 days	0 to 4°C.	88% 12.5%*	100% 8%*	81% 26%*

M E D I U M

Tyrode's solution	Earle's solution	Earle's soln. + precolost serum.	Earle's + Lactalbumin hydrolysate.	Earle's + Yeast + Lactalbumin.
97% 2%*	98% 15%*	100% 11%*	0%	0%
48% 22%*	69% 14%*	0% These had a heavy precipitate	0%	0%
47% 16%*	85% 8%*	0% These had a heavy precipitate	0%	0%
88%	93%	100%	57%	85%
10%	14%	0%	0%	0% (very decomposed)
0%	0%	0%	0% (Very decomposed)	0%
94%	100% 16%*	0%	0%	0%
35%	0%	0%	0%	0%
92% 32%*	100%	0%	0%	0%

* = Coiled, and probably of lowered infectivity.

The conclusion of this part of the experiment was that the first five of the solutions under experiment all seemed capable of maintaining third stage D. viviparus larvae, within the range of 0 to 4°C. as the optimum storage temperature.

It was also thought useful at this stage to make a brief morphological study of Dictyocaulus viviparus.

Eggs. These were selected in two ways:

1) from the middle of the uterus of gravid female worms

2) from bronchial mucus from the freshly opened lungs of recently killed culture calves. Once collected, the eggs were washed in normal saline and examined in a drop of saline. Prior to examination, the slides were kept at 37°C. on a hot plate. The egg wall consists of an inner vitelline membrane and an outer elastic membrane. The larva inside each egg could usually be seen to move, sometimes very vigorously. The mean egg length was 82.12 ± 2.01 microns. The mean breadth was 47.85 ± 1.23 microns. These means were obtained from counts on 300 eggs from five worms from each of six calves.

First stage larvae. Most of the body of first stage larvae is occupied by coarse granules, beginning from 80 to 105 microns behind the head to 40 microns from the tip of the tail. The mean length of first stage larvae was 352.5 ± 16.4 microns. First stage larvae are very active relative to third stage larvae although neither is comparable in activity to that of intestinal helminth larvae, such as Trichostrongylus or Haemonchus species.

Third stage larvae. Larvae at this stage have a double cuticular membrane which is best appreciated on the inner curvature as the worm bends. The larval outline is similar to the previous stage but the internal structure is much more visible as the granules are much diminished.

The oesophagus is seen faintly as a thin tube. The excretory pore is seen as an S-shaped structure approximately 75 microns from the head. The rectal tube and anus are approximately 30-40 microns from the tail tip.

Adult males. Fifty males from 10 calves were measured. All were smaller than the females present in the same lung, all had a yellow tinge and spicules were visible to the naked eye as short brown streaks at the posterior end. The mean length of the worms was 40.4 ± 6.5 mm. Their mean breadth was 495 ± 30.2 microns. The width of the buccal capsule was 19.5 ± 1.8 microns and its mean depth was 10.4 ± 0.8 microns.
The length of the oesophagus was 945.2 ± 61.4 microns. Its minimum breadth was 69.2 ± 5.4 microns while the maximum breadth was 147.4 ± 11.7 microns.

The excretory pore could usually be seen fairly easily in the middle third of the oesophageal length and less frequently the nerve ring could be observed around the oesophagus in the region of the pore.

The spicules were thick and brown and their mean length was 225.0 ± 15.8 microns.

Adult females. Fifty worms were measured from the same 10 calves. The females were always larger and more opaque than the males and were always white. The opacity and colour were due to the uterus' being full of eggs. Their mean body length was 55.5 ± 7.1 mm. and their mean breadth was 559.2 ± 35.1 microns.

The buccal capsule, which similarly was without teeth, measured 21.2 ± 3.1 microns in width and 9.6 ± 1.5 microns in depth.

Oesophagus. The mean length was 985.4 ± 73.1 microns. The minimum breadth was 78.2 ± 7.8 microns. Maximum breadth was 151.8 ± 13.7 microns. The excretory pore and nerve ring were fairly easily visible as in the males, and were similarly positioned with relation to the oesophagus.

Vulva. This was situated slightly posterior to the mid-point of the worm having a mean distance from the tail of 24.2 ± 3.1 mm. The anus was clearly visible at 438 ± 31.2 microns, from the tip of the tail.

Anal papillae were clearly visible. These were approximately opposite each other and 174± 14.1 microns from the tip of the tail.

Section II.

This section of the thesis describes a series of experiments designed to test the immunising powers of x-irradiated D. viviparus larvae under a wide range of conditions as the vaccine was translated from a laboratory tool to an applied product.

The main factors under consideration were:-

1) quantity of irradiation
2) number of larvae per single immunising dose
3) number of doses per calf
4) interval between dosing
5) the size of the challenge
6) the method of applying the challenge
7) the timing of challenge after vaccination
8) the timing of the final kill after challenge

The handling of these factors is discussed under the individual experiments, but one factor remained constant - the quantity of irradiation.

Previous experiments (vide supra) had indicated that less than 20,000 roentgens did not inactivate the larvae sufficiently - they reached the lungs in numbers and activity adequate to produce lesions which result in coughing and quite markedly increased respiratory rates in the calves. 60,000 roentgens over-attenuated the larvae so that they either failed to make the initial intestinal penetration or did penetrate but died before eliciting an appreciable immune response.

The effective range for Dictyocaulus viviparus lay between 20,000 and 40,000 roentgens and, as larval inactivation appeared equally effective at each level, the higher quantity was selected on grounds of safety.

A discussion on irradiation quantity would be incomplete without a brief consideration of irradiation rate. The upper level of the rate was partly determined by the output of the X-ray unit originally available - a Newton Victor GX 10 - which had an output in the order of 200 roentgens per minute. As this rate had proved satisfactory it was not changed in further experiments on Dictyocaulus viviparus, even when machines of higher output became available, because there were other more urgent variables to be considered.

Experiment 3. Assessment of the efficacy of a single vaccinating dose of 1,000 irradiated larvae under simulated challenge conditions.

In order to estimate the utility of vaccination as a field procedure it was necessary to expose vaccinated calves to natural challenge while simultaneously employing some quantitative standard of measuring resistance. The first way in which this was done was as follows:-

A number of calves was infected artificially and was then allowed to graze on a pasture located within the grounds of the Veterinary Hospital and which was subsequently grazed by groups of vaccinated and control calves. These latter groups were clinically examined three times weekly.

Materials and methods.

Forty-five Ayrshire bull calves were bought when two to seven days old and were reared parasite-free by housing in individual isolation pens and feeding artificially. They were randomised into three groups of 15, viz: carriers, vaccinates and controls.

Carriers. The carrier calves were divided into two sub-groups of five and 10, one lot being infected with 4,000 normal third stage larvae 45 days after the other.

Vaccinates. Fifty days before being turned out to graze, the vaccinate group were each given 1,000 X-irradiated larvae as a single oral dose.

Controls. These calves received no treatment of any kind before being turned out to graze.

The Design of the Experiment. Five carrier calves were put on to a three acre pasture for 40 days while their faeces contained large numbers of larvae. These calves were then withdrawn and the remaining carriers, vaccinates and controls were allowed to graze the paddock. (The control group now numbered 12 since three had been disposed of by accident.)

At a conservative estimate, 200,000,000 larvae were deposited on the pasture by the carriers, giving an approximate concentration of 1,300 larvae per square foot. This was, of course, considerably augmented by the calves of the other groups which became infected during the experiment. All the calves were gathered together for clinical and faecal examinations three times weekly; the faeces were subjected to a modified McMaster technique.

Results.

The average faecal larval levels in the control and vaccinate groups are shown in graph 1. The highest levels were reached at 44 days after their grazing of the pasture began and the difference between the two groups is highly significant at this point. The difference would have been even greater had not four of the most highly infected controls died before this point. The successive deaths of controls after this time caused an unusually sharp drop in the average level of the group. The peak larval counts and the duration of the larval counts in the two groups are shown in Tables I and II. The average respiratory rates of the two groups are given in graph 2.

Ten of the 12 controls died between the 27th and 73rd day after being turned out; the specific day on which deaths occurred and the

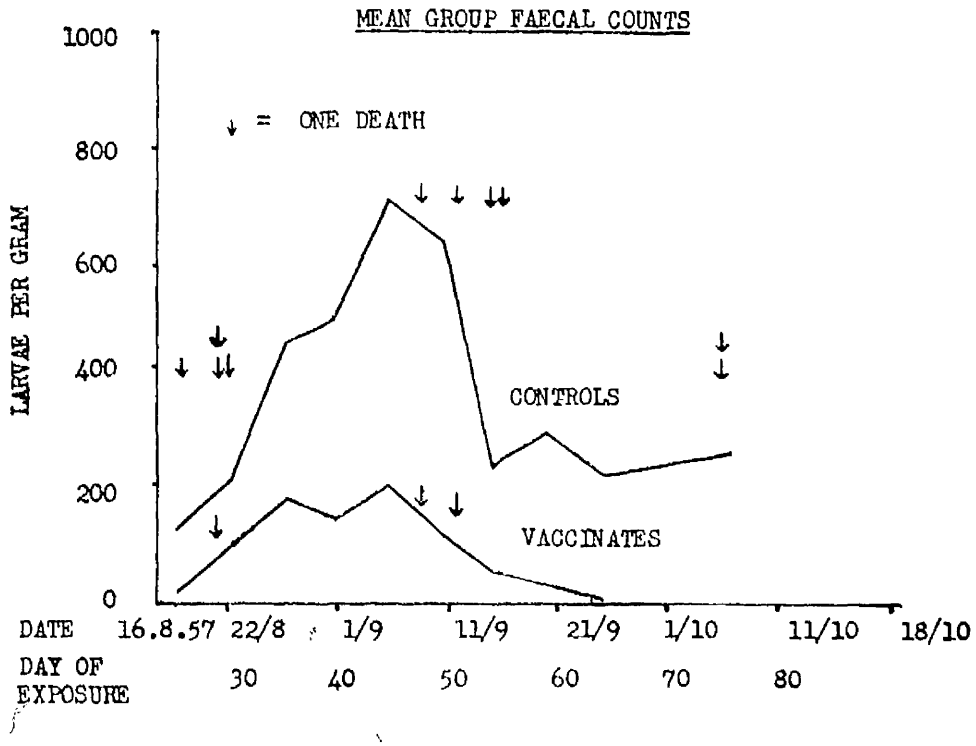
worm burdens are recorded in Table III. The mortality rate is 83%. Three of the 15 vaccinates died, a mortality rate of 20%; details are given in Table III.

At post mortem and subsequent histological examination, the lungs of calves dying during the experiment had lesions typical of parasitic bronchitis. In addition, the vaccinate which died 50 days after exposure showed widespread acute interstitial pneumonia.

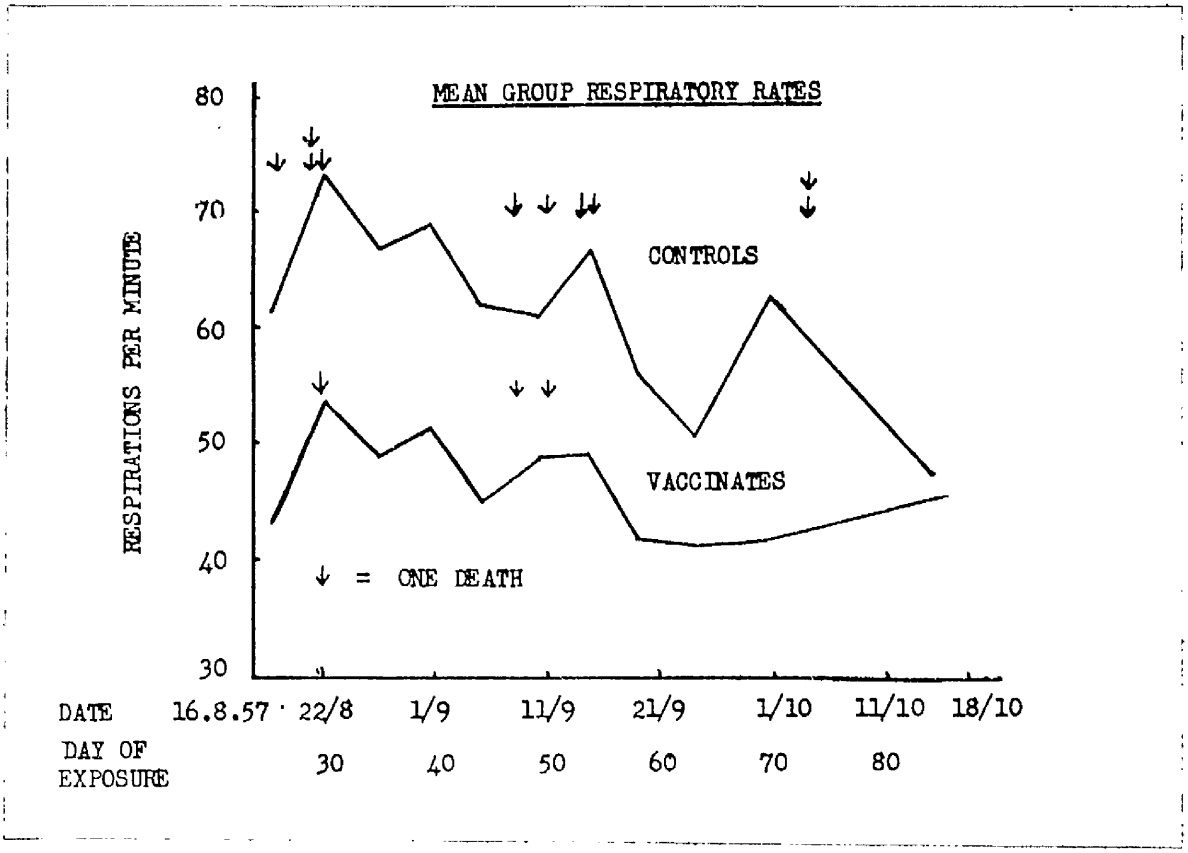
The survivors were killed when all faecal counts became negative. One vaccinate had two lungworms and the remainder none; one other had a small area of consolidation in a diaphragmatic lobe. Both of the controls showed some consolidation but in neither was it severe.

Discussion.

Two larval variations are possible in this type of attenuated vaccine, the amount of X-irradiation to which infective larvae were subjected and the number of such larvae used to immunise each calf. Both of these variations would reflect in the potency and safety of the vaccine. Irradiation factors have been discussed in the introduction. Concerning larval number, in the preliminary experiments the vaccinating dose was 4,000 irradiated larvae and this proved to be 99% effective under certain conditions, viz. if calves were challenged with 4,000 normal infective larvae 50 days after vaccination and killed after a further 30 days, the worm burdens were reduced from an average of 1,000 in controls to about 10.



Graph 1. Mean faecal larval levels and days on which deaths occurred in vaccinate and control groups.



Graph 2. Mean respiratory rates in vaccinate and control groups.

This was a very acceptable degree of protection but it was felt that the vaccine dose for use in the field during early trials should be reduced to 1,000 larvae since, if a failure of attenuation did occur, this number of larvae would not produce fatal disease. This dose was used in the present experiment to assess its utility under field conditions of challenge.

The weather conditions during the course of this trial were very conducive to maintaining a high level of infective larvae on the pasture and it is unlikely that the levels produced by the 15 carriers, all severe cases of husk, are often obtained under commercial farming conditions. It is considered that this system effected a very vigorous challenge to a vaccine designed for field use.

Success of such a vaccine should be judged by three criteria: it must reduce or abolish mortality; it should diminish the number of larvae being deposited on the pasture so that, after a few years, any individual farm should have been largely cleared of the disease; thirdly it should remove the need both to house and to supply supplementary feeding to susceptible animals during the grazing season.

The results leave little doubt that a very high degree of resistance was produced. Whether enough immunity would be produced by a vaccine of this strength to save all calves subjected to infection under field conditions was as yet unknown, but it seemed that the vaccine would be capable of considerably reducing mortality.

The reason for the apparent failure of the vaccine to protect three of the vaccinate group is unknown. Pathological examination of the lymph nodes and lungs failed to reveal the reactions usually seen in vaccinated calves.

On the second criterion of utility there is no doubt; the larval output of the controls was very significantly greater than the vaccinates, both in numbers of larvae at any one time and the length of time over which larvae were excreted.

The third criterion is difficult to assess in this trial because of the severe and prolonged nature of the challenge and the fact that calves, carriers and controls, were maintaining pasture levels which would not occur on a farm on which all calves were vaccinated. It was easily seen, however, a fortnight after the trial began that the vaccinates were in much better physical condition than the controls. This became less appreciable in the middle period but recovery in the vaccinated group was fairly rapid. The clear difference in the respiratory rates between controls and vaccinates shows the benefit conferred by vaccination.

When surviving vaccinates were killed there were virtually no pulmonary lesions. Apart from the obvious point that the animals had been protected in great measure against the effects of the parasitic pneumonia, this demonstrated that the vaccinating dose of irradiated larvae had produced no permanent harmful changes in the lungs.

It appeared from the experiment that this form of vaccination might have an important part to play in the control of husk in the field, both in reducing morbidity and mortality and in reducing pasture contamination with larvae on the farms.

TABLE 1 Number of days on which faeces contained larvae. Peak larval counts obtained in the control group.

Calf number	Days Positive/ / Days sampled	Highest count recorded
1	2/2	450
2	2/2	100
3	2/3	250
4	2/3	1,900
5	11/11	1,900
6	12/13	2,650
7	12/13	1,850
8	9/12	550
9	16/19	1,150
10	15/16	600
11	10/21	700
12	8/20	120

TABLE II Number of days on which faeces contained larvae. Peak larval counts obtained in the vaccinate group.

Calf number	Days Positive/ /Days sampled	Highest count recorded
13	3/4	250
14	8/9	650
15	10/11	1,550
16	16/22	1,600
17	16/22	800
18	12/21	250
19	8/22	250
20	7/22	200
21	6/18	250
22	8/22	100
23	5/22	100
24	5/22	100
25	3/22	150
26	2/22	150
27	2/22	100

TABLE III Time of death, and number of worms found in lungs at post-mortem.

Calf number	Group	Days after Exposure on which death occurred	Worm Count
1	Control	26	1,200
3	"	29	930
2	"	29	1,560
4	"	30	4,670
5	"	47	2,100
8	"	50	910
6	"	53	1,210
7	"	54	2,250
9	"	73	2,600
10	"	73	3,000
13	Vaccinate	30	1,660
14	"	47	840
15	"	50	1,300

Experiment 4. Controlled trial of a vaccinating dose of 1,000 irradiated larvae under conditions of natural challenge.

The second way in which the utility of vaccination as a field procedure was assessed was in a large scale farm trial. Through the liaison of their own local veterinary surgeons, 40 farmers expressed willingness to co-operate. Most of their farms were located in Ayrshire, an area where parasitic bronchitis was known to be endemic; moreover the disease had been present on each farm in the previous year.

The husbandry of farms in this region is such that calves are born in two fairly confined waves in winter and spring, giving a dichotomous calf population divided by an age gap of approximately 3 months. The farmers, who were very co-operative throughout, had been fully informed of the nature of the trial and they had been requested to keep the calves indoors from birth until otherwise directed. As a rule in the south west of Scotland many farmers did not allow their calves out to grass until six or even 12 months of age because of the parasitic bronchitis risk, so this restriction was in no way an imposition on them by us.

Materials and Methods.

Most of the calves were of the Ayrshire breed and were reared under varying conditions of husbandry, usually receiving colostrum for two or three days before going on to bucket feeding. The larvae for use in this trial were harvested fresh each vaccinating day at 5 a.m. Irradiation with 40,000 roentgens was carried out between 6 and 10 a.m. then the larvae were taken back to the laboratory for counting. Doses of 1,000 larvae were made up in 50 ml. of tap water and were taken by car to the

farms immediately, so that vaccination was always performed on the day of manufacture. Samples of unused vaccine were always examined for viability on return and were 95 to 100% viable in each case. The calves were not turned out to graze until four weeks after vaccination. Faeces examinations were carried out by the Baermann, Zinc sulphate flotation and modified McMaster methods (see technical appendix).

Experimental procedure

The trial was performed during March to November of 1957. All farms were visited once each month, when the calves were individually faeces sampled per rectum and their clinical status noted with particular reference to thoracic auscultation and respiratory rate. In the laboratory the faeces samples were bulked in groups not usually exceeding five for Baermann examination. This was necessary in view of the large number of faeces samples and the relatively limited laboratory facilities available. However, if one Baermann funnel was positive, the component samples, whose numbers were known, could if required be subjected to individual zinc sulphate flotation and modified McMaster examinations. All the calves throughout the trial were readily identifiable by numbers tattooed in their ears or in some cases by metal ear tags. Most calves were vaccinated over 12 weeks of age and the vaccinations were performed between the 12th March and 19th April, as the crop of winter calves came of immunisable age, and between the 4th and 27th of June for the spring calves. On each farm only half of the calves of each wave were vaccinated. These were selected at random and the remainder served as controls. Altogether 396 of 833 calves were

vaccinated in the first run, and 135 of 289 in the second. The dosing was carefully performed to ensure that each calf received all its larvae.

Results. From the experimental view-point 1957 was a poor year for husk and, although the disease had occurred on all the farms the previous year, only 23 of the trial farms showed evidence of infection and of these only six could be said to have experienced overt outbreaks of parasitic pneumonia in the clinical sense. Summarised details of these six farms are shown in Table I. In two of these, AG and AK in the table, the experiment was technically interfered with when the farmers, quite understandably, called in their veterinary surgeon to treat the outbreak at its onset. One of the drugs used was a cyanacethydrazide compound which may provide some alleviation by causing the dislodgement of some of the adult worms in the main bronchi. As the only quantitative criterion for assessing the parasitic status of the calves in this particular experiment was based on the faecal larval counts, such treatment nullified quantitation on farms AG and AK. Nevertheless controls died on the two farms. It was unfortunate that there was no opportunity to examine post mortem the two vaccinated calves which died. In particular it would have been interesting to see if their lungs showed histological evidence of reaction to the vaccine.

Table I. Distribution of cases on the six outbreak farms as assessed by modified McMaster technique.

Farm Code	number of vaccinates.	number McMaster positive	number of controls.	number McMaster positive	deaths
Z	18	0	13	8	0
AI	8	1	8	6	1 (vaccinate
AJ	12	0	13	6	0
AP	9	2	7	5	1 (vaccinate
AG, AK	On these two farms 1 and 2 controls died, respectively. The other calves had been 'Helmox'* treated and were not positive.				3 (controls
Total	47	3	41	25	5

* 'Helmox' - I.C.I. cyanacethydrazide.

Calves on a further 17 farms had faecal levels of larvae which were demonstrable on Baermann examination only. An initial breakdown of these figures in shown in Table II.

Table II. Distribution of cases which were Baermann positive only.

Number of farms.....17
Number with controls only positive..... 4
Number with vaccinates only positive..... 3
Number with both +ve.....10

A further breakdown of the Baermann figures reveals that out of the vaccinated wave of winter calves on these farms there was a total of 15 positive Baermann examinations compared to 31 positive in the corresponding controls. In the spring wave of

calves the picture is rather different in that six of the vaccinates were positive and seven of the controls.

In the seven farms where only one of either group contained positive cases, the number of positive Baermanns did not exceed two on either side.

Discussion and Conclusion

It was disappointing from the experimental point of view that clinical outbreaks of the disease occurred on only six farms. However, the results here were encouraging in that they indicated a significant degree of protection in the vaccinated calves, as shown in Table III.

Table III. Percentage morbidity in vaccinated and control calves on 4 farms, Z, AI, AJ and AP.

Group	Number at risk	Number with clinical disease	Morbidity
Vaccinates	48	3	6%
Controls	43	27	62%

These results are highly significant if only the relevant four or six farms are used to obtain data. They are not significant if all 40 farms are included; however, as each individual farm could be considered as a closed experiment it was thought reasonable to include only those farms with the clinical disease.

Regarding the 17 Baermann positive farms, it was interesting that the results of the winter calves appeared to show a trend favourable to the vaccine whereas vaccinates and controls in the spring calf groups appeared equally affected.

Detailed examination of the individual records shows that the spring calf figures are obtained from five farms, where the calves in question were all vaccinated on 4 to 7/6/57 and showed larvae in their faeces on 3 to 14/8/57. Subtraction of the 24-day prepatent period of D. viviparus gives a mean period between vaccination and challenge of 44 days for these affected spring calves. It must be borne in mind that this is a theoretical maximum vaccine/challenge interval and assumes that the calves were sampled on the first day of patency. The true vaccine/challenge interval probably lay between 30 and 40 days, which is possibly too short as complement fixing antibody attains a maximum level between 80 - 100 days after single infection.

In addition the later vaccinated calves were turned onto pasture on which the larval levels had already appreciated from calves of the winter group, so the former did not have the benefit of a more gradual parasitic acclimatisation. The main conclusions from this trial were:-

1) that the vaccine itself appeared safe to use under normal farming conditions. No breakdowns in attenuation were found and no untoward vaccine reactions occurred.

2) that the vaccine could confer an appreciable degree of protection to a natural challenge infection.

3) that the spring calves might be at a temporal disadvantage, which could possibly be overcome either by increasing the vaccine dose or by giving two spaced doses.

Experiment 5. To investigate the protection of single and double vaccination, including the effect varying the size of the second dose, against a high challenge infection.

The dose of vaccine (1,000 larvae) used in experiments 3 and 4 was probably sufficient to protect the majority of animals at risk, but it was known that in the endemic areas, such as south-west Scotland, some calves are exposed to very large infections (as many as 6,000 worms being found at post-mortem) and these would require a greater degree of protection. For this purpose the use of double vaccination was next explored since it had been shown (Jarrett, Jennings, McIntyre, Mulligan, Thomas and Urquhart 1959) that an important factor in the immunity against D. viviparus is the time taken for the anamnestic response to develop. After an initial infection (as was mentioned above) the complement fixing antibody titre does not reach its maximum point until approximately 80-100 days; with subsequent reinfections this time is reduced to 10 - 14 days. The aim in double vaccination was to have an animal, when turned out to graze, capable of attaining a high level of immunity within a fortnight of ingesting infective larvae, thus preventing the establishment of adults in the bronchi. In preliminary experiments (Jarrett, Jennings, McIntyre, Mulligan and Urquhart, 1960b) an acceptably high degree of immunity had been produced with a single vaccinating dose of 4,000 irradiated larvae, but this dose gave rise to pulmonary complications which, though mild, might have caused some alarm to stockowners.

Materials and Methods.

Fifty Ayrshire calves were bought when three days old and

were raised parasite-free in individual isolation stalls.

Third-stage infective larvae of D. viviparus were cultured and subjected to a dose of 40,000 roentgens from an X-ray source.

The methods of administering larvae to the calves, estimating faecal larval outputs, counting the number of worms in the bronchi and examining the calves for clinical and pathological changes are as described in the technical appendix.

The fifty calves were allotted to five groups of 10. At the commencement of the experiment (Day 0), each calf in group one was given 1,000 irradiated larvae. Each calf was given a further dose of 4,000 irradiated larvae 42 days later. On day 93, each calf was challenged with 10,000 normal infective third-stage larvae. The experiment was terminated by killing the calves on day 126. At this time final faecal larval examinations were made, the number of worms in each pair of lungs was counted, and an assessment of the degree of pulmonary consolidation was made using a lesion score method. (vide technical appendix). Histological examination was made of affected lung tissue.

The calves of groups 2 and 3 were treated similarly except that their second vaccinating doses were 2,000 and 1,000 irradiated larvae, respectively.

The calves of group 4 were given only the initial irradiated 1,000 larvae at day 0, with no further dose, and were challenged with 10,000 normal larvae as before on day 93.

Group 5 was divided into two sub-groups, A and B. Each of the five calves in group A was given 10,000 normal larvae on day 93 and the five calves of group B were given 5,000 normal larvae. Animals in the subgroup which were infected with 5,000 normal larvae were included in case a heavy mortality should result among the 10,000 challenge group during the prepatent period; in the latter case a quantitative assessment of response to challenge would have been impossible, as an accurate worm count could not have been obtained.

During the course of the experiment, a clinical examination was carried out on each calf twice weekly; the respiratory rate and the degree of coughing was noted. Each calf was weighed once weekly from two weeks before challenge until the end of the experiment.

In addition to these calves, two other groups (6 and 7) were used to control the viability of the larvae used in making the two batches of vaccine and to give comparisons between group respiratory rates. Each calf in group 6 was given 4,000 infective larvae at day 0 and the calves of group 7 were given 4,000 normal larvae on day 42.

The design of the experiment is summarised in Table I.

Results.

The respiratory rates of the corresponding groups are shown in graphs 3 to 8. In response to the first vaccination, there was an increase in respiratory rate in each of these groups which reached its maximum about the 18th day.

There was no significant statistical difference in response between these groups. This transient rise in respiratory rate has been noticed in almost all calves vaccinated by this method and is due to a few irradiated larvae reaching the lungs and producing the quickly resolving alveolar and bronchial lesions, which are described in Part III of this thesis. The respiratory rate fell away to normal by about the 25th day, whereas in the control group (Group 6) the respiratory rate continued to rise, reaching a maximum between the 30th and 40th day, when a fully patent infection had developed.

After the second vaccinating dose in groups 1, 2 and 3 there was an apparent rise in respiratory rate reaching a peak between 60 and 70 days, but this is not significantly different from the respiratory rate of calves in group 5 (graph 7) which reached 50 per minute at that time during a spell of warm weather. The calves of this latter group (destined to be the challenge control) had not at that time been interfered with experimentally in any way. After challenge with 10,000 larvae on the 93rd day, there was no rise in respiratory rates of calves in groups 1, 2 and 3, whereas in groups 4 and 5 there was a marked rise. On auscultation at this time, no abnormality was detected in any calves in the first three groups; marked clinical respiratory abnormalities were present in calves in groups 4 and 5. The calves of group 5A were considered to be moribund shortly before the termination of the experiment.

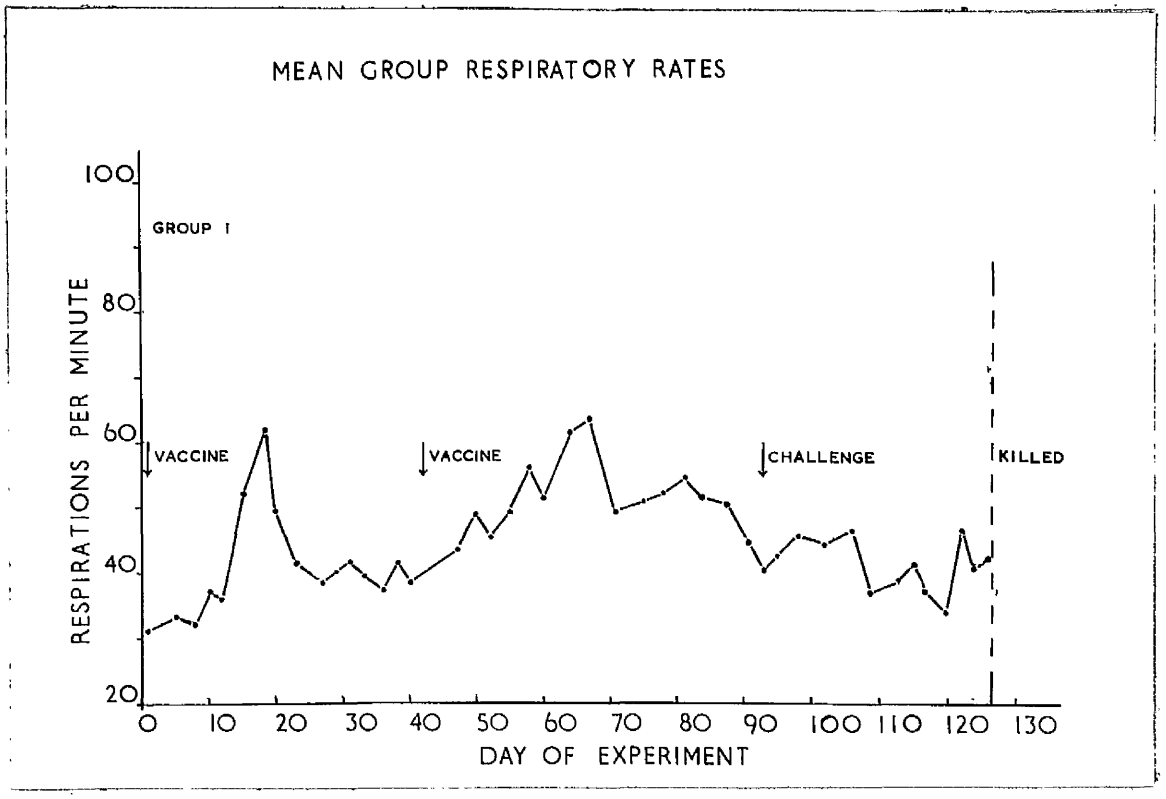
The total and percentage weight gains of the groups are shown in Table II. The calves in the double vaccination groups gained approximately 18% in weight during this time. Those in the single vaccination group gained 7.6% and the challenge controls gained 5.0%.

Parasitological Results. A high degree of protection against the challenge infection was produced as shown in Table I. In groups 1, 2 and 3 no worms were found in the lungs at necropsy and no larvae appeared in the faeces of these animals. In group 4, patent infections developed and a mean of 820 worms was found at necropsy after challenge; this result, however, must be interpreted in the light of the pathological findings. In groups 5A and 5B severe infections developed; it was impossible to assess the total number of worms present in 5A as many were immature and were visible only on histological examination.

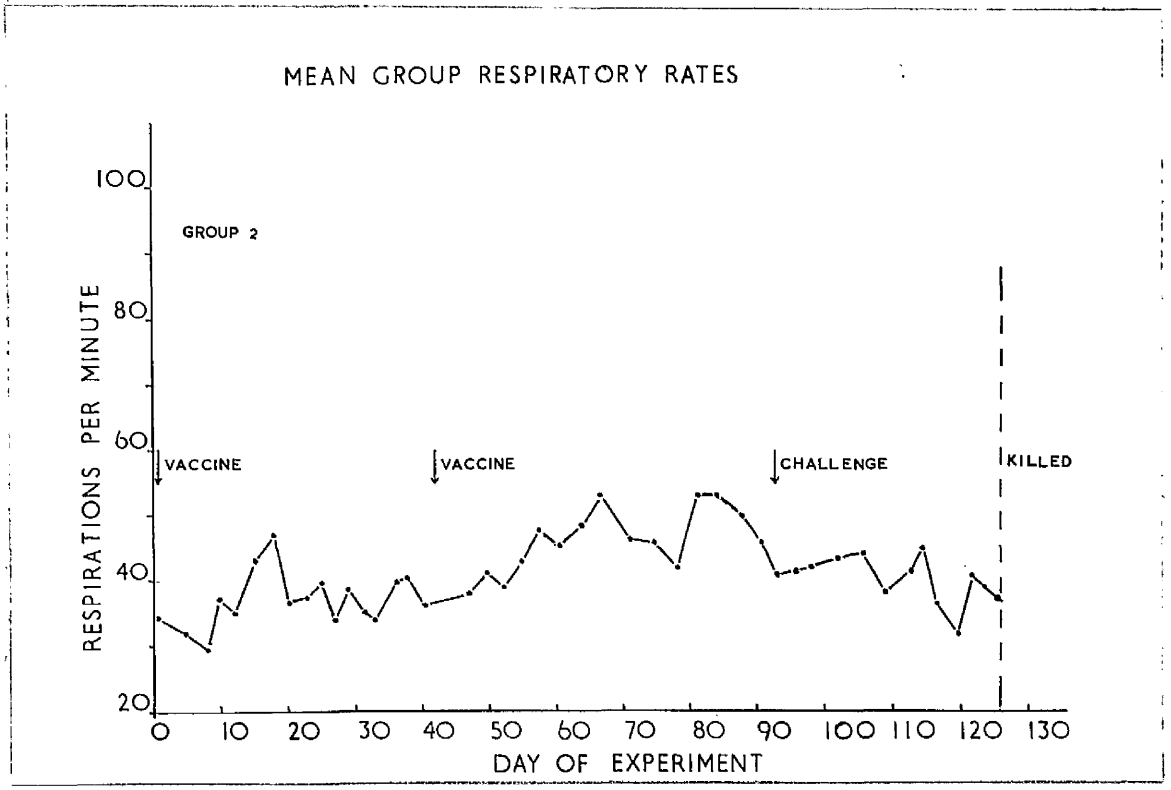
Pathological Results. In groups 1, 2 and 3 there were virtually no lesions at post mortem and histological examination of the lungs failed to show any signs of an infection having been established. In group 4, the lesion score was almost the same as that in groups 5A and 5B indicating that, macroscopically, the degree of consolidation was the same in the group given one dose of vaccine as it was in the challenge controls. However, histological examination showed the lesions to be qualitatively different in the two groups.

Microscopic examination of sections from calves in group 4 revealed that the majority of the worms in the bronchi were dead or dying; the lesions produced were those of marked eosinophil leukocyte infiltration into the bronchi and desquamation of the bronchial epithelium. There was little evidence of alveolar consolidation of the type normally found in primary lungworm infections. The collapsed lobules, which accounted for the

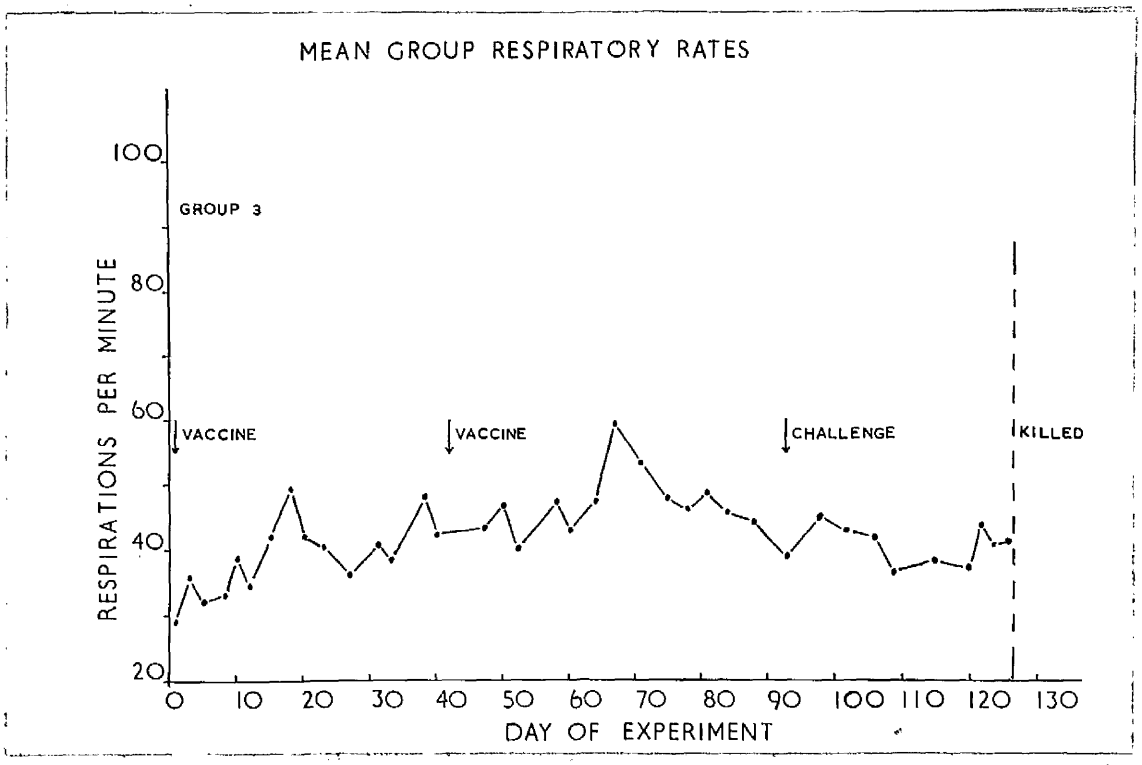
high lesion score, were caused by bronchial plugging with degenerate eosinophil pus. This atypical, exaggerated response to worms dying in situ in the lungs will be described in greater detail in Part III. It is known to resolve in a much shorter time than the lesions produced by a normal infestation of D. viviparus. The severe pneumonias recorded in groups 5A and 5B are in keeping with previous experience of the experimental disease (Jarrett, McIntyre, and Urquhart, 1957).



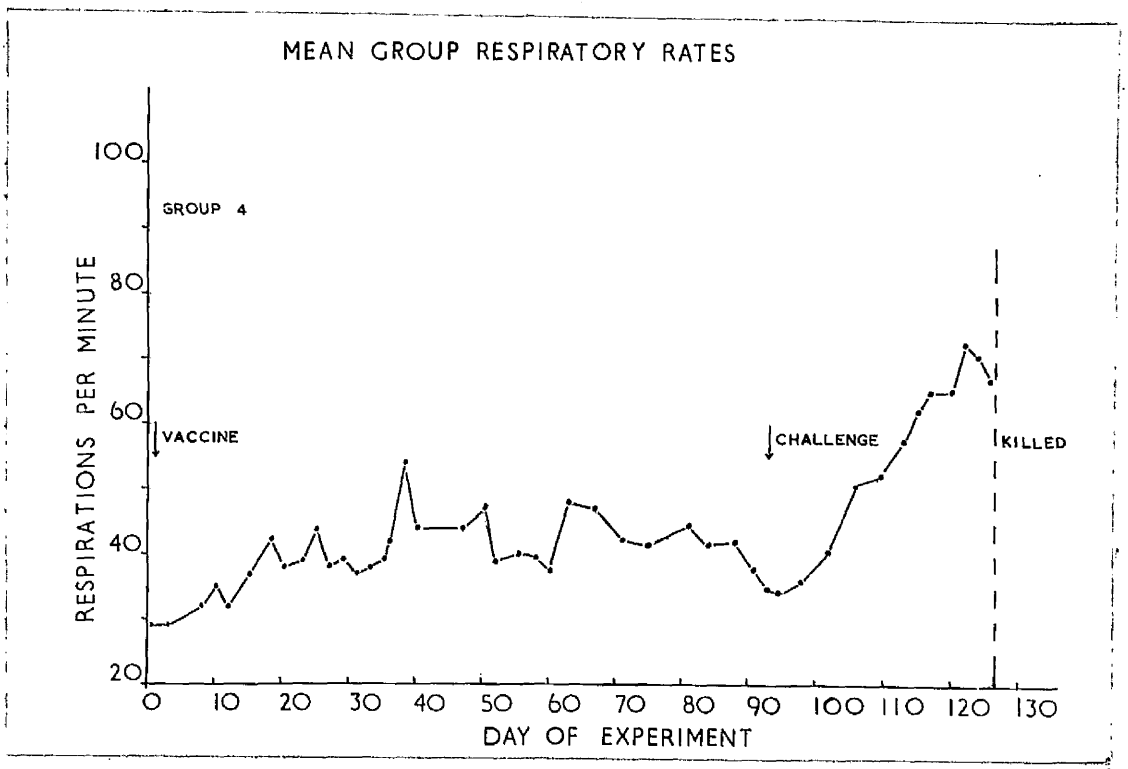
Graph 3 - Mean respiratory rate of Group 1.



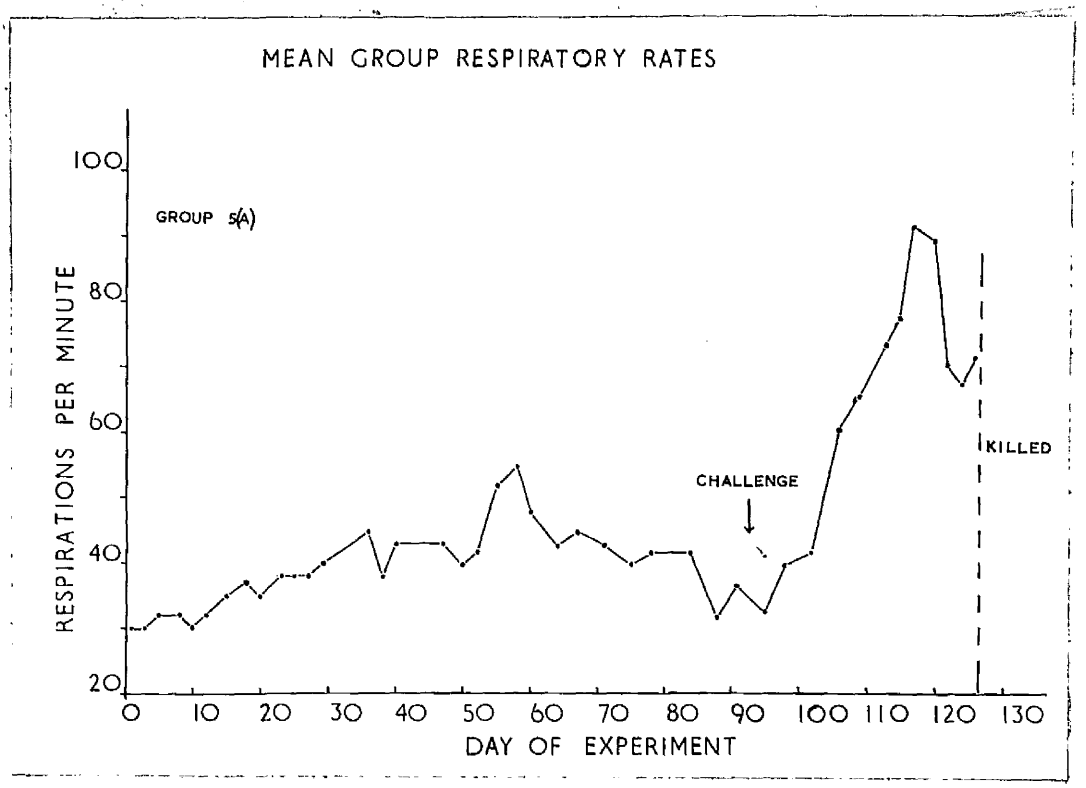
Graph 4 - Mean respiratory rate of Group 2.



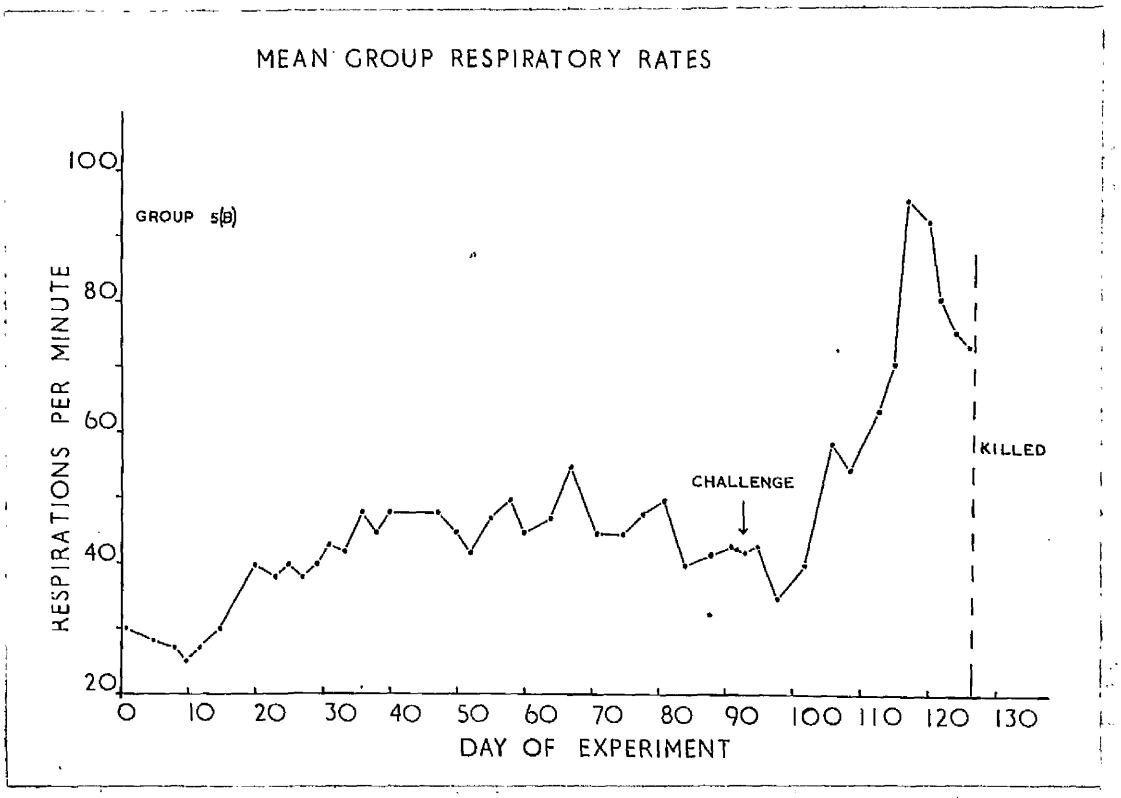
Graph 5 - Mean respiratory rate of Group 3.



Graph 6 - Mean respiratory rate of Group 4.



Graph 7 - Mean respiratory rate Group 5A.



Graph 8 - Mean respiratory rate of Group 5B.

Table I. Parasitological and Pathological Results of the Challenge Infection.

Group	Number of larvae			Mean larvae per gm of faeces	Mean number of adult worms at necropsy	Mean lesion score.
	1st vaccine	2nd vaccine	challenge			
1	1,000	4,000	10,000	0	0	0.1
2	1,000	3,000	10,000	0	0	0.1
3	1,000	1,000	10,000	0	0	0.2
4	1,000	-	10,000	285	820	7.3
5A	-	-	10,000	454	897	7.2
5B	-	-	5,000	300	1,020	7.2

Table II. Total group weights and percentage weight gains.

Group	Total Group Weights at challenge (lb)	Total Group Weights at slaughter (lb)	Group weight gains %
1	2,720	3,227	18.6
2	2,632	3,110	17.2
3	2,636	3,092	17.3
4	2,457	2,664	7.6
5	2,607	2,738	5.0

Discussion.

The results given above indicate that the administration of two doses of infective larvae of D. viviparus, partially inactivated by irradiation, produced a level of immunity which enabled the calves to withstand a high challenge infection. The clinical effects of vaccination were mild even in Group 1 in which the second dose was 4,000 larvae. The transient rise in respiratory rate and occasional cough would pass unnoticed under practical farming conditions.

A most pertinent comparison is that between Groups 3 and 4; the calves receiving only a single vaccination with 1,000 larvae were overwhelmed by the challenge, whereas those receiving a second dose of 1,000 were almost completely refractory to the challenge used. No difference was found in response to the challenge between the three groups receiving double vaccination. Had the challenge been greater it probably would have separated these three groups.

The difference in weight gains between the double vaccine groups and the control and single vaccine groups is significant. Weight gains in his stock are one of the prime considerations of the farmer; it is probable that even a mild adult lungworm burden could depress the rate of growth without causing overt signs of disease in cattle, whic burden would not exist in the immune animal. In one sense, and without confining discussion to the lungworm, the parasitised animal which shows obvious signs of disease is a fairly extreme case. Much of parasitism is confined in its effect to the no-man's-land between health and disease wherein also lies the farmers' profit.

Experiment 6. Large-scale uncontrolled field trial using two vaccinating doses of 1,000 irradiated larvae.

This experiment was performed during the summer and autumn of 1958 on lines very similar to the farm trial described in Experiment 4. However, there were several major points of difference.

1) This trial was very much larger, involving over 8,000 calves on 204 farms scattered throughout Scotland in areas ranging from Crieff, Perthshire, to Stranraer, Wigtownshire.

2) All calves on the farms were vaccinated and all vaccination was double.

3) Routine faeces sampling was not performed unless clinical suspicions were aroused.

4) The vaccine was not irradiated in Glasgow. This was because the process was being scaled up for possible commercial production. Consequently the vaccine was prepared by the scientific staff of Allen & Hanburys Ltd., under the direction of Dr. D. Poynter.

In the previous experiments described, the numbers of animals vaccinated were relatively small, the largest batch of vaccine prepared at any one time by the author being only 500 doses. This quantity of larvae could be cultured fairly easily on a laboratory scale. Considerable work was involved in adapting the process for commercial use and this trial was designed largely to test two factors:

1) The safety of the vaccine under a wide range of farming husbandry and under the altered conditions of factory production.

2) The practicability of factory production, packing and transport.

Materials and Methods.

The calves were from most of the common British breeds of both dairy and beef types. As before the farmers, who had all been contacted through their own veterinary surgeons, were instructed to keep the calves indoors until after the completion of vaccination. The vaccine was contained in 30 ml. bottles in cardboard boxes of 12. Eighteen such boxes were crated in wood with one of three types of packing material, wood-wool, straw, or cork, each together with several polythene ice-bags. The crates were always sent by night-train from London and were collected from Glasgow Central station at 6.00 a.m. All crates were opened and 10 temperature measurements were made of the vaccine in different parts of the crate. The temperatures were within the range 6 to 10°C., irrespective of the packing material used, and this range was deemed quite satisfactory. Larval counts and viability checks were performed on approximately 5% of the vials, randomly selected, from each crate and were also satisfactory.

Experimental procedure.

The 204 farms selected had a history of past severe parasitic bronchitis. The calves were vaccinated twice. For practical reasons the vaccine interval was reduced from 42 to 28 days. All farms were visited on average once every month within the experimental period. As stated above, routine faeces sampling was not performed.

Results.

1958 was a year in which there was a widespread high incidence of parasitic bronchitis. Despite this, however, there was no major breakdown in immunity where the calves had been given two doses before being exposed to challenge.

Many of these farms, especially in the Ayrshire and Wigtownshire regions, were free of parasitic bronchitis for the first time for many years. Quite apart from the drop in morbidity and mortality, the farmers reported reduced feeding bills since supplementary feeding had been cut; calves did not require to be reared or removed indoors and animals could be left longer at pasture than in previous years.

Discussion.

As this experiment was uncontrolled there was no way of so quantifying the results that an acceptable degree of objectivity could be applied. For this reason the trial is only briefly discussed here. However, its primary object was to act as a safety test and it was very important both from this aspect and from the fact that it satisfactorily showed that the vaccine was capable of being produced and distributed on a scale large enough for possible commercial manufacture.

Earlier in this section of the thesis it was shown that a high degree of immunity could be gained in laboratory, paddock and farm experiments. The work presently under discussion was complementary to the previous results in that it demonstrated the capability of their being applied as a practical measure on a wide scale.

Experiment 7. A controlled pasture trial of the irradiated larval vaccine using two immunising doses against a natural challenge infection.

Experiment 5 indicated that if a primary dose of vaccine was followed by a second dose given six weeks later, complete protection against a heavy challenge resulted. The challenge might be considered an 'artificial' one in that the larvae were administered as a single large dose, although it is probable that it provided a more severe test of resistance than if a similar number of larvae was spread over several days.

However, an opportunity arose to test the two dose procedure under natural field conditions when a heavily infected pasture became available. In the late summer and autumn of 1958, 38 out of 40 calves on this field had died of parasitic bronchitis; this mortality rate is seldom seen under farm conditions and it was through that this pasture might give a rigorous challenge to the vaccine under natural conditions of ingestion of larvae. Despite the fact that it was rather late in the season, the following experiment was carried out.

Materials and Methods.

Calves: Ten Ayrshire calves of about eight weeks of age were used. It had been standard practice throughout the parasitic project to buy calves for experiment when under a week old and to rear them worm-free until required. As the present trial was undertaken at short notice this could not be done, so the animals were bought from commercial herds. We had, therefore, only the farmers' word that they had not been in contact with grass or diseased stock.

Nevertheless careful faecal examination by the Baermann technique of each calf on three occasions prior to the commencement of the experiment revealed no evidence of lungworm larvae.

Pasture: This was four acres of permanent pasture. Much of the grass had already been eaten off so that concentrate and hay had to be supplied; each calf received 2 lb. of rearing concentrate each morning and 2 lb. of hay each evening.

Vaccine: Five of the calves were given two doses of vaccine prepared by the author. There was an interval of 30 days between dosing. Each dose consisted of 1,000 third stage larvae which had been subjected to 40,000 roentgens of X-rays.

Parasitology: Modified McMaster and standard zinc flotation, Baermann and worm counting methods were used.

Results.

On the 20th October the 10 calves were turned out to graze on the infected pasture 20 days after the vaccinates had received their second dose. Respiratory symptoms were noticed 15 days later and one control calf died on the 18th day from severe pre-patent husk. A portion of one lung was Baermannised and 265 immature adult lungworms were recovered together with an approximately equal number of early fifth stage forms. Many more were seen on histological examination, which also revealed severe pulmonary oedema and hyaline membrane formation, i.e. the typical lesion of the severe pre-patent stage of the disease.

Between the 18th and 20th day on pasture the mean respiratory rates of both groups had risen to 65 per minute. (Graph 1). That of the vaccinate groups then began to fall and by day 47 the vaccinate

mean rate was 45 while the controls reached a peak of 83 per minute.

The vaccinates were not observed to excrete larvae in their faeces at any time during the experiment. On the 30th day two of the four surviving controls had counts of 200 and 400 larvae per gram, respectively, and on the 44th day the group mean rose to 500 per gram (Table I). One control remained apparently uninfected throughout.

The calves were killed 50 days after being put on the pasture and the worms in their lungs were counted; the results are shown in Table II. Mature worms were not found in the vaccinates whereas the controls had a mean burden of 442. Immature worms were found in two of the vaccinates.

Over the whole trial there was a mean weight gain of 58% in the vaccinates against 38% in the controls: in the final four weeks the former gained 9% and the latter 0.7%.

Discussion.

One difference between this double vaccine experiment and that described as experiment 5 was that the present challenge infection was 'natural', i.e. it was established by the intake of larvae from grass over a period. This challenge, however, must have come soon after the calves were turned out as one died in the prepatent phase on the 18th day; this death is compatible with a massive infection in the first few days. The majority of the calves were reacting clinically in the third week and this also indicates early ingestion of infective larvae.

Four acres is a fairly large field for 10 calves and it is quite possible that there was a considerable quantitative variation in larval intake, particularly in view of the supplementary feeding required. Nevertheless it seems unlikely that control calf 2, which had neither larvae in its faeces nor adults or immature forms in its lungs, failed to ingest infective larvae. It is possible that it had some immunity from the start since, as explained above, the detailed history of each animal was not known for certain.

The immature worms found in two of the vaccinates may have resulted from ingestion of larvae late in the experiment but it is more reasonable to conclude, from the facts already outlined, that they were picked up soon after the calves went out and that inhibition of development occurred. Inhibition arises from an appropriate balance between the degree of immunity possessed by the individual and the size of the infecting dose; it has been well documented experimentally in Nippostrongylus muris infections (Taliaferro and Sarles, 1939). This phenomenon has been noted after challenge of calves vaccinated by a single dose of irradiated larvae but not when double vaccination has been followed by a high (10,000 larvae) challenge (experiment 5). It is therefore likely that calves 7 and 10, whose lungs contained immature worms, ingested a very large number of infective larvae. This is substantiated by the clinical findings. It has been noticed regularly, as in experiment 5, that when vaccinated animals are exposed to heavy infections there is a short sharp rise in respiratory rate. This is due to some larvae reaching the lungs but soon dying there; it will be amplified in Part III. The degenerating worms set up a marked eosinophil leukocyte reaction which leads to some bronchial plugging and alveolar collapse.

This usually passes off quickly and the calves return to normal. Several farmers involved in the field trials thought that husk was about to break out in vaccinated calves but had their fears allayed within a week.

A respiratory response of this type was observed in vaccinated calves 7 and 10, whose respiratory rates suddenly increased to 110 and 85 per minute, respectively, during the third week on pasture. One of the other three vaccinated calves, number 8, showed a moderate and transient respiratory increase at this time.

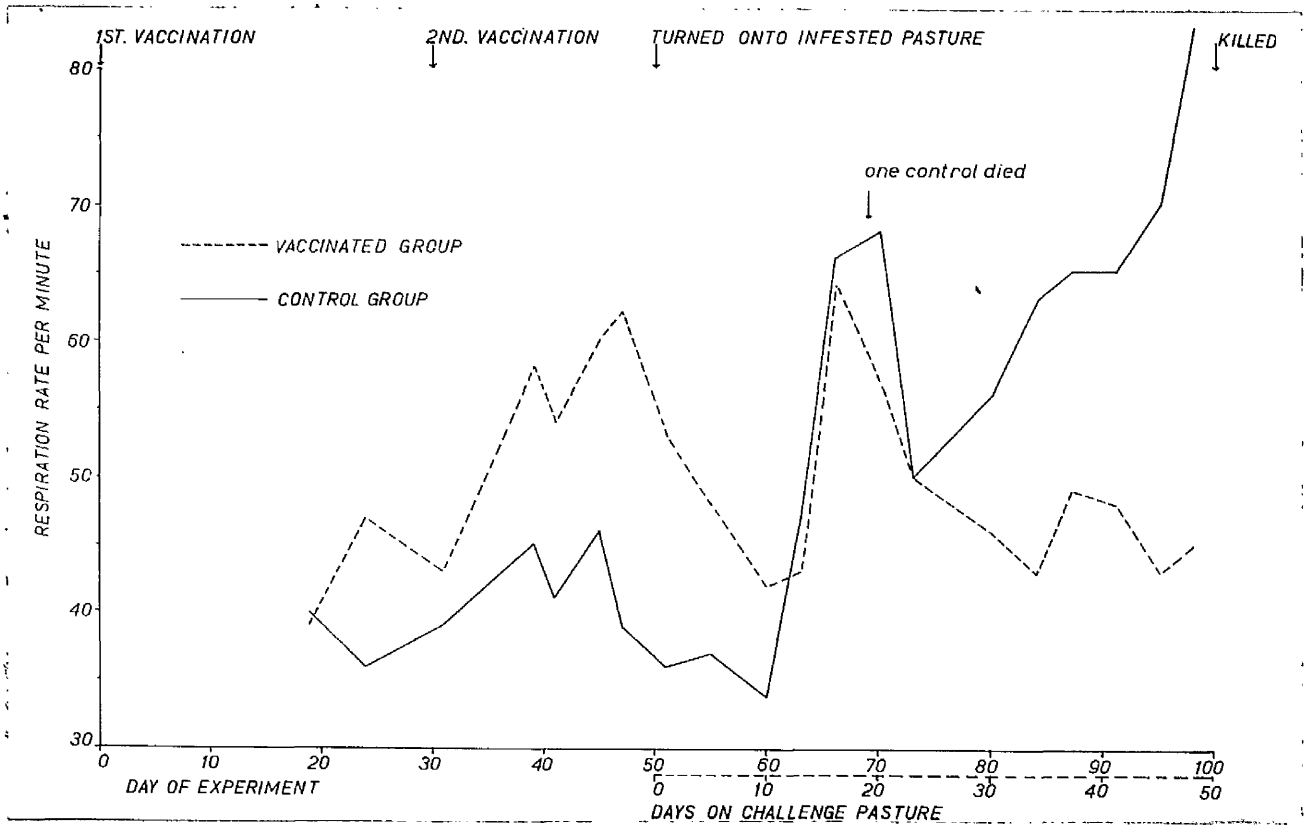
A factor which might be of considerable importance in the development of adequate immunity is the interval between the vaccine doses. Serological studies of complement fixing antibodies (and it should be noted that these do not necessarily correspond with the absolute level of immunity possessed by an animal) indicate that the highest titre is reached 100 days after primary infection of the susceptible calf (Jarrett, Jennings, McIntyre, Mulligan, Thomas and Urquhart 1959). Subsequent re-infections are associated with a secondary response which reaches a peak after about 14 days. Two doses of irradiated larvae with a six-week interval between do not themselves give rise to a high level of complement fixing antibody, but the pattern after challenge is that of a full secondary response. In the controlled dosage challenge experiment with double vaccination (experiment 5) an interval of 42 days was decided upon, whereas in the present experiment the seasonal time factor necessitated the second dose of vaccine being given 30 days after the first.

It is impossible so far to draw a final conclusion as to the optimum time interval between vaccine doses as this could be done only when a uniform challenge dose is employed against calves twice vaccinated at different intervals. It is often necessary on individual farms to use the shorter time interval of the present experiment and it was encouraging to note that it appeared to be associated with considerable protection.

In experiment 3, where a similar type of paddock trial was described using single vaccination, one significant difference between vaccinates and controls was their faecal larval outputs. This difference was even more marked in the present case since the vaccinates did not have larvae in their faeces on any of the six days on which examinations were made. On the farm this would have two main consequences: first, the overall pasture level of infective larvae would be reduced and in areas where over-wintering of larvae on the pasture occurs it is possible that one of the main sources of new outbreaks would be reduced; secondly, the build-up of infection by autumn calves in the spring would be markedly lowered and the susceptible spring calves would not be exposed to the high levels of infection which often cause severe outbreaks where mixed-age grazing is practised.

To the farming industry the total cost of parasitic bronchitis is made up of a number of components including mortality, extra feeding, veterinary expenses and the lowered growth rate of many affected animals. The latter is often obvious for many months after the acute stage of the disease has passed. The benefit of vaccination in the last respect was underlined in this experiment by the fact that in the final four

weeks the vaccinates gained a mean of 9% of their body weight, representing about 16.5 lbs. each, while the surviving controls gained only 0.7%.



Graph 1. Mean respiratory rate of vaccinated and control groups, correlated with time on challenge pasture.

Table I. Group mean faecal counts in larvae per gram.

Number of days the calves had been on the infected pasture.	Vaccinates	Controls
30	0	162
35	0	275
37	0	188
40	0	287
44	0	500
49	0	412

Table II. Individual worm counts at post mortem.

Calf Group and number	Number of mature worms	Number of immature adult worms
Controls		
1	1,250	0
2	0	0
3	367	0
4	150	0
5*	0	265
Vaccinates		
6	0	0
7	0	70
8	0	0
9	0	0
10	0	55

* Died after 18 days on pasture. 265 immature adults and approximately 250 early fifth stage forms recovered from Baermann examination of one diaphragmatic lobe only.

SUMMARY OF PART II

Section I.

Experiment 1. Longevity tests on unharvested first and third stage D. viviparus larvae indicated that storage of larvae in faeces was not a practical and reliable technique.

Experiment 2. Longevity tests on harvested third stage larvae stored in eight different media at varying temperatures, indicated that the control solution of Glasgow tap water (GTW) preserved the larvae approximately as well after 28 days at 0 to 4°C. as did phosphate buffer solution, Tyrode's solution, Earle's solution and GTW with added antibiotics. GTW ('neat') was therefore chosen as the medium for all larval handling procedures.

Section II. (All the experiments in this section carry their own discussion, which is cumulative from one to another.)

Experiment 3. A small-scale pasture trial of a parasitic bronchitis vaccine is described. The vaccine was made by subjecting third stage infective larvae of D. viviparus to 40,000 roentgens of ionising radiation from an X-ray source. A single dose of 1,000 larvae, so attenuated, was given to each of 15 calves with appropriate controls. A considerable degree of protection was conferred on the vaccinates as assessed by clinical and pathological findings, mortality, morbidity, faecal larval counts and worm burdens.

Experiment 4. A larger scale trial, using a single dose of similarly irradiated larvae was carried out on 40 farms on each of which only half of the calves at risk were vaccinated. A total of 531 calves received vaccine and there were 591 controls.

Clinically appreciable parasitic pneumonia occurred on six of the farms with a morbidity of 6% in the vaccinates and 62% in the controls. This was a result highly significant in the vaccine's favour.

Experiment 5. There were indications on theoretical and practical gounds that the immunity conferred by a single dose of 1,000 irradiated larvae might not stand up to either an unusually high challenge or to a modal challenge coming too soon after vaccination. Accordingly, a procedure of double vac cination was applied against the high challenge level of 10,000 infective larvae. The vaccine was given to three groups of calves in two doses; all calves were given 1,000 irradiated larvae as the first dose and 4,000, 2,000, and 1,000 respectively as the second dose. A fourth group of calves received a single vaccine dose. All the twice vaccinated calves were completely resistant to infection on challenge with the 10,000 larvae. The single vaccine group and the controls were heavily infected. The challenge was nevertheless not high enough to differentiate the three double-dosed groups.

Experiment 6. This is a brief description of a large-scale field trial using two vaccinating doses of 1,000 larvae. In this experiment the vaccine was not prepared by the author. All of over 8,000 calves on 204 farms received the course of vaccine. The trial was designed largely to test two factors.

1. The safety of the vaccine under a wide range of farming conditions.
2. The practicability of factory production, packing and transport.

This was partly a test of the scaling up procedure involved in adapting the process to possible commercial production.

From this viewpoint and that of preventing the disease, the trial was very satisfactory although criticisms of its objectivity are discussed.

Experiment 7. An opportunity arose to test the procedure of double vaccination against a natural challenge of great magnitude. Two groups of calves, one appropriately vaccinated, were put to graze on a pasture on which a severe epizootic of parasitic bronchitis had recently occurred. There was a marked difference in clinical signs, faecal larval outputs (all the vaccinates were McMaster negative throughout), worm burdens (the vaccinates contained no adults at post-mortem), growth rates and mortality in favour of the vaccinated group.

Parasitic bronchitis - general illustrations

Figure 1. Infective third stage larva of Dictyocaulus viviparus showing double cuticular sheath, opening of oesophageal gland and terminal excretory pore. X 650.

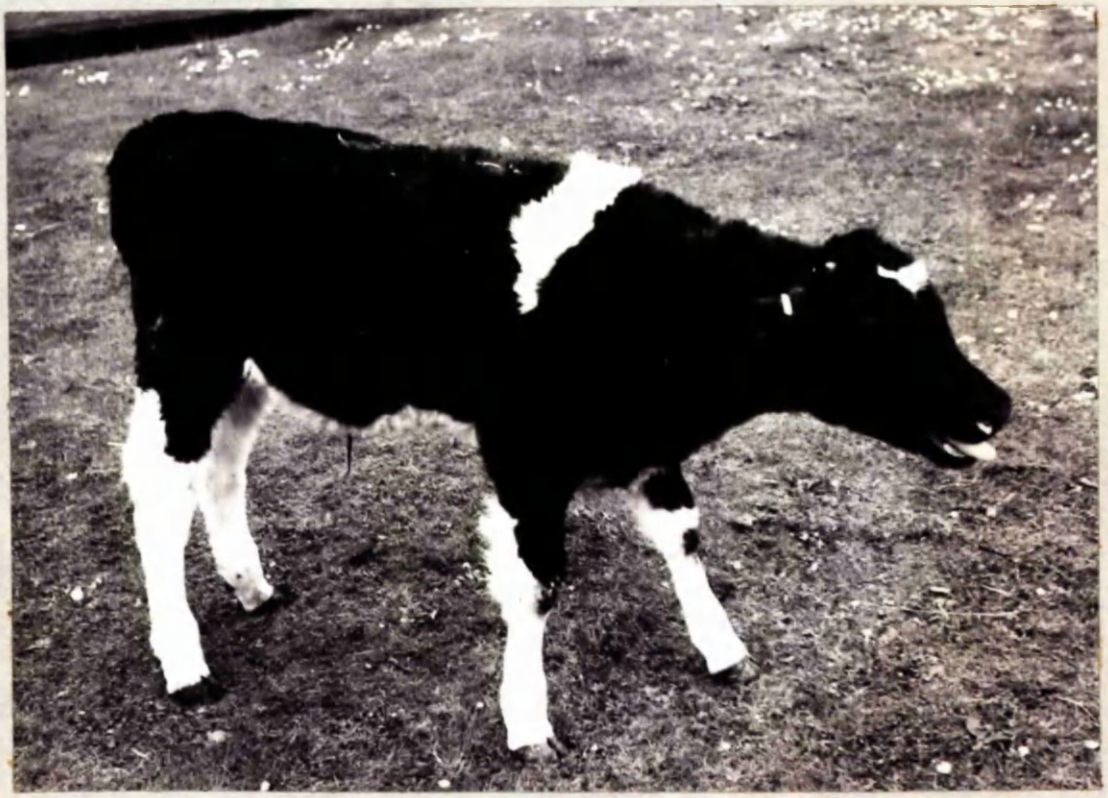


Figure 2. Calf with severe patent parasitic bronchitis standing in the typical 'air-hunger' position.

Figure 3. Opened bronchi from a fatal case of patent parasitic bronchitis. Several hundred D. viviparus adults are present in the lumena. X ½.

PART III

PART III

Section I

Introduction

The preceding part of this thesis described the development of a vaccine against the pneumonia caused by Dictyocaulus viviparus. In 1957 Jarrett, McIntyre and Urquhart described the course of events caused by infection of calves with normal infective larvae of this parasite; this information on the pathogenesis of the disease was obtained by killing experimentally infected calves at fairly close intervals. During the development of the vaccine, due to the nature of the experiments, a similar technique could not be applied and the changes produced could only be studied at a fairly late stage: therefore, only tentative hypotheses could be advanced as to the mode of action of and tissue reactions to the vaccine. The experiments reported in this section were designed to obtain more details of these changes and of the reaction of the immune host to re-infection. This work is described in two papers: Jarrett, McIntyre and Sharp (1962) and Jarrett and Sharp (1963).

Discussion of these three experiments is postponed until each has been separately described because their implications are so closely correlated.

Experiment 8. The relative distribution of normal and irradiated D. viviparus larvae present in the lungs of calves at various times after infection.

Materials and Methods.

The methods of culturing the larvae, irradiating them and administering them to artificially raised worm-free calves were similar to those used previously (vide technical appendix).

The procedure for recovering larvae from lungs, which is described below, was adopted only after extensive experiments had been made in recovering larvae from calves given a wide range of larval dosage; for the present purpose acid and enzymatic digestion techniques were not found to be superior. The method used was as follows. The calf was shot with a captive bolt pistol and the throat was cut to include the oesophagus and trachea; this was successful in preventing the ruminal contents from being aspirated into the bronchial system. The work bench was warmed and kept near body temperature with a hot water hose. Two litres of normal saline at 37°C. were put into a bucket on which the warm hose was playing. The lungs were removed from the body and cut into their seven lobes and each lobe was opened down to the smallest bronchioles using fine probe pointed scissors. The lobe was then shaken and massaged in the saline and immersed in it for two to three hours, the surface with the opened bronchi being placed facing down; during this time the bucket was kept in a warm room at 25°C. The saline was then poured into a Baermann funnel. Each bronchus was washed with one litre of water to which 10 gm. of salt had been added and this fluid was poured into the original funnel.

Four litres of warm normal saline were poured into each of two more funnels, and the lung lobes were apportioned between these to float with the bronchial side down. These funnels were left overnight in the warm room and in the morning 60 ml. was run off from the bottom of each, into two flat-bottomed 30 ml. tubes. This fluid contained the larvae which were counted in squared petri dishes under a dissecting microscope and transferred to glass slides with coverslips for microscopical examination.

Experimental Procedure.

Thirteen calves were each given 1,000 normal larvae and 13 were each given 1,000 larvae which had been subjected to 40,000 roentgens of X-rays delivered at approximately 200 roentgens per minute. The calves were killed in pairs, one each from the 'normal' and 'irradiated' groups on the following days after infection: 1,2,3,4,5,7,9,11,13,15,17,25 and 35. The lungs were subjected to the opening, washing and Baermann processes immediately after killing.

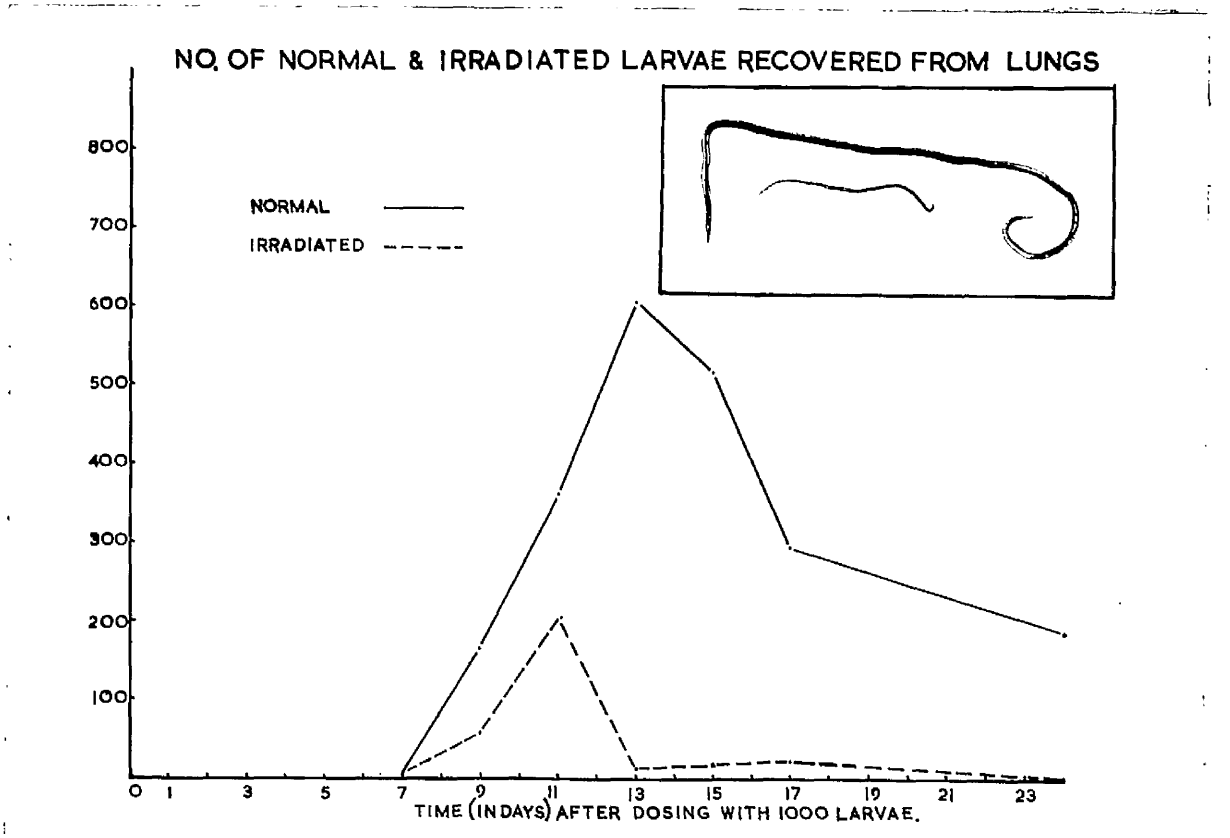
Results.

The numbers of worms recovered are shown in Table I as are their sex ratios. All of the worms recovered up to the eleventh day were at the fourth moult or early fifth stage; after this, the normal larvae followed the usual course of development while the irradiated larvae remained stunted, the largest specimen recorded being 4.8 mm. in length at 20 days. The pictorial insert on Graph 1 shows the size comparison between a 13 day normal early fifth stage female worm and its irradiated contemporary, while the graph illustrates the numerical comparison between the worms recovered.

Table 1. A Comparison between the Worms found in the Lungs of Calves Given Normal Larvae and Those Given Irradiated Larvae.

Days after infection.	Normal Larvae		Irradiated Larvae	
	Number recovered.	Female: male ratio.	Number recovered.	Female: male ratio.
1	0	-	0	-
2	0	-	0	-
3	0	-	0	-
4	0	-	0	-
5	0	-	0	-
7	5	-	7	-
9	181	8:2	65	9:1
11	372	6:4	203	8:2
13	608	5:5	23	8:2
15	535	5:5	34	9:1
17	288	5:5	43	9:1
25	181	5:5	74	10:0
35	231	5:5	0	-

The relative distribution of normal and irradiated larvae.



Graph 1. Numerical comparison between the worms recovered at different days after infection. The pictorial insert shows a typical size comparison between a 13 day normal early-fifth-stage female worm and its irradiated contemporary. X10.

Experiment 9. The pathogenesis of the lesions in calves resulting from the administration of irradiated D.viviparus larvae.

This was designed to study the histological reactions to the larvae as they matured in the lymph nodes and lungs. Part of the material for this was obtained from the previous experiment but the numbers of tissue blocks which could be taken there was limited by the fact that the main data required were quantitative. In order to make a more detailed qualitative study calves in this experiment were given irradiated larvae and successively killed, and all the pulmonary tissue was available for histological examination.

Materials and Methods.

The calves were reared worm free as in previous experiments. Larvae were similarly X-irradiated at 40,000 roentgens. Each calf was killed by shooting and at necropsy approximately 100 tissue blocks were taken from the lungs and 100 from the mesenteric nodes in each case. Fixation was by formol-sublimate; the blocks were dehydrated and cleared in an alcohol-amyl acetate-benzene series under reduced air pressure. Paraffin embedding under reduced pressure was also employed. Routine staining was by haemalum and eosin; other methods used for special purposes were picro-Mallory, periodic acid-Schiff, Feulgen, phosphotungstic acid-haematoxylin, carbol chromotrope, Weigert's fibrin stain and pyronin-methyl green.

In selecting blocks, an attempt was made to incorporate all of the main broncho-pulmonary units; lobes were labelled separately but as there were no obvious predilection sites references to

individual lobes have not been made in the text. Similarly, the mesenteric lymph node blocks were labelled as to position.

Experimental Procedure.

One thousand irradiated larvae were given as a single dose to each of eight calves, which were then killed in pairs on post-infection days 3,7,14 and 21. As it was not known if histological methods would locate such very small numbers of larvae in the relatively great volume of the calf's lung, further calves were given much larger single doses as follows: Four received 50,000 irradiated larvae and were killed 3,5,7 and 11 days later; two more calves were given 250,000 irradiated larvae while two controls received 250,000 normal larvae; all four were killed on day 7. The experimental design is shown in Table II.

Table II. Design of Experiment 9.

Day of Expt.	0	3	5	7	11	14	21
8 calves; nos.27-34	each received 1,000 irrad. Larvae	Nos. 27&28 killed.		Nos. 29&30 killed.		Nos. 31&32 killed	Nos. 33&34 killed
4 calves; nos.35-38	each received 50,000 Irrad. Larvae	No. 35 killed	No. 36 killed	No. 37 killed	No. 38 killed		
2 calves; nos.39&40	each received 250,000 Irrad. Larvae			Nos. 39&40 killed			
2 calves; nos.41&42	each received 250,000 Normal Larvae			Nos. 41&42 killed.			

Results.

One and two days after infection.

No lesions were found in these calves.

Three days after infection.

Lesions were present in the mesenteric nodes and the greatest concentration was in the posterior group which lie in the general region of the ileum, caecum and the first part of the colon. They consisted essentially of small necrotic tracts surrounded by a narrow zone of macrophages and eosinophil leukocytes; When cut longitudinally they extended from the outer cortex to the medulla. Live larvae were not found in them but an occasional tract, on serial section, showed a dead larva which had lost its staining affinity and which was structurally disintegrating.

Neither lesions nor larvae were found in the lungs.

Five days after infection.

The findings were essentially similar to those at three days. Pulmonary changes were absent even in the 50,000 larvae infection.

Seven days after infection.

The lesions described above in the lymph nodes were well-marked in these calves, especially those given 250,000 larvae; giant cells and macrophages lay around the few disintegrating larvae seen.

Pulmonary lesions

1,000 larvae. Of 170 sections examined, only three had lesions and these were of the type associated with the breakthrough of larvae into alveoli. (Fig.4). These foci usually involved four or five alveoli and in these were found fibrin and some erythrocytes from capillary haemorrhage.

There was usually a small fibrinoid necrotic centre surrounded by a light infiltration of neutrophils, eosinophils, macrophages and one or two foreign-body type giant cells. Larvae were present in a proportion of these lesions.

50,000 larvae. In addition to an increased number of breakthrough lesions, two bronchi contained a larva and this was surrounded by a light exudate of eosinophils.

250,000 larvae. Two of the four calves in this group were given normal larvae for comparison of lesions as this size of infection at this stage had not been used in the earlier work. (Jarrett, McIntyre and Urquhart 1957). Whereas in the 1,000 and 50,000 larvae infections described above there were no visible changes at post mortem, in the present calves there were many areas of lobular collapse scattered over all lobes. These were especially well-marked around the subsegmental bronchi. There was also sub-pleural emphysema which was present in greatest amount on the surfaces of the posterior part of the diaphragmatic lobes. The broncho-mediastinal nodes were obviously enlarged and oedematous.

There was no difference in appearance between the calves which had been given normal and those which had been given irradiated larvae. Microscopically, in one of the two normal infections larvae were found with ease in almost every section; most were situated in the bronchi and some were found in the trachea. There was an obvious increase in eosinophils in the bronchial lamina propria and they could be seen migrating between the epithelial cells in large numbers.

An eosinophil infiltrate was also present in the interlobular and peribronchial septal tissues and the latter were often oedematous. In the other calf, larvae could easily be found in the bronchi but they were not present in such great numbers. The alveolar reactions were very much more marked in this animal, breakthrough lesions being present in almost every section. It was obvious that this infection had not progressed as far as the first.

In the calves given irradiated larvae the picture was quite different in three respects.

First, although many normal breakthrough lesions were found, a large number showed a more marked eosinophil infiltrate in them. Secondly, there was a large number of lesions which represent the first stage in a process which results in what will be called the lympho-reticular broncho-occlusive lesion (L.B.L.) and which is discussed in detail later. In lesions of this first stage, the components from within outwards are:

1. An immobilised, dying or dead parasite in the lumen and usually in direct contact with the epithelium (figs. 5 and 6).
2. When the parasite is intact, has just started to lose its staining affinity or is mildly damaged structurally, the epithelium with which it is in contact may show bizarre hyperplasia (fig. 7) in which can be seen multi-nucleated and hyperplastic cells which have lost their normal polarity in the epithelial sheet.
3. The lamina propria is usually increased in thickness especially in the larger bronchioles and bronchi and in

addition to an infiltrate of eosinophils shows multiplication of the reticulum cells and swelling of endothelial cells (fig 7).

4. When the epithelium is ulcerated, fine granulomatous tissue can be seen growing out of the lamina propria into the eosinophil exudate surrounding the dead or dying parasite, (fig.8). These lesions take up a much larger part of the lobe than does a normal bronchiole.

The third point of difference between the lungs of the calves receiving irradiated larvae and those receiving normal larvae was the considerable difficulty in finding living larvae in the bronchi compared to the ease with which this could be done in the normal infections.

In the broncho-mediastinal lymph nodes there was oedema of the hilar area and infiltration of eosinophils into it; these were concentrated particularly around the small blood vessels and extended with them into the substance of the lymph nodes. The cortices of the nodes showed increased activity of the germinal follicles, thickening of the cortical layer and an increase in the number of immature plasma cells in the medullary cords. The medullae showed hypertrophy and hyperplasia of the sinus cells. In a few cases disintegrating larvae were found surrounded by a foreign-body reaction.

9 days after infection.

1,000 larvae. There were no significant qualitative differences from the day 7 infection although there were more breakthrough lesions.

50,000 larvae. Many breakthrough lesions were present as well as occasional larvae in bronchi and bronchioles surrounded by exudates of eosinophils. There was no obvious alveolar collapse associated with these lesions.

11 and 13 days after infection.

As these show merely a transition between the 9 and 14 day infections and since the latter were studied in considerably greater detail no further description of these two cases will be given.

14 days after infection.

At necropsy in one case a few collapsed lobules were obvious while in the other this lesion was more marked and dark red partially collapsed lobules were found, particularly in the diaphragmatic lobes. Microscopically, in the first case, no larvae were found but the areas of alveolar collapse were associated with occlusion of the related bronchioles by plugs of disintegrating eosinophil leukocytes (fig. 9). The epithelium of these bronchioles was intact and no L.B.L.'s were seen. The eosinophil exudate extended in some cases to the respiratory bronchiole but seldom below this level. In other words, there was no pneumonia present but merely alveolar collapse distal to plugged bronchioles. There was no qualitative difference in the lesions in the second case but there were more of them and a few of the bronchi contained living larvae.

In the bronchial nodes, the germinal centres were very obvious with an apparent depletion of cells between them.

The reticulum cells of the sinuses of the medulla were very prominent and there was again marked oedema and eosinophil infiltration of the hilar connective tissue and trabeculae.

15 days after infection.

In this calf, very few lesions were found and these consisted of bronchioles plugged with dead eosinophil leukocytes.

17 days after infection.

This represents an intermediate stage between the 14 and 21 day infections.

21 days after infection.

At necropsy, only an occasional lobule of collapse could be found. In addition to these, a few lobules showed a central grey nodule of about 2 mm. diameter which on pressure yielded a small green plug, (fig. 10). Most of these were obviously developed from the broncho-occlusive lesions described earlier and showed a central dead larva surrounded by a focus which sometimes contained epithelium but sometimes did not and of which the main feature was the multiplication of reticulum cells, haemocytoblasts and the presence of immature plasma cells; the increase in number of these cells had begun to obliterate the architecture of the affected bronchiole. One lesion was found in which the worms had reached young adult stage before dying and there was disruption of the epithelium, growth of young connective tissue and proliferation of reticulum cells and haemocytoblasts, many of which had pyroninophilic cytoplasm. Both of these lesions had the appearance of a developing antibody-producing site.

Apart from these few localised nodules there were some lobules which represented a resolution of the 14-day picture, i.e. fewer eosinophils in the bronchi and bronchioles and re-expanding lobules with a general regression of exudative reaction.

25 days after infection.

This was very similar to the 21 day infection.

35 days after infection.

In this case there was no evidence of bronchiolar plugging or alveolar collapse. A few L.B.L. lesions were seen macroscopically and were examined histologically (fig. 11). These showed a further extension of the development of this lesion and consisted of a central dead eosinophilic larva surrounded by tissue indistinguishable from lymph nodal tissue (fig. 12). Germinal centres were present and these and their surrounding lymphocytes and plasma cells obscured the normal bronchiolar structure almost completely. Fragments of bronchiolar muscle could be found among the lymphoid aggregates. These lesions occupied up to 70% of the area of the lobule. No living parasites were found in the sections.

Experiment 10. The pathogenesis of the lesions of re-infection with D. viviparus in calves previously given two doses of either irradiated or normal larvae.

The general design of this experiment was to follow a double vaccination procedure similar to that described earlier for immunising calves and then to challenge them with a fairly heavy infection; the course of that infection was then investigated by killing the calves at intervals. An identical group was given normal larvae at each vaccine time to compare this treatment with the former.

Material and Methods.

These were the same as those of the previous experiment.

Experimental Procedure.

The number of larvae in each immunising dose was 1,000 for both groups. Those given to the 'irradiated' group had been subjected to 40,000 roentgens as before. The first dose was given on day 0, the second on day 42, and the challenge, of 10,000 normal infective larvae, on day 93. One pair of calves from each group was killed on the 3rd, 7th, 14th and 21st day after challenge.

Results.

Before describing the results in detail, two general points can be made to avoid repetition. In all of the calves previously given normal larvae, as would be expected, lesions of the late stage of uncomplicated parasitic bronchitis were found; these have been described previously by Jarrett, McIntyre and Urquhart (1957).

Other lesions common to animals of both groups were well developed lymphoreticular nodules as described in experiment 9; these presumably were derived from the second infection in the case of the group having normal larvae and from both infections in the group having irradiated larvae. They were few in number and were selected macroscopically at necropsy.

3 days after infection.

Normal larvae. At post mortem, the lungs of one of the calves showed occasional, and the other widespread, lesions of old parasitic bronchitis. Microscopically, apart from the old lesions most sections showed a little deviation from normal; one contained a very large lymphoid nodule forming around a dead adult worm. There was no evidence of the challenge infection having reached the lungs.

Irradiated larvae. Apart from a few grey-pink lentil-sized broncho-occlusive lesions scattered throughout the substance, these lungs were normal, macroscopically and microscopically.

7 days after infection.

Normal larvae. At necropsy the lungs from both calves were about one-third consolidated. In the first, the lesions were distributed widely in both diaphragmatic lobes and the main bronchi contained much partially inspissated green pus. No worms were found in this case. The lungs of the second calf were enlarged in volume, mainly due to the emphysema which was present on the under-side of the diaphragmatic lobes. Both apical and cardiac lobes were almost completely consolidated. When these lungs were

dissected the distribution of lesions was seen to be of the usual pattern with the majority of the consolidated lobules lying immediately around the segmental bronchi.

Irradiated larvae. These lungs were identical with those from the three day infections.

Microscopically, both 7-day groups had similar appearances. There were many breakthrough lesions in alveoli, as described under experiment 9, but the eosinophil infiltration was of a more intense degree. Larvae had reached some of the small bronchioles and epithelial hyperplasia and necrosis as described above (L.B.L.) were prominent (fig. 13). Near these lesions there was heavy eosinophil infiltration into the septal tissues and a similar infiltration was very marked in the hilar and trabecular areas of the broncho-mediastinal lymph nodes.

14 days after infection.

Normal larvae. The macroscopic appearance of these lungs was the same as in the 7-day infections; in one, 12 adult worms were found, eight being female and four male.

Irradiated larvae. There was no consolidation in either of these cases. Nodules of the L.B.L. type were present over the surface of both diaphragmatic lobes, (fig. 14) and on the ventral surface of these lobes were several green translucent plaque-like areas, 1.0 x 0.5 cm. in size, located subpleurally. Worms were not found in either of these calves.

The histological findings were the same in both groups, and the type reaction in these cases was the middle stage of the development of the lymphoreticular broncho-occlusive lesion. The dead worm in these lesions was situated centrally and this was

surrounded by giant cells, some of which were of the mesenchymal foreign-body type, while others obviously consisted of residual areas of hyperplastic bronchiolar epithelium. Immediately external to this was a zone in which there was a mixture of eosinophils, proliferating endothelial cells, macrophages, immature plasma cells, pyroninophilic haemocytoblasts and reticulum cells. External to this was a band of lymphocytes and plasma cells with the beginning of germinal centre formation. These lesions were quite numerous throughout the sections and occupied from a half to a third of the area of the lobular cross-section. The broncho-mediastinal lymph nodes showed eosinophil infiltration particularly in their hilar areas and dead larvae were not uncommon in the cortices. These were surrounded by giant cells and early fibrosis.

21 days after infection.

Normal larvae. The lungs of one calf showed a few scattered areas of lobular collapse and a few small patches of interstitial emphysema. Grey-pink miliary nodules were present on the surface and in the substance of the lungs. The second calf had about one-third of its lungs consolidated, the affected areas being largely in the mid-ventral segments of the diaphragmatic lobes and in the intermediate lobes. Numerous L.B.L. nodules were present throughout the lungs. One adult female worm was found in a bronchus. An attempt was made in these cases to tease larvae out of the new L.B.L. nodules for examination, but this was unsuccessful.

Irradiated larvae. One calf showed a few scattered areas of individual lobule collapse. Many L.B.L. nodules

were present subpleurally and throughout the substance (fig.15). This was especially marked in the cardiac lobes. The other calf had areas of collapse in the posterio-ventral segment of both diaphragmatic lobes. Many L.B.L. nodules were present throughout the lungs and several small bronchi had plugs of green pus. No worms were found in either of these calves.

Microscopically, all of these lungs showed a similar appearance and the type lesion was again the development of lymphoreticular nodules. At this stage, most of the eosinophil and giant cell reaction around the worms had disappeared and the whole area had taken on the appearance of lymph nodal tissue with a centrally placed larva. Germinal centres were well-developed and some of these nodules had the appearance of lobulation (fig. 16).

DISCUSSION.

The original object in irradiating larvae was to attenuate them so that they would not be pathogenic but would still be able to stimulate an immune response which would be capable of protecting the host against the naturally occurring disease. In the field the latter is associated with the pulmonary part of the helminth's life cycle; the commoner form of the condition is a bronchitis and pneumonia caused by sexually mature adult worms but many fatal cases are associated with heavy burdens of fifth stage larvae (Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart 1960).

We had speculated that the mesenteric nodes might be a site of protective antibody formation and our original intention was to prepare a vaccine the larvae of which would not progress further than this. It was obvious, however, from the mild and transient clinical signs seen during the immunising period (Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart 1959) that some larvae were reaching the lungs. The results recorded above give some indication of the fate of the attenuated larvae and of the host's reaction to them.

From Table 1 of experiment 8, it will be seen that the larvae started to appear in the lungs seven days after dosing and that the highest number in 'normal' infections occurred at the 13-15 day period. The count then fell to about 250 and this ratio of infecting dose to final worm burden of approximately 1:4 is the general rule under our experimental conditions.

It is possible that these larvae which migrate into the large bronchi and trachea while they, the larvae, are still small, are washed out by the mucus and cilia and this may account for the drop in numbers noted;(if, during latter half of the prepatent period, calves are killed and scrapings of tracheal mucus made almost immediately, many active larvae can be found even in the upper half of the trachea).

In the irradiated group the highest larval count recorded was 203 so it appears that the majority either did not reach the lungs or were quickly expelled if they did. The histological evidence is in favour of the former view. This maximum count was recorded 11 days after infection and it is interesting that, as recorded earlier, the peak clinical reaction is found to occur a little later. The histological findings probably explain this; the clinical signs, of which tachypnoea is the most prominent, are almost certainly caused by the areas of lobular collapse. These latter are associated with plugging of the related bronchioles by an exudate of eosinophils and this lesion was most obvious at 14 days (fig. 10). The eosinophils had disappeared shortly after this and the consequent re-expansion of the alveoli explains the quick clinical resolution. It should be emphasised that these reactions, both clinical and pathological, are very mild and are of no practical significance.

Also from Table 1, it will be seen that the sex ratio of the lung population resulting from the irradiated larvae is markedly in favour of the females. This phenomenon has been observed in the survivors of irradiated larvae of other parasites, e.g. H.contortus

and T. colubriformis, and it will be commented upon in Part IV.

The exact fate of the irradiated larvae is still uncertain. Dead larvae were found in the mesenteric nodes on histological examination but this is in no way quantitative. We have repeatedly attempted, both by maceration and by enzymatic digestion, to evolve a method by the use of which larvae could be recovered from the mesenteric nodes and which would yield results capable of being replicated and handled statistically; but although some larvae can be recovered the yield is neither large nor constant. The large volume of these nodes and their structure are probably largely responsible for this and in this respect the large domestic animals are inferior to the small laboratory rodents which are more usually employed in parasitological work.

The results show that the important immunogenic larval stages are probably the third and fourth, but the main antibody-producing site in the host cannot yet be definitely recognised. The mesenteric nodes probably play a part but the marked broncho-mediastinal lymph node enlargement always associated with immunity in parasitic bronchitis, and the concommitant increase in cells of the plasma cell series almost certainly indicates the participation of these nodes. It is, of course, likely that both the mesenteric and thoracic nodes are involved and, in the hyperimmune animal, reactions in the intestinal wall may even contribute. The fluorescent antibody technique could well be used to explore this further.

It is germane at this point to digress and consider the life cycle of D. viviparus. Previous work (Jarrett, McIntyre and Urquhart, 1957) had indicated that larvae do not reach the lung in any significant number until the end of the first week after infection and, as the first experiment in this section shows, they are by then at the fourth stage. Douvres and Lucker (1958) and Poynter, Jones, Nelson, Peacock, Robinson, Silverman and Terry (1960) observed that in the guinea pig, third stage larvae could be found in the lung within 24 hours of dosing, and that the third moult took place in that organ. This experimental system is, of course, highly artificial as the guinea pig is not a normal host of the parasite. Nevertheless, very important results have been obtained by its use; Douvres and Lucker (1958) gave a morphological description of the various stages of the parasite, and Poynter, Jones, Nelson, Peacock, Robinson, Silverman and Terry (1960) evolved a method of testing the invasiveness of normal and irradiated larvae by utilising the guinea pig instead of the much more expensive calf. We feel, however, that it is dangerous to assume that the life cycle in the bovine can be adduced from the behaviour of the parasite in the guinea pig. We have been able to recover small numbers of third stage larvae from calf lungs 24 hours after infection by giving very large doses, but most of our findings indicate that this is not the normal course of events. Poynter, Jones, Nelson, Peacock, Robinson, Silverman and Terry (1960) gave doses of 200,000 larvae to calves (4,000 larvae is approximately a 25% lethal dose) and recovered a range of from four to 5,225 larvae from the lungs of individual animals in the first three days after infection;

their mean recovery was four, 1,109 and 3,348 on days 1,2 and 3 respectively, representing 0.00002%, 0.5% and 1.5 respectively of the dose administered. They reach the conclusion that in calves, the larvae "reach the lung where subsequent development occurs and that they do not dwell as fourth stage larvae in the lymph nodes" yet in a calf killed by them 2 days after infection, only 6 larvae were recovered from the lungs and 5 from the mesenteric lymph nodes;in another 705 were found in the lungs and 905 in the lymph nodes; it is neither stated nor speculated upon where the rest of the 200,000 larvae were. It would appear at least possible that these workers, like ourselves, have not yet found a reliable and repeatable method of recovering larvae from the mesenteric lymph nodes of the calf.

By analogy, if we had obtained a proportionate recovery of larvae from the lungs with the dose (1,000) used, we should have had the expectation of finding 0.5, and 15 larvae on the first three days, and we consider that such evidence in no way leads to any alteration in the conception of the life cycle as we have outlined it. The histological evidence from both this and earlier work (Jarrett, McIntyre and Urquhart 1957) is also very much in favour of the great majority of the larvae reaching the lungs later than 1 week after infection.

From data presented, Poynter, Jones, Nelson, Peacock, Robinson, Silverman and Terry (1960) also reached the conclusion that "irradiated larvae reached the lungs and no evidence of a mesenteric lymph node hold up was seen".

Their figures, after giving 200,000 irradiated larvae, are as follows: one day after infection, six larvae were seen in the lungs and one in the mesenteric nodes; at two days after infection three calves were examined and the respective figures for lungs and nodes were 1,480 and 50, 185 and 40, and 5 and 0; at three days one calf had 105 and 0; at four days the findings in three calves were 255 and 5, 80 and 0, and 15 and 0. The combination of these low percentage recoveries from the lung and failure to demonstrate almost the whole of the infecting dose elsewhere leads one to doubt very seriously whether these small numbers reaching the lung in the early part of the cycle play any major part in the immunising process. They give no data on any calves killed later in the life cycle.

A query that could be made is; do the methods used preclude the recovery of larvae in the first few days after infection because they might be trapped in foci of cellular or exudative reaction in the lungs? We failed to find such foci histologically and we also failed to recover appreciably larger numbers of larvae by macerating or mincing the lung tissue and then subjecting it to trypsin and pepsin digestion, which might have helped free trapped larvae.

The second main point to emerge from the experiments described is the development of lympho-reticular nodules in the walls of bronchi and bronchioles. These foci had the morphological characteristics of antibody-producing sites and it may be asked, do they play any part in the formation of protective antibodies? This cannot be answered definitely at the moment but they were not found in any great numbers after the initial dose of irradiated larvae although they were

present after the second dose. We know (Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart 1959) that two doses of 1,000 larvae produce a much greater resistance to reinfection than one dose so it is possible that these lesions may play such a part. It is equally possible that the increased number seen after the second dose merely resulted from larvae killed by the immunity derived from the first dose as this is the typical lesion seen when the immune host deals with invading larvae; this is clearly shown in experiment 10. It is also possible that this is one of the sources of antibody to 'somatic' antigens; these nodules are obviously in close contact with the disintegrating bodies of the parasites andit could be hypothesised, purely speculatively, that the irregular results obtained in complement fixation tests using 'somatic' antigens might be related to the presence or absence of an appreciable number of such antibody-producing sites. Poynter, Jones, Nelson, Peacock, Robinson, Silverman and Terry (1960), Cornwall (1960) and ourselves have each found that the expected secondary response in complement fixation titre after the second dose of vaccine does not always take place, although the resistance to reinfection is much higher, whereas a rise is almost invariable after a challenging infection. Large numbers of lympho-reticular lesions are found after such a challenge. Cornwall (1960) suggested that the serological response was caused by the larvae which reached the lungs and that in those animals which did not show it the larvae had been stopped in the mesenteric node. From our work, it would appear that this is partly true, but it seems possible that in most cases larvae do reach the lungs and that those which become trapped in broncho-

-occlusive lesions produce antibodies detectable by the 'somatic' antigen. It must also be borne in mind, however, that we have repeatedly found disintegrating larvae in the broncho-mediastinal lymph nodes and this large volume of antibody-producing tissue can by no means be neglected as a possible source of 'somatic' antibodies.

What appears to be beyond doubt is that this lesion is at least partially responsible for the clinical signs seen when an immune animal is subjected to a high level of challenge and we have encountered several cases of death in adult cows from this cause (Jarrett, Jennings, McIntyre, Mulligan Sharp and Urquhart 1960). Identical lesions have been found in calves challenged after being protected with immune serum (Jarrett, Jennings, McIntyre, Mulligan and Urquhart 1955) or with killed vaccine (Jarrett, Jennings, McIntyre, Mulligan and Urquhart 1960) and in infected calves, the larvae in whose lungs have been killed by diethylcarbamazine (Jarrett, McIntyre and Sharp 1962) as is described in the next experiment. It would appear that the common factor in all these is the presence of dead or injured larvae or worms trapped in the air passages so that they make contact with the respiratory epithelium. It is not intended to convey the impression that all larvae killed in the lungs are dealt with in this way; most are probably shed via the air passages and only these retained lead to this response.

We are very much aware of the fact that the data presented in this section were obtained from single calves or pairs of calves killed at given points and that these data are not capable of adequate statistical treatment for their practical evaluation.

It should be said, however, that such work, carried out in calves which have to be bought soon after birth and reared individually parasite-free in isolation, is very expensive and that the techniques described, both histological and parasitological, are extremely time consuming so that only a small number of animals can be dealt with each day in the required detail. The most that can be said is that the general trend of the data permits certain limited and qualified conclusions to be made and that these add to the understanding of the mode of action of vaccines of this type.

Illustrations for Experiment 9.

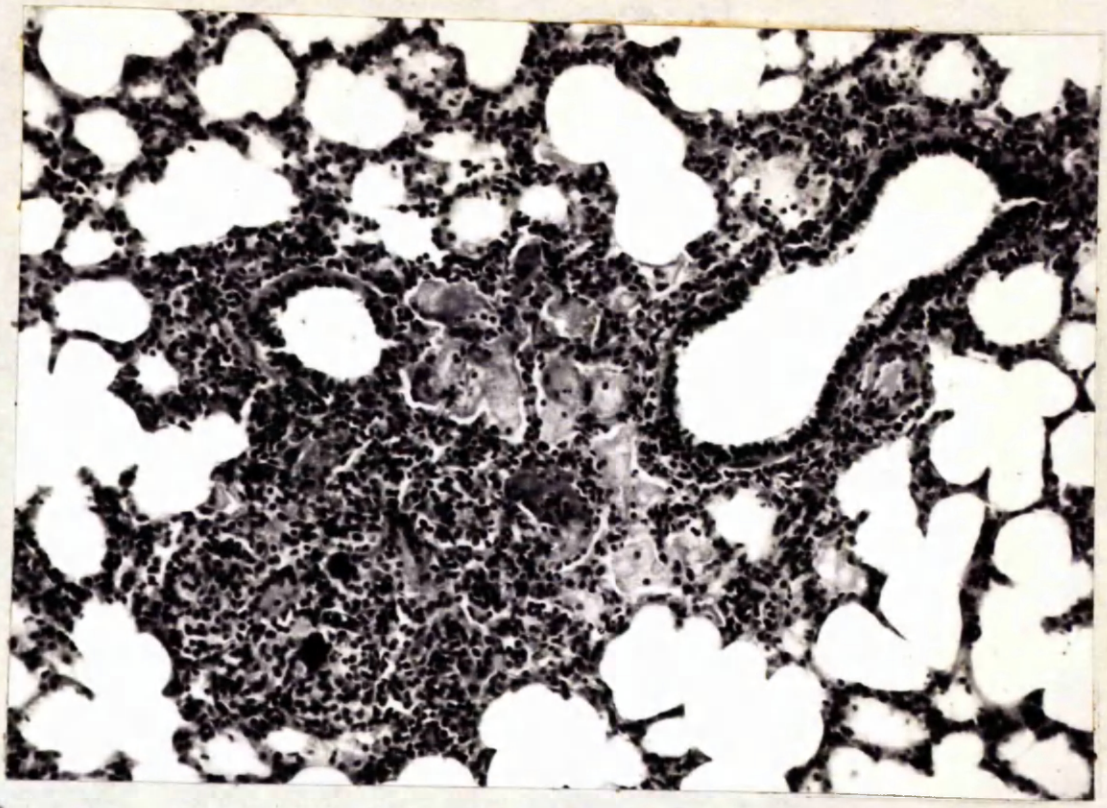


Figure 4.
The breakthrough lesion. A few alveoli containing fibrin, foreign-body giant cells and leukocytes. X 180.

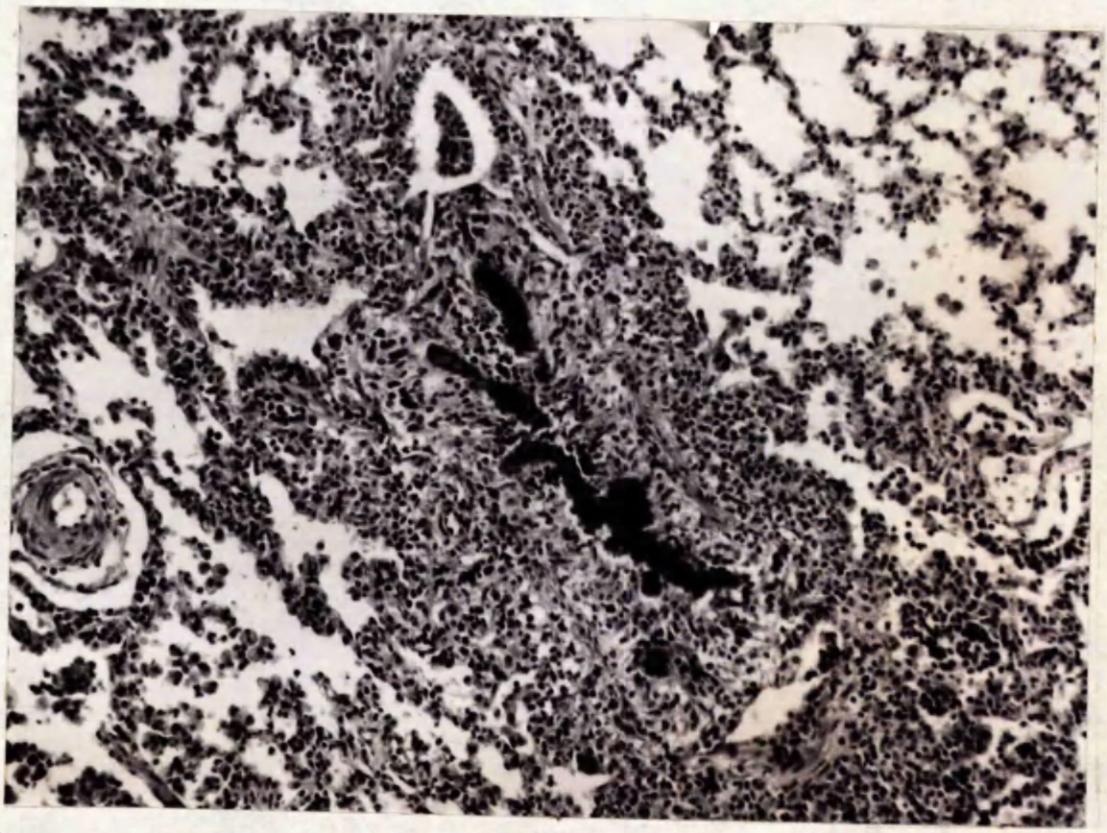


Figure 5.
Disintegrating parasite in bronchiolar lumen. Early bizarre epithelial hyperplasia. X 150.

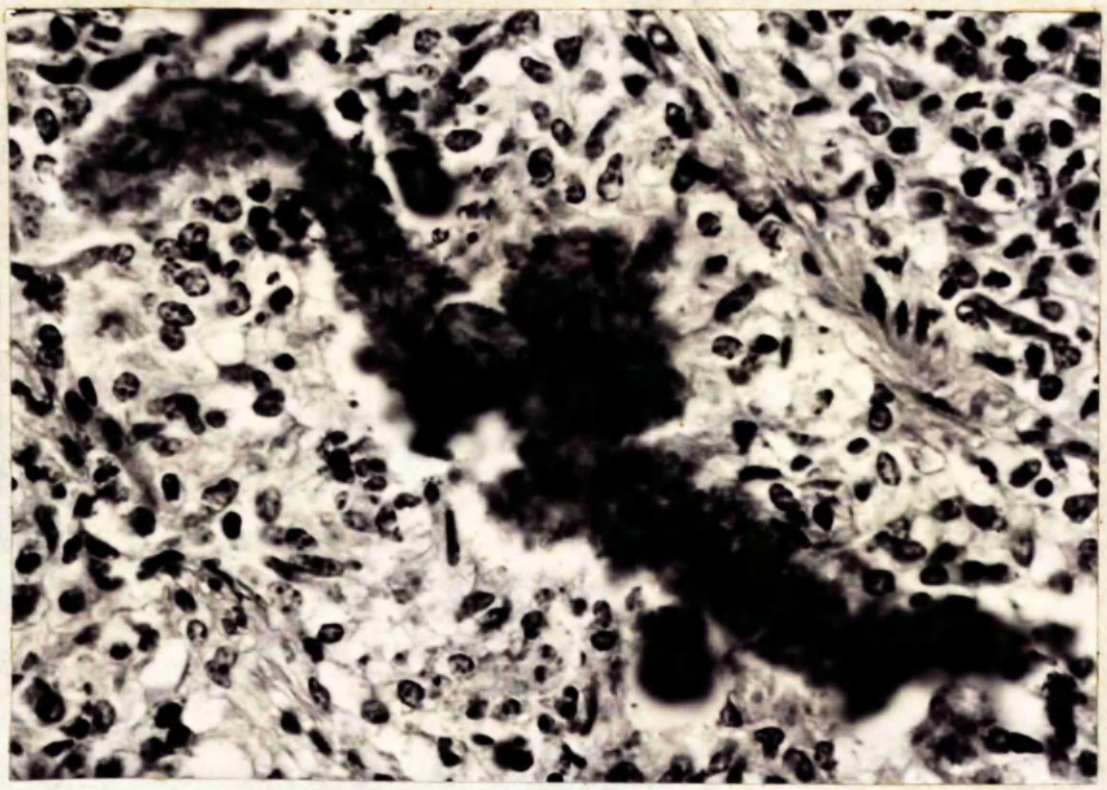


Figure 6.
Detail of figure 5. Early bizarre epithelial hyperplastic response to disintegrating parasite. X 560.

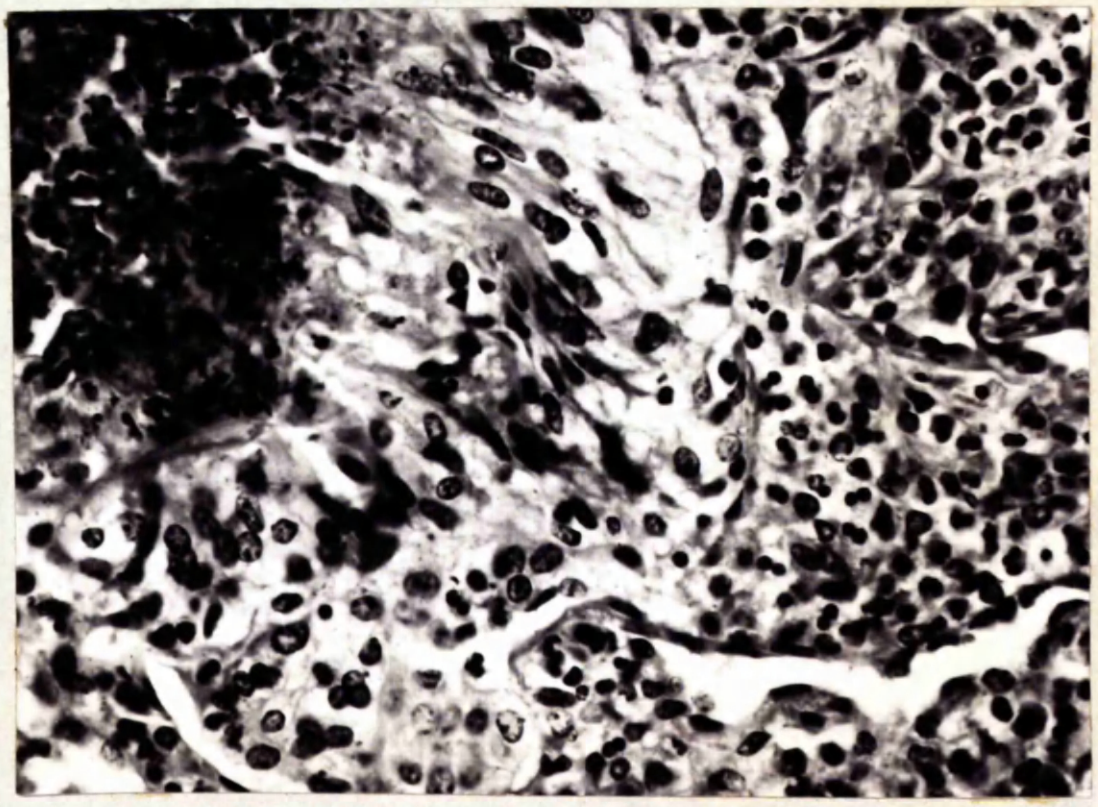


Figure 7.
Irregular hyperplasia of bronchial epithelium, infiltration with eosinophils, and activation of reticulum cells of lamina propria. X 560.

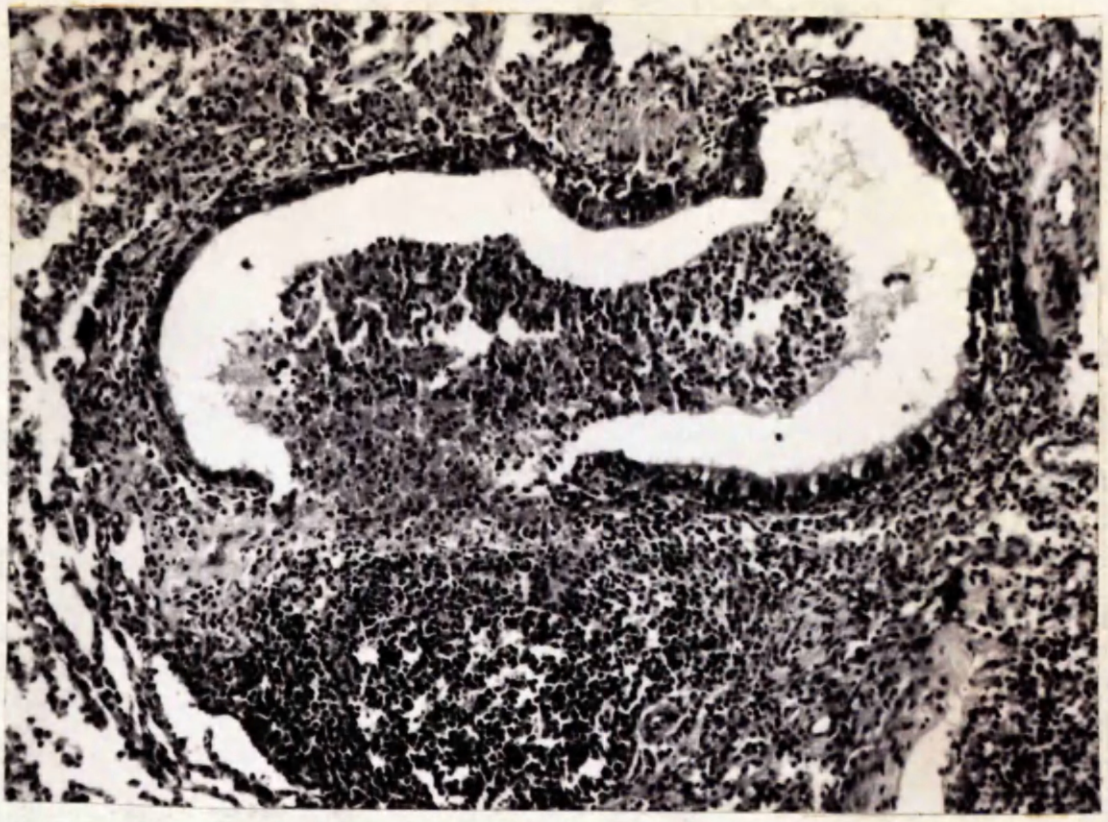


Figure 8.
Ulceration of bronchiolar epithelium and early organisation of exudate. X150.

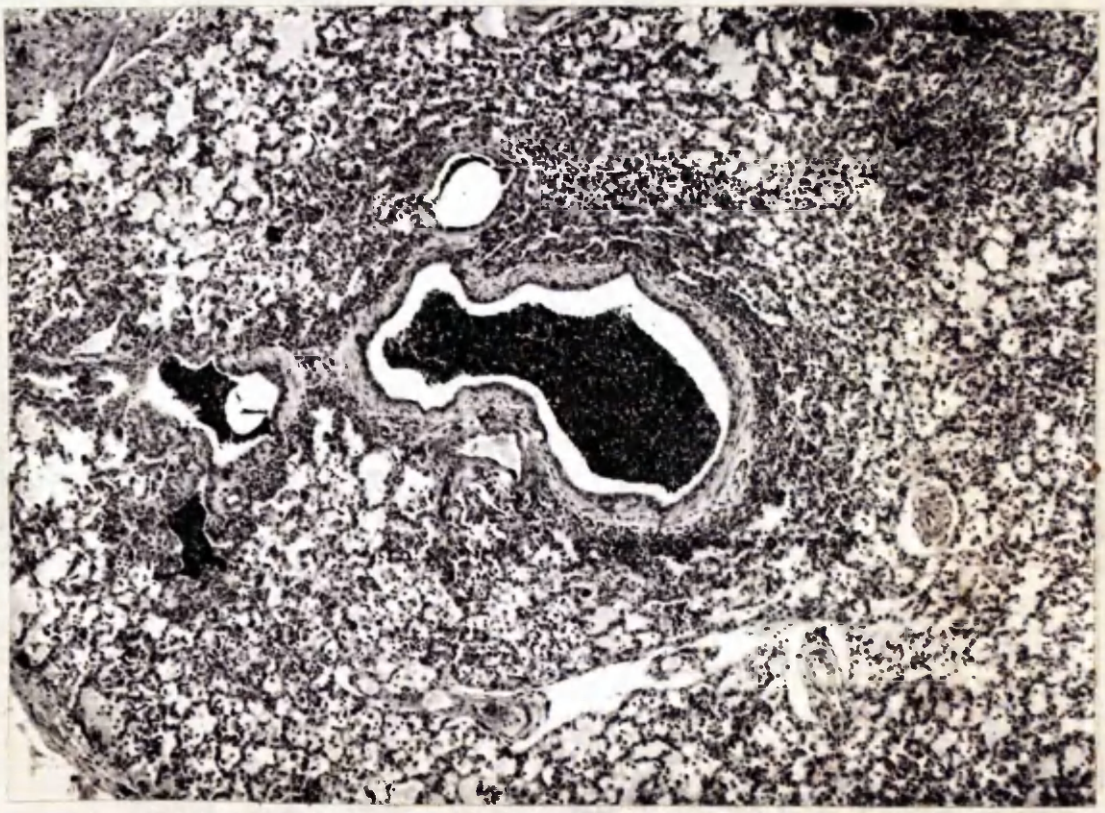


Figure 9.
Occlusion of undamaged bronchiole by plug of degenerating eosinophils, and associated partial collapse of lobule. X50.

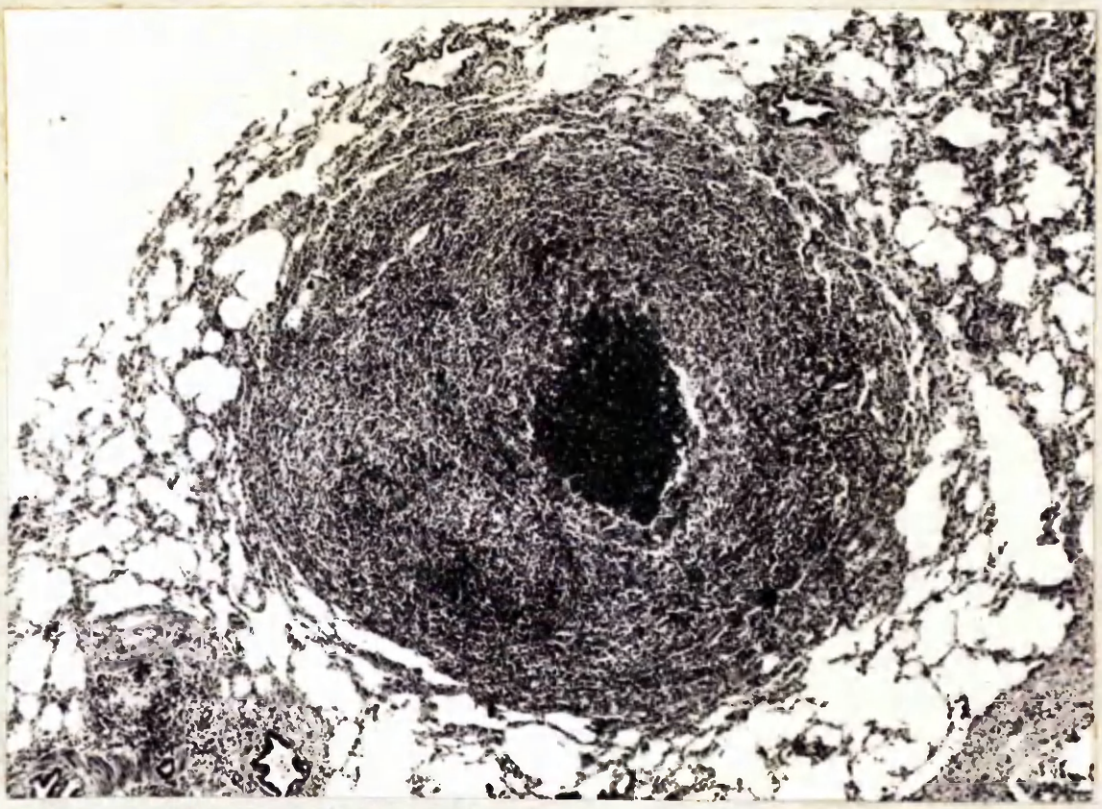


Figure 10.
Development of broncho-occlusive lympho-reticular nodule. Centre of degenerating eosinophils surrounded by activated reticulum cells, haemocytoblasts, plasma cells and cells of the lymphoid series. X 50.

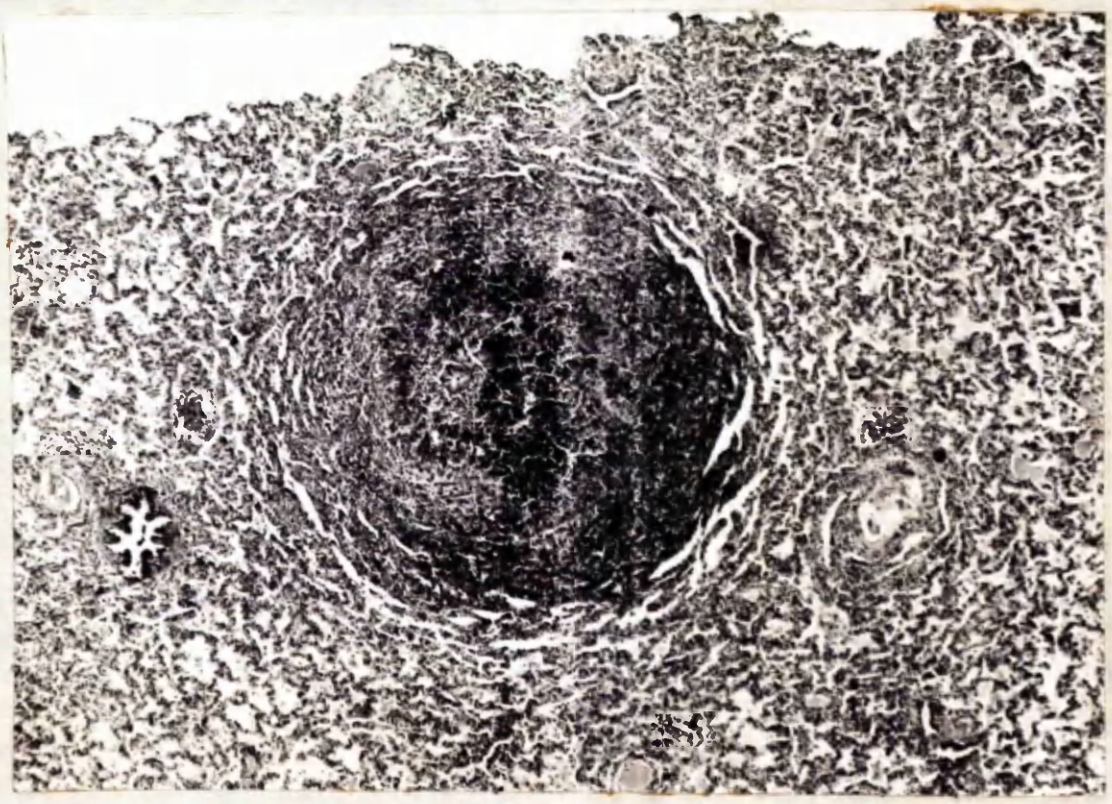


Figure 11.
Further development of lympho-reticular nodule. Central dead nematode surrounded by lymph-nodal type tissue. X50.

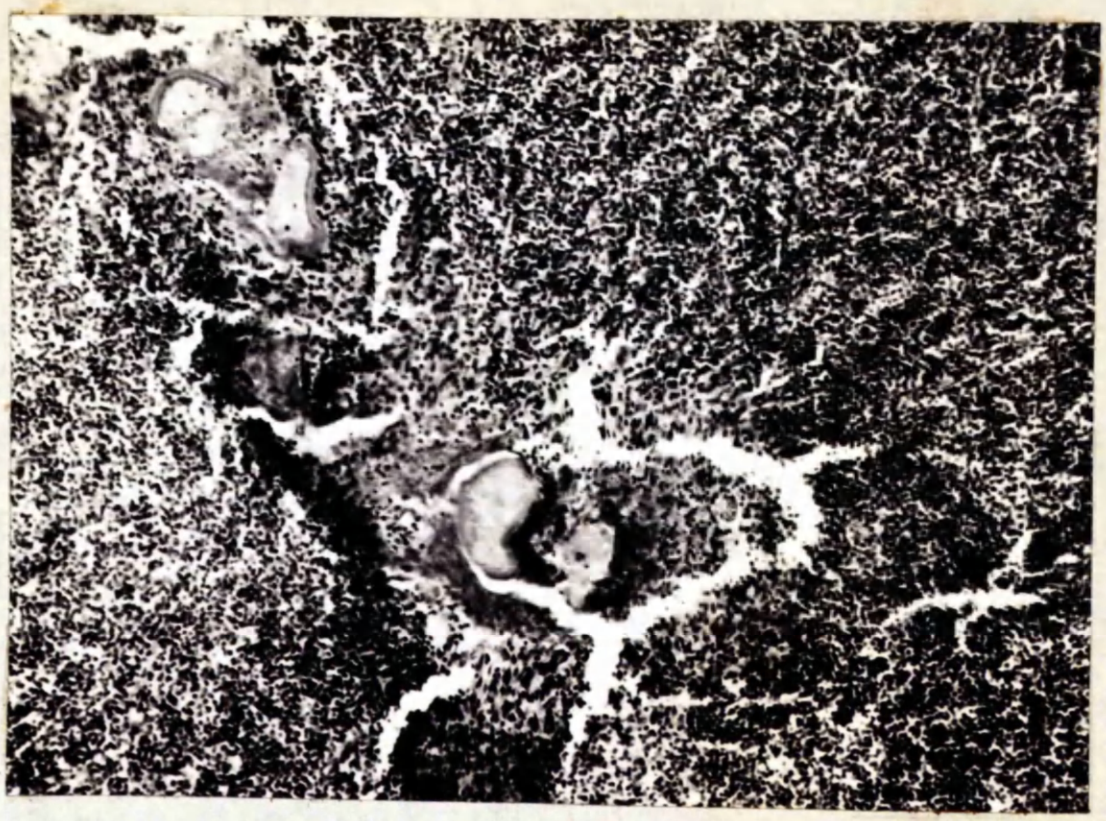


Figure 12.
Detail of centre of developing lympho-reticular nodule. Disintegrating nematodes which have lost their normal staining affinities. Worms now intimately surrounded by lympho-reticular tissue. X50.

Illustrations for experiment 10.

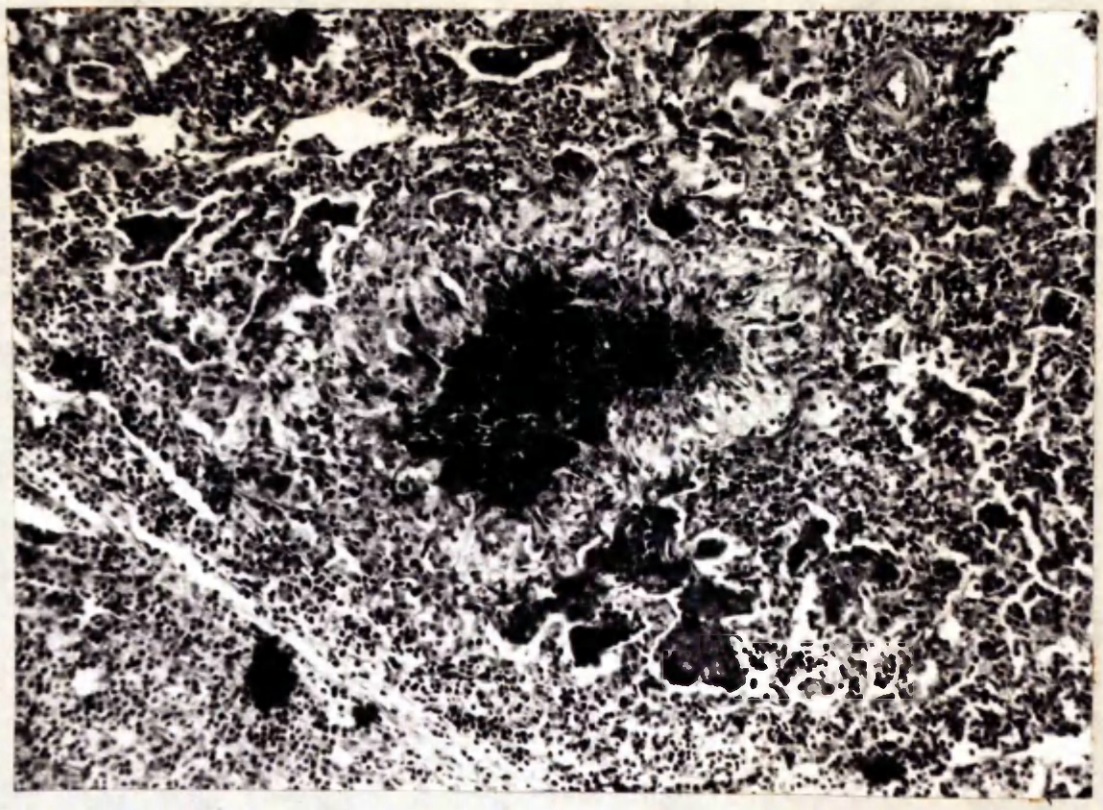


Figure 13.
Bronchiole in re-infected case with eosinophils in lumen, and epithelial hyperplasia. X 150.

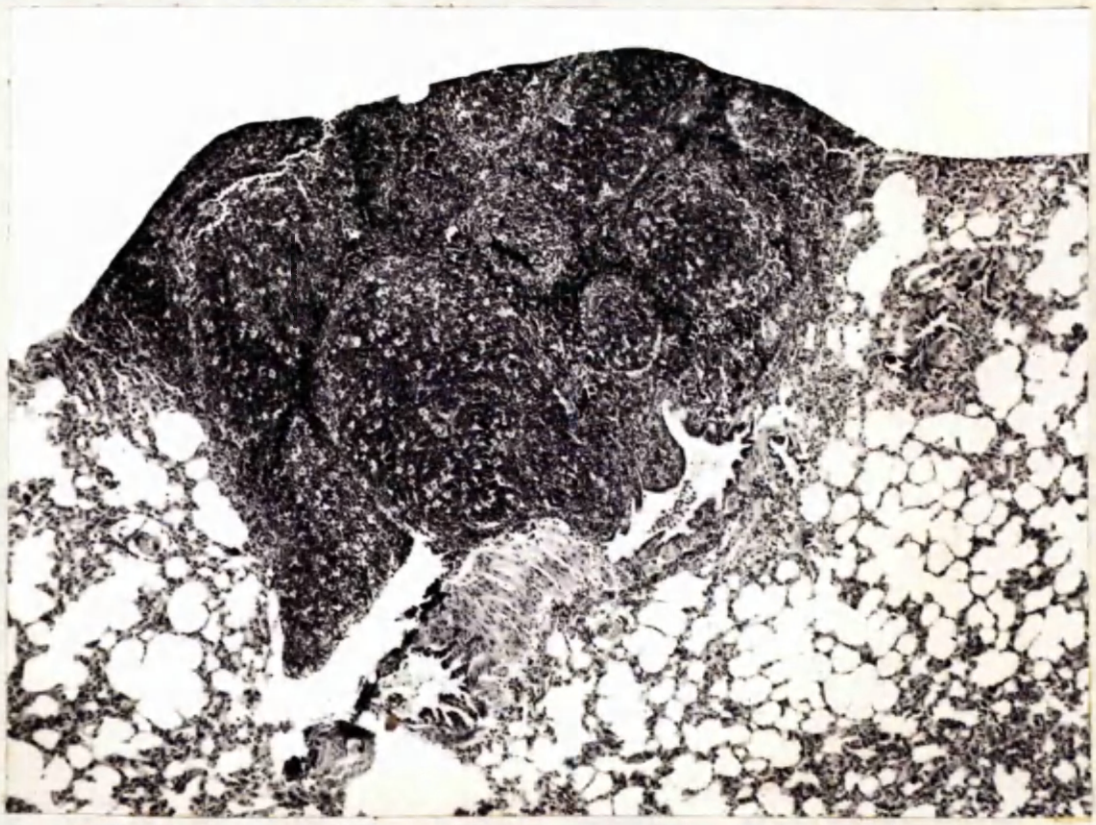


Figure 14.
Fully developed lympho-reticular nodule, with germinal centres, protruding from pleural surface of lung. X50.

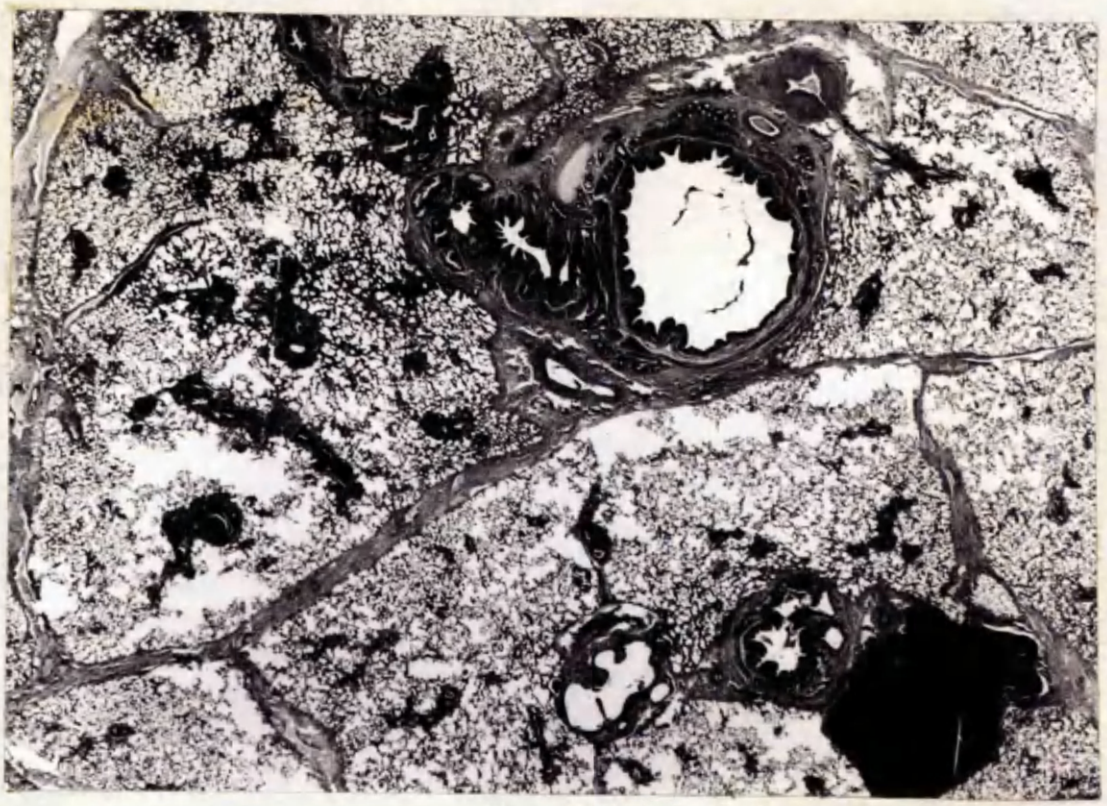


Figure 15.

Lympho-reticular nodule showing size and position in relation to the various pulmonary structures. X16.

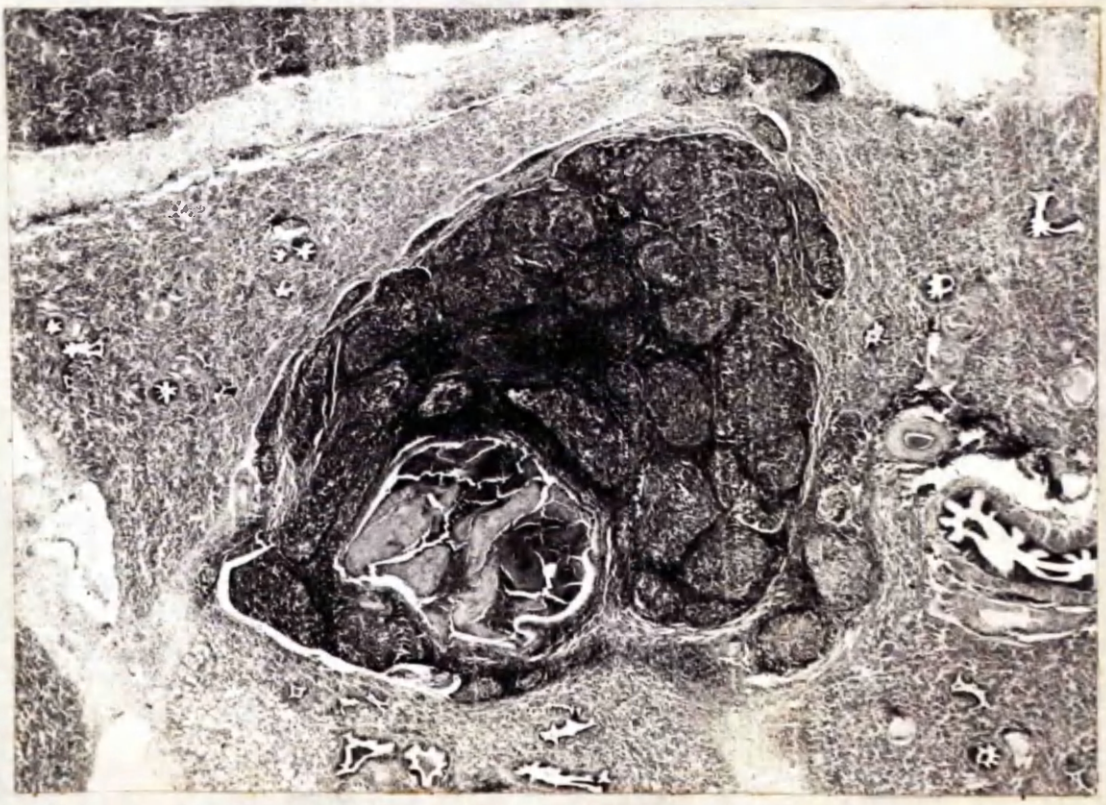


Figure 16.

Well-developed lympho-reticular nodule showing lymph nodal structure with germinal centres and large part of lobule occupied. X25.

Section II.

Experiment 11. A trial of the effect of diethylcarbamazine on prepatent and patent parasitic bronchitis in calves.

Introduction.

This section describes an experiment concerning the effects of diethylcarbamazine (DECZ) on prepatent and patent parasitic bronchitis in calves. DECZ was developed as an anthelmintic agent against human filariasis during the second world war. It has an effect primarily on the microfilariae. In vitro they can live many hours in a 1% solution of DECZ in plasma, serum or saline without consequence. However, in vivo, the drug, if given intravenously to cotton rats infected with Litomosoides carinii, reduces the numbers of circulating microfilariae by 80% in two to three minutes. Histology shows that they are collected in the liver, which contains most of the fixed reticulo-endothelial system in the cotton rat. In a few hours the microfilariae are surrounded by phagocytes and in a further few hours they are obviously disintegrating. The mode of action favoured by Bangham (1955) is that the drug may modify the surface of the filarial larva and act as an opsonin. Results of his own work with carbon-14-labelled DECZ support the opsonin theory. The effects of the drug on adults is in dispute. Parker (1957) was the first to report the use of DECZ against D. viviparus.

The trial described here was performed for two main reasons. 1. Any drug which can arrest larvae during early development has a great potential in the study of helminth immunity as it might

allow termination of infections at known times and thus assist in the determination of the relative immunogenic effect of the various developmental stages of the parasite.

2. There appeared to be some dubiety about the relative efficacy of DECZ when used at different stages of the bovine disease. Parker (1957) and Parker and Roberts (1958) reported that when treatment of experimentally infected calves was started at about the middle of the prepatent period, suppression of the infection or a considerable amelioration of the disease occurred, but when treatment was initiated on the 19th day post-infection, all of the calves died subsequently of parasitic bronchitis. On the other hand, the manufacturers (Burroughs Wellcome & Co. London) of the drug claimed that adult lungworms are expelled and that further lung damage is stopped immediately.

A further point of great interest arose during the experiment - when the calves were killed certain of the lung lesions appeared potentially very interesting in that they were similar to those seen grossly at post-mortem in experiment 9.

The present experiment was designed to afford a direct comparison of the effects when the drug is administered at the beginning of the last third of the prepatent period and also when the disease was fully established.

Materials and Methods.

Eighteen Ayrshire bull calves, purchased when three to ten days old were housed in individual metal huts which were cleaned twice weekly, the bedding used being oat straw.

Their ration consisted of 1½ lb. of powdered milk (Ostermilk No. 1, product of Glaxo Ltd., Greenford, Middlesex), suspended in one gallon of water at approximately 34 to 38° C., half of this amount being given twice daily. During the third and fourth weeks, hay and calf weaner pellets (produced by B.O.C.M. Ltd., London) were introduced, and powdered milk feeding ceased at the end of the fourth week. From then until the end of the experiment the calves were given 3 lb. of the pelleted ration daily.

When the calves were eight to nine weeks old, they were allotted at random into three groups of six calves on the basis of their body weights. Throughout the five weeks of the experiment, the calves were weighed and blood samples were withdrawn, for haematological examination, twice weekly, giving a total of 11 estimations for body weight and 10 for blood. Total and differential leukocyte counts and haemoglobin estimations were carried out, the latter being measured with a colorimeter (Evans Electroselenium Ltd., St. Andrews Works, Essex, England), using a number 3625 filter. For the differential eosinophil counts, at least 200 cells (400 cells when available in the blood film) were counted.

Larval culture was as before, as were the parasitological and pathological techniques.

Experimental Procedure.

On day 1, each calf was given an oral dose of 4,000 infective D. viviparus larvae. Calves of group 1 were each given 0.2 ml. per Kg. of 10% diethylcarbamazine citrate (Franocide, product of Burroughs Wellcome & Co., London) injected intramuscularly into

the gluteal region on day 15 and again on days 16 and 17. From day 22, daily faecal counts were made on all calves.

On day 31, the 12 untreated calves were allotted to two groups on the basis of their faecal larval outputs on that day. When this had been done the respiratory rates and body weights were inspected to determine if any major disparity existed between groups. No significant difference between any of these parameters was found to exist in the two groups. The calves in group 2 were then given DECZ for three days (days 31, 32 and 33) in the same manner as Group 1.

All calves were slaughtered on day 37, and the worms in each pair of lungs were counted; blocks were taken for histological examination and the usual lesion score method was applied to the individual pairs of lungs.

Results.

A summary of the relevant clinical, parasitological and pathological results is given in Table I.

Respiratory Rate. The mean respiratory rate of each group is shown (graph 1) and the values obtained on each day are given in Table 2. The means were between 30 and 35 per minute on the first day of the experiment. By the end of the week following infection, there was a slight rise in each group to between 40 and 45. On day 15, when treatment in group 1 was started, the mean respiratory rate of group 1 calves was 64, while those of group 2 and 3 calves were 72 and 73, respectively. There was a brief halt in the rise in respiratory rate between days 15 and 17 following the very rapid rise which took place between days 12 and 15.

This is characteristic of the clinical response to a single dose of 4,000 larvae.

From day 18, 72 hours after treatment with DECZ had begun, there was a marked difference in the clinical condition of the calves in group 1 as compared with those in the two untreated groups. For the remainder of the experiments, the mean respiratory rate of the early treated calves remained between 45 and 55, whereas in the calves of the other two groups, the rates rose steadily to about 100 during the fourth week and continued to fluctuate in this region during the fifth week of the experiment. In marked contrast, there was no significant effect on the respiratory rate or on the general clinical condition of the calves in group 2 following treatment on days 31, 32 and 33. Although there was a drop of 11 in the mean respiratory rate of group 2 calves between day 33 and day 34 (graph 1), it had risen again by day 36 to a level within 10 of the control rate. The accuracy of the method of counting respiratory rates is insufficient to attach significance to these variations.

To summarise the clinical condition of the calves in groups 2 and 3 it may be said that most were characteristic of severe cases of husk, and the treated calves could not be distinguished from the untreated controls.

Weight Gains. There is no statistically significant difference between the mean weight gains in the three groups (graph 2).

Appetite. During the fourth week of the experiment, the majority of the calves in the three groups did not finish their daily ration of 3 lb..

the usual residue being ½ to 1 lb. per day. This may have been due to the hot weather prevailing at the time. Apart from this general comment, there were several variations in appetite which are worth noticing. Calf 60, in the early treatment group, was the smallest calf in the experiment and, on 13 of the 36 days it did not finish its feed allowance. This may have been due to its size. In the late treatment group, four of the six calves did not finish their food on day 15 but finished it on day 16. On days 33 and 34, five of the six calves in this group did not eat all of their ration, these being the second and third days after treatment.

In the control group, three of the six calves did not eat all of their ration on the 15th day but did so on the following day. On days 33 and 34 two of the six calves left some food as compared with five of the six in group 2.

Faecal Larval Output. Larvae were not found in the faeces of any of the calves in group 1, on any of the 15 days on which examinations were carried out. The fluctuations in the mean larval output in group 2 and 3 calves are shown in graph 3. It can be seen that in calves in the later treatment group the faecal larval output was lower than that of the control group when treatment began on day 31, but that by the end of the experiment this position had been reversed. However, there is no statistically significant difference between the mean larval outputs in the two groups.

Lungworm Burden. Only one worm was found in the lungs at necropsy of calves in the early treatment group. Although there appears to be a difference in the numbers of worms found in groups 2 and 3 calves this is not significant because of the large standard deviation.

Haematology. The mean values for haemoglobin, leukocytes and eosinophils of the three groups are shown in graphs 4 to 6.

Pathology (Group 1). At post mortem examination there was no consolidation found in the lungs of any animal in this group except in a few lobules associated with the presence of the single worm in calf 56. One notable feature, however, was the presence in all lungs of multiple grey-pink nodules, 1 to 2 mm. in diameter. These were found both subpleurally and in the substance of the lung (fig. 17), and there were approximately 50 visible on thepleural surfaces in each calf. Histologically these nodules consist of a central dead immature lungworm which had lost its normal staining affinity and was surrounded by well-developed lymphoid tissue (figs. 18 and 19). These nodular lesions are identical to those found in experiment 9, on the pathogenesis of irradiated larvae, and described as lymphoreticular broncho-occlusive lesions.

Pathology (Group 2). There was no macroscopic difference between the lesions in group 2 calves and those in group 3 calves, and the lesion scores (Table 1) show that there was no quantitative difference in extent of consolidation.

Histologically, all of the calves had typical lesions of patent parasitic bronchitis but, in addition to these, there were other changes which are infrequently seen in uncomplicated primary infections; In calf 48, which had only 19 worms at post mortem, every histological section contained these lesions. The lesions had three main components:

1) The luminal contents,
2) the reaction and breach of the epithelial lining,
3) the reaction in the lamina propria.

The lumena of the affected air passages were plugged with an intensely cellular exudate consisting largely of eosinophils which appeared to be dead or dying, as many had a waning staining affinity and a degree of apparent fusion of granules.

The worms embedded in this exudate were dead and disintegrating (figs. 20 and 21). They had become generally more eosinophilic, all outlines were blurred, and the cuticle was ruptured and separated off from the underlying tissue, allowing the entrance of leukocytes.

Pieces of dead helminth somatic material, apparently when in contact with the epithelium caused it to undergo a local hypertrophy and hyperplasia and to slough off, leaving a breach in the wall (fig. 22). There is then a giant cell reaction at this site; the component multinucleated cells appear to be of both epithelial, endothelial and the more normal mesenchymal foreign body giant cell types. Where a female worm has ruptured and the eggs lie against the wall of the bronchiole, the reaction is particularly intense.

The cells of the lamina propria become very much increased in number; this is due partly to the foreign body reaction described and partly to an infiltration with eosinophils, but a marked feature is the occurrence of many cells of the plasmacyte series. It is seen by pyronin-methyl green staining that many of the reticulum cells are activated and have strong cytoplasmic pyroninophilia, and that immature and young mature forms are prominent. This is probably representing the start of an anti-body-producing system. Mast cells aremoderately increased in number, there being five to six per high power field. It is known from the histological series described in experiment 9 that the further progress of this lesion is the occlusion of the bronchiole and the multiplication of lymphoreticular cells in the wall, so that ultimately an area of new lymph nodal tissue is formed around this segment of the air passage. This is strictly analogous to the lesions produced by the dead larvae in group 1 calves and these lesions were in the lymph nodal development phase since the death of the parasites occurred about 14 days earlier than group 2 calves.

Discussion.

The results of this experiment fully support the original discovery of Parker of the efficacy of DECZ against the immature forms of D. viviparus and also throw some light on the mode of action of this drug and the changes produced in the host. They do not support the view that it has the beneficial effect when used to treat fully developed infections.

Group 1 - Prepatent Treatment: When the treatment is started 14 days after infection, as in group 1, the majority of infecting larvae have developed to the fifth or immature adult stage. They are found as was shown in experiment 8, in bronchioles, in bronchi and in the trachea. In light infections, few clinical signs are seen at this time. In moderately heavy infections, such as in the present experiment, some tachypnoea is present and there may be coughing. The stockmen did not notice that these calves were "ill" in the present experiment. In heavy infestations, e.g. 50,000 larvae, (Jarrett, McIntyre and Urquhart 1957), calves may be moribund and do not usually survive the third week. The important underlying point is that pneumonia is not a marked feature at this stage of the disease. The associated lesions are an eosinophil bronchitis with possibly lobular collapse distal to plugged bronchioles. Death in heavily infected animals is caused by pulmonary oedema and hyaline membrane formation. It is to be expected, therefore, that if the larvae are killed and expelled and if there is no marked disruption of the bronchopulmonary architecture, the less severe forms of the prepatent disease will resolve quickly.

This rapid resolution appears to have taken place in the early treatment group. Virtually no worms were found at necropsy and the absence of larvae in the faeces indicates that there was no significant development to the adult stage. This is confirmed by the absence of the consolidating lesions always found in patent husk, and by the clinical resolution after treatment.

A fact of great interest is the development of bronchial and bronchiolar lymphoreticular nodules in the calves treated early. Similar lesions have been described after challenge in calves immunised by any of four methods:

1. Hyperimmune serum.(Jarrett, Jennings, McIntyre, Mulligan and Urquhart, 1955).

2. Whole worm vaccine. (Jarrett, Jennings, McIntyre, Mulligan and Urquhart, 1960).

3. Previous normal infection, as in experiment 10.

4. Irradiated larval vaccine, as in experiment 10.

The lesions have also been found in non-immune calves which have been given one dose of irradiated larvae. As described in experiment 9, it was possible in the latter case, by serial killing, to study the development of these lesions, and the suggestion was made that they are formed in response to inhibited, dying or dead parasites, the parasitocidic or parasitostatic agent being either antibodies in the case of immune calves, or radiation effect in the irradiated larvae. This is an important point in parasitic immunity, as the relative roles of humoral and cellular responses have not yet been clearly elucidated. The findings in the present experiment support the hypothesis we have put forward (Jarrett and Sharp,1963) that, at least in the case of certain parasites, any set of circumstances which will adversely affect or kill the helminths will initiate the formation of (1) bizarre bronchial epithelial hyperplasia and disruption of the cell sheet, (2) eosinophilic leukocyte infiltration of the lamina propria and epithelium,

(3) multiplication of lymphoreticular cells in the walls of air passages leading to (4) the formation of cuffs of nodules of lymph nodal type tissue with germinal centres surrounded by lymphocytes and plasma cells. The contribution of such foci to the protective antibodies is as yet unknown; this cellular proliferation may be in response to somatic antigens which may or may not give rise to protective antibodies.

Since, as was shown in experiments 8 and 9, irradiated larvae, the majority of which do not progress beyond fourth or early fifth stage, can stimulate immunity and also produce similar lesions, it is probable that calves in which these larval stages have been killed by DECZ will also become immune. This appeared to be the case in two calves which were reinfected by Parker.

When these lymphoid lesions, which are broncho-occlusive in nature, are present in very large numbers, they may give rise to clinical signs and even death. It is common experience to find some coughing and tachypnoea when immune animals are exposed to heavy challenge; cases have been seen in the field and in the veterinary hospital, where death from these lesions has occurred in adult, naturally exposed animals. One farm lost 17 cows this way. It is important to note that some animals with a heavy prepatent infection might be killed by DECZ treatment. It might be said that if they are killed by this reaction, then it is only anticipating by a few days death due to the disease proper; this is not necessarily the case. The results of the quantitative experiment (number 8) indicate, without providing definite proof,

that when 1,000 larvae are given to a calf approximately 600 reach the lungs and persist until about the end of the second week after infection, when there is an expulsion of immature forms so that only 200 to 250 are retained to form the adult population found at the patent stage. It may well be that a calf which could survive pulmonary damage required to produce a crop of 'n' number of adults in the bronchi, could not survive the effects of 3n broncho-occlusive lesions.

The significance of the results of experiment 8 quoted is, as was there stated, only in doubt because of the small numbers of calves (two) which were killed at each serial time interval after infection; this does not permit a valid statistical analysis to be made. However, it may be that many of the immature forms killed or immobilised by DECZ are in fact removed from the bronchi without trace, and only those in the air passages of small diameter cause lesions. Parker and Roberts (1958) challenged the immunity of two calves which had previously been successfully treated and noticed a severe clinical reaction during the second and third weeks; this was almost certainly due to such bronchial lesions.

Group 2 - Patent Treatment: Group 2 calves which were treated during the patent period had no improvement over the controls. The clinical conditions of the calves and the extent of the pneumonia at necropsy were those of severe husk. The mean larval output of the two groups was statistically similar and there was no statistically significant difference in the mean worm burdens, although the mean of the treated calves was lower.

Several points must be borne in mind concerning the latter. First, the mean larval output in the treated group was consistently, although not significantly, higher than in the control group, indicating that there was at least numerical parity in worm burden between the two groups. Second, there is normally a fair scatter in the worm burdens of any ten calves and the difference in means may be due to chance as Student's "t" test indicates. However, there is no doubt that at least in calf 48 (and probably also in calf 58) DECZ seems to have caused death of adult worms. Calf 48 previously had a faecal larval output of 1,100 eggs per gram and had the most severe degree of consolidation of any of the calves in the experiment, yet it had only 19 worms at necropsy. It might be argued that this was merely one of those occasional animals which throws off infection early, but here the histology is most illuminating.

In every calf in the late treatment group, and in every section taken from the lungs of calf 48, there are bronchial lesions of the type described previously and which are the adult worm analogue of the lesions discussed in the early treatment group. When the lesions are caused by adults they are, as one would expect, of greater severity and affect a bigger area of pulmonary tissue. The presence of these lesions plus the normal husk lesions explains the severe clinical state of the animals and gives rise to grave doubts about the beneficial effect of this drug when used against heavy patent infections.

A small but interesting point arises from a consideration of the mean eosinophil percentages of the groups (graphs 5 and 6). Group 1 shows a very rapid rise to over 7% on the 18th day, i.e. on the third day of DECZ treatment. By analogy with the timing of the development of the lymphoreticular broncho-occlusive lesion in experiment 9, the eosinophil blood rise most probably reflects the first stage of the lesion, viz. an eosinophil bronchitis with occlusion as shown in figure 9 of experiment 9. Both other groups show a similar trend, reflecting a probably similar lesion but one of a lesser degree.

When the relative and absolute numbers of eosinophils were declining or low in the other two groups after the 30th day, group 2 is interesting in that its eosinophil percentage rose fairly steeply from the time of DECZ treatment until death. The increase here probably reflects the eosinophil component of the exaggerated reaction to dead and disintegrating adult worms as shown in figures 20 and 21.

It is doubtful whether the eosinophilic response could be used by the clinican as a guide to the pathological events occurring under DECZ treatment in the field, but it does seem worthy of further investigation.

The facts presented above probably explain the apparent variations in efficacy of this drug in the field. If the treated animal is in the prepatent stage or if it has a mild infection associated with the regular uptake of small numbers of larvae, one might expect good and sometimes spectacular results. In the developed disease, however, the lung damage may even be increased

in extent and severity.

One possible objection to the present experiment is that the calves were killed too soon after treatment, but it was necessary to kill at the height of patency in order to get a valid comparison of worm numbers in the treated calves and the controls.

TABLE 1 - Summary of the Respiratory Rates, Weight Gains, Larval Outputs, Worm Burdens and Lesion Scores in the Calves Infected with Parasitic Bronchitis.

	Calf No.	Peak respiratory rate	Group mean respiratory rate peak *	Weight gain	Group Mean
GROUP 1	45	80	70	51	37 ± 11.5
	54	65		45	
	55	60		41	
	56	60		39	
	57	90		19	
	60	90		29	
GROUP 2	46	120	100	16	24 ± 11.0
	48	130		33	
	50	100		27	
	58	120		32	
	59	140		6	
	61	105		32	
GROUP 3	47	130	120	18	25 ± 12.4
	49	140		17	
	51	130		24	
	52	100		49	
	53	130		25	
	62	130		16	

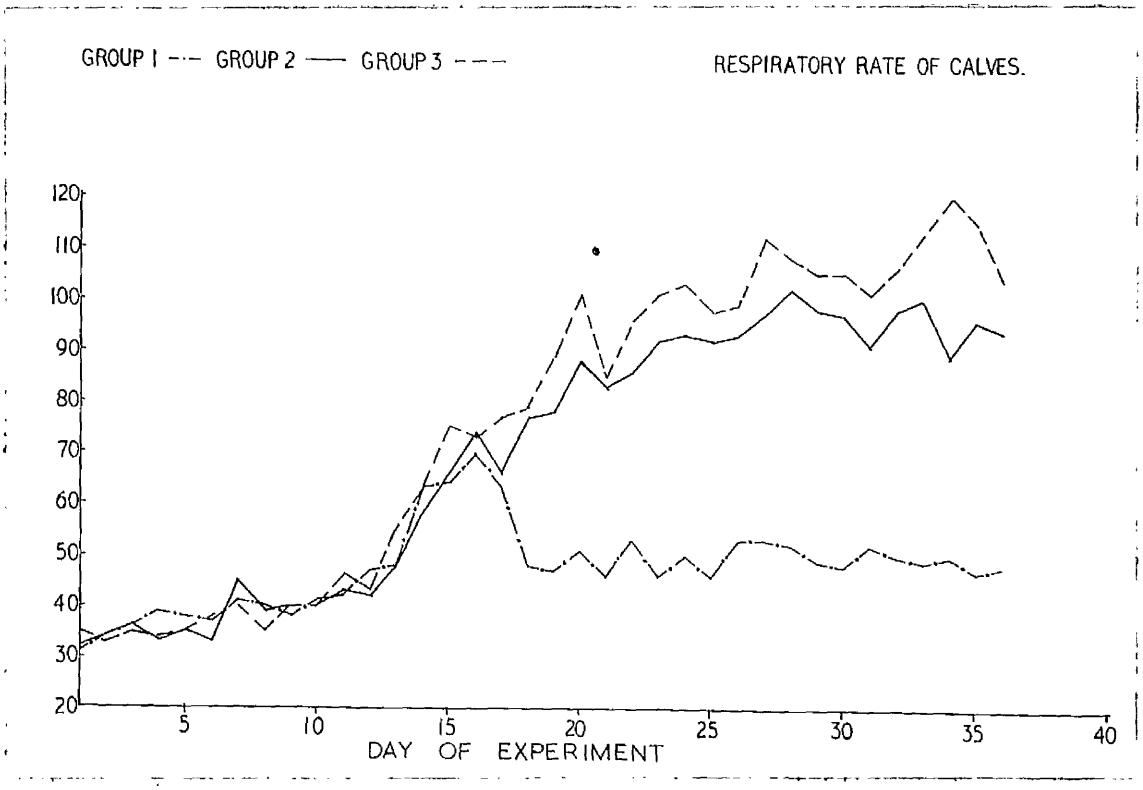
* These are not means of the preceding column; they represent the group mean on the day it was highest.

Peak Larval Output	Group mean peak* (larvae/Gm.)	Worm Burden	Mean	Lesion Score	Mean
0	0	0	0.2	0	<0.2
0		0		0	
0		0		0	
0		1		<1	
0		0		0	
0		0		0	
800	520	1,042	275±391	5	6.0
1,100		19		10	
250		284		8	
50		0		6	
1,750		302		4	
25		105		6	
850	340	508	630±468	5	6.5
900		1,361		10	
350		483		6	
350		157		3	
500		252		6	
850		1,020		9	

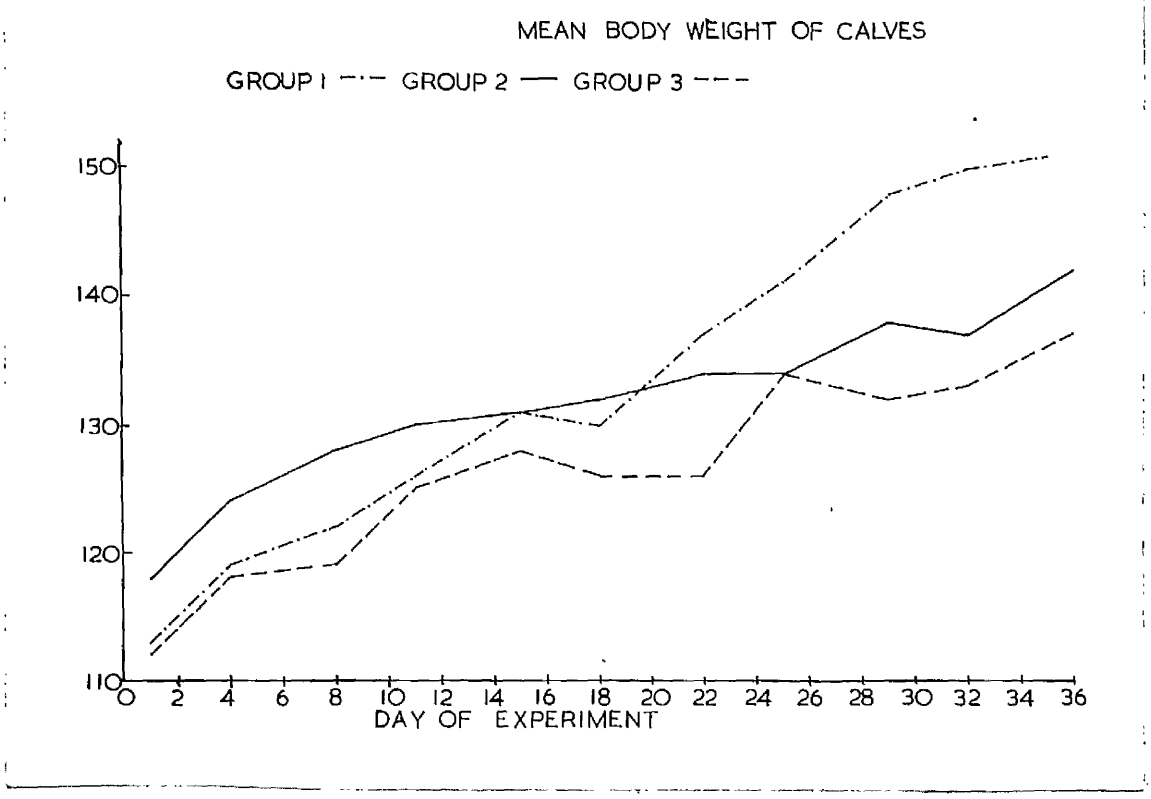
TABLE 2 - Respiratory Rates per Minute of the Calves in Group 1

Day	Calf Number						Mean	S.D. ±
	57	55	56	45	54	60		
1	20	20	30	40	30	45	31	11.32
2	30	25	30	45	30	45	34	8.61
3	35	25	40	50	30	35	36	8.61
4	40	30	35	50	35	45	39	7.36
5	30	30	45	50	30	40	38	8.82
6	40	25	35	50	30	40	37	8.76
7	50	30	35	55	40	35	41	9.76
8	40	25	35	50	30	60	40	13.04
9	45	25	40	50	35	35	38	8.76
10	50	40	35	50	30	40	41	8.01
11	40	35	50	45	40	40	42	5.18
12	60	35	40	50	35	60	47	11.7
13	50	50	50	50	40	65	48	8.59
14	60	55	60	65	65	70	63	5.27
15	70	60	60	70	60	65	64	5.92
16	90	50	65	80	65	70	70	13.78
17	70	60	55	50	50	90	63	12.24
18	55	35	50	50	35	60	48	10.8
19	45	35	50	50	40	60	47	8.8
20	50	45	55	60	40	55	51	7.36
21	45	45	45	60	35	45	46	8.01
22	60	50	50	60	35	60	53	9.9
23	50	45	40	55	40	45	46	5.31
24	60	45	45	50	40	60	50	8.36
25	45	40	35	60	35	60	46	11.6
26	55	50	50	55	50	55	53	2.5
27	50	45	45	65	45	70	53	11.2
28	60	40	50	60	40	60	52	9.6
29	45	40	50	60	40	60	49	9.2
30	50	40	45	50	50	50	48	4.2
31	50	50	50	55	45	60	52	4.3
32	65	45	50	50	45	45	50	7.7
33	45	50	50	55	45	50	49	3.9
34	40	50	50	60	45	55	50	7.07
35	40	40	55	50	45	50	47	6.06
36	50	50	45	50	45	50	48	2.6

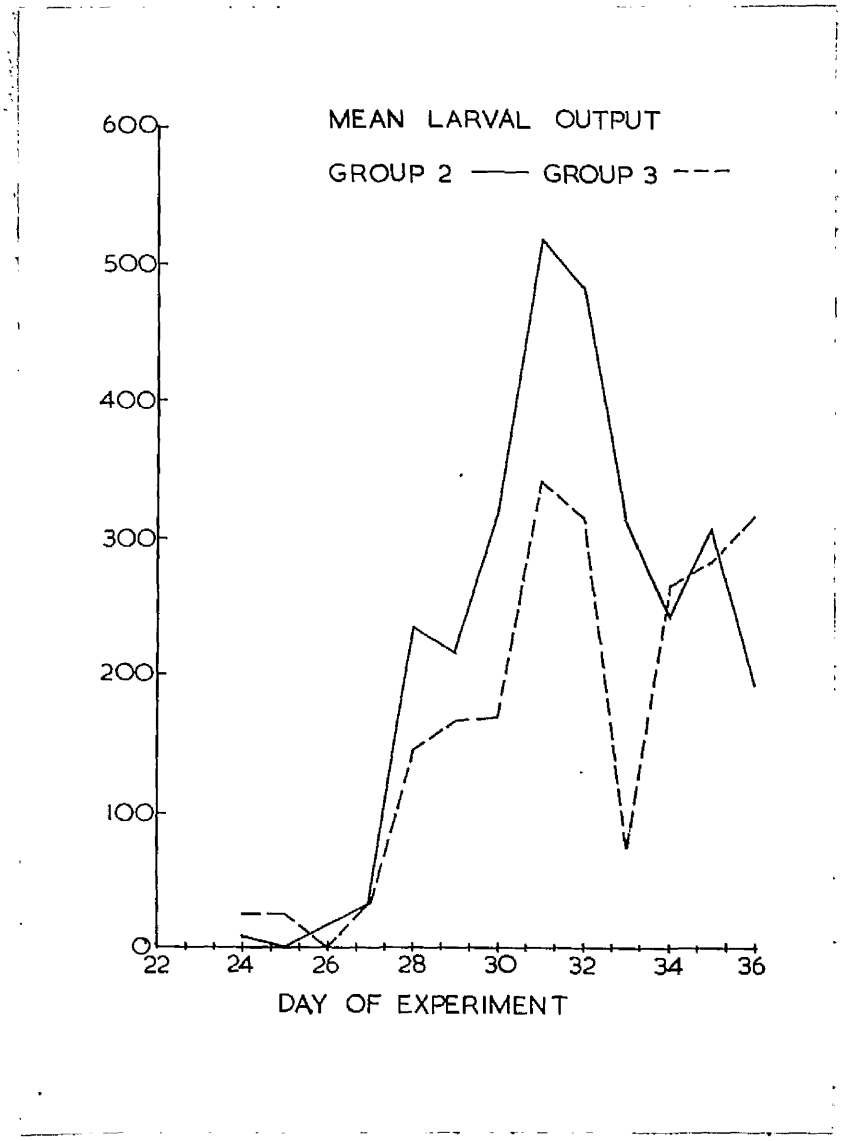
The effect of diethylcarbamazine on parasitic bronchitis - graphical results.



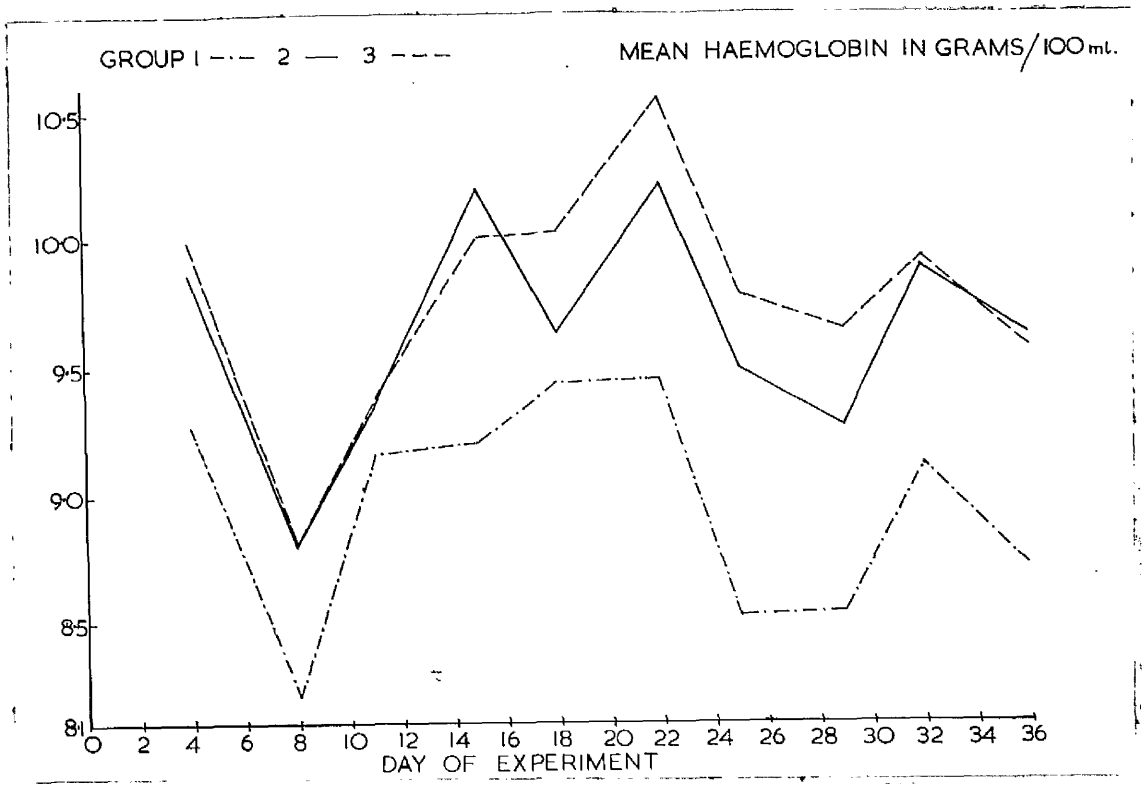
Graph 1. Mean group respiratory rates (per minute). (Group 1 early treated, group 2 late treated, group 3 controls.)



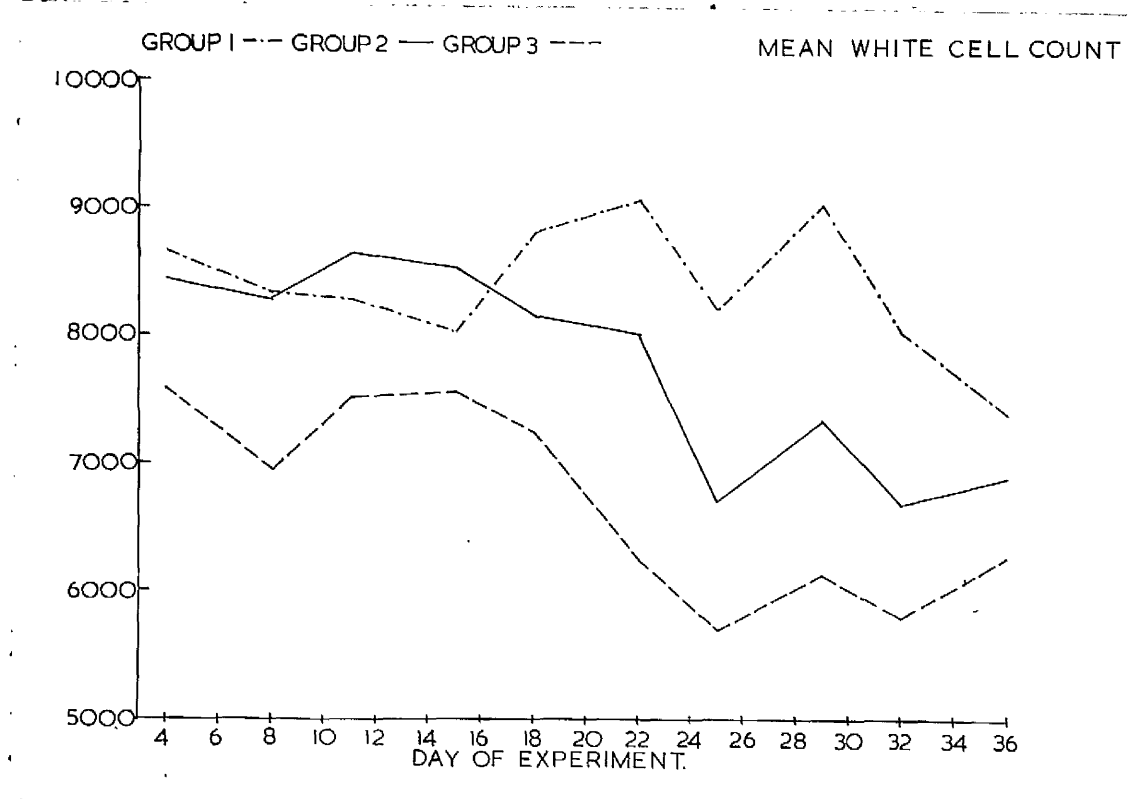
Graph 2. Mean group body weights in lbs. during the experiment. (Group 1 early treated, group 2 late treated, group 3 controls).



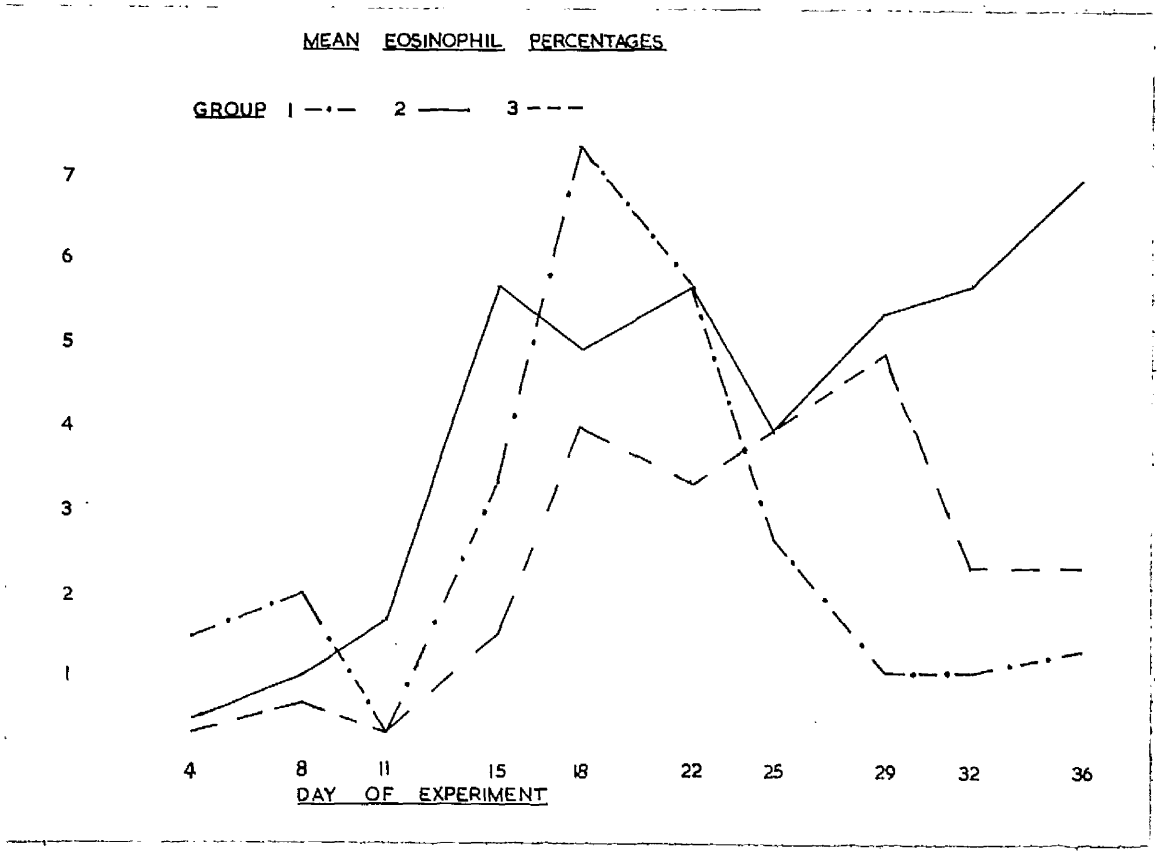
Graph 3. Group mean larval outputs per gram of faeces. (group 2 late treated, group 3 controls).



Graph 4.
Group mean haemoglobin levels (Gm./100 ml.).(group 1 early treated, group 2 late treated, group 3 controls).



Graph 5. Group mean leukocyte counts (cells per cu.mm.). (Group 1 early treated, group 2 late treated, group 3 controls).



Graph 6.
Group mean blood eosinophil percentages of total white cell count. (group 1 early treated, group 2 late treated, group 3 controls).

Illustrations for experiment 11.

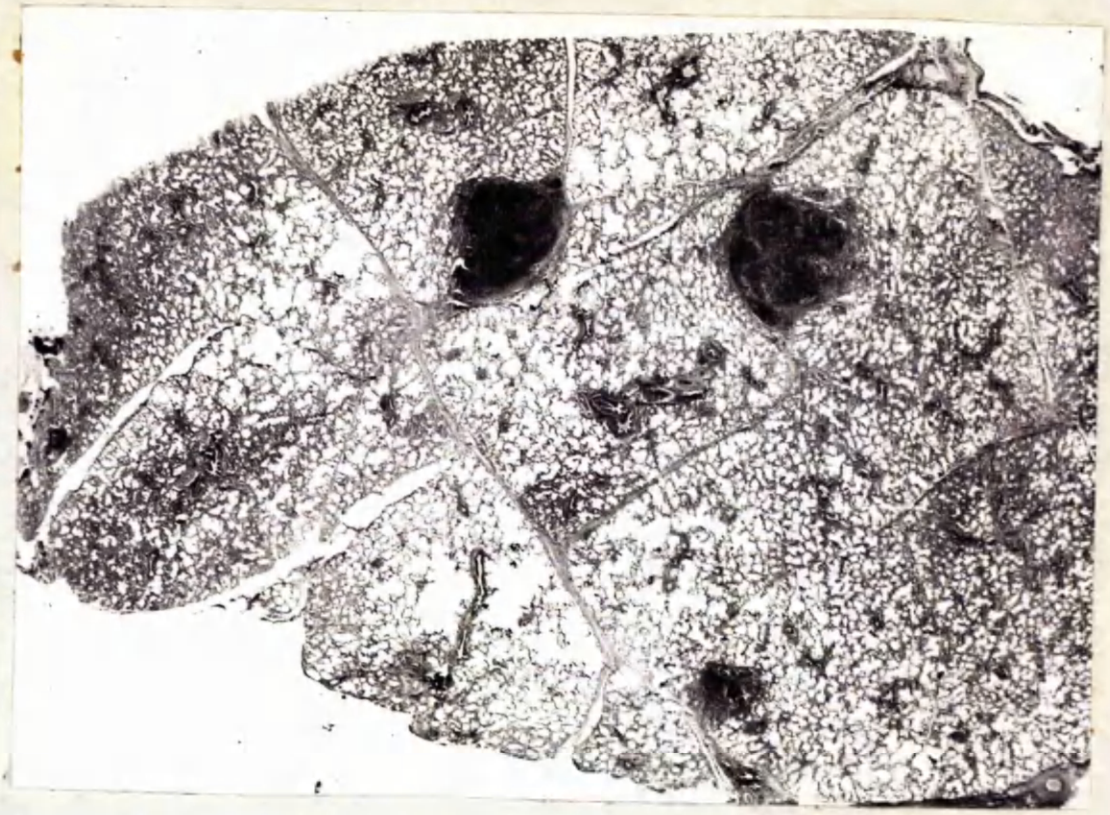


Figure 17.

Lymphoid nodules in substance of lung. X8.

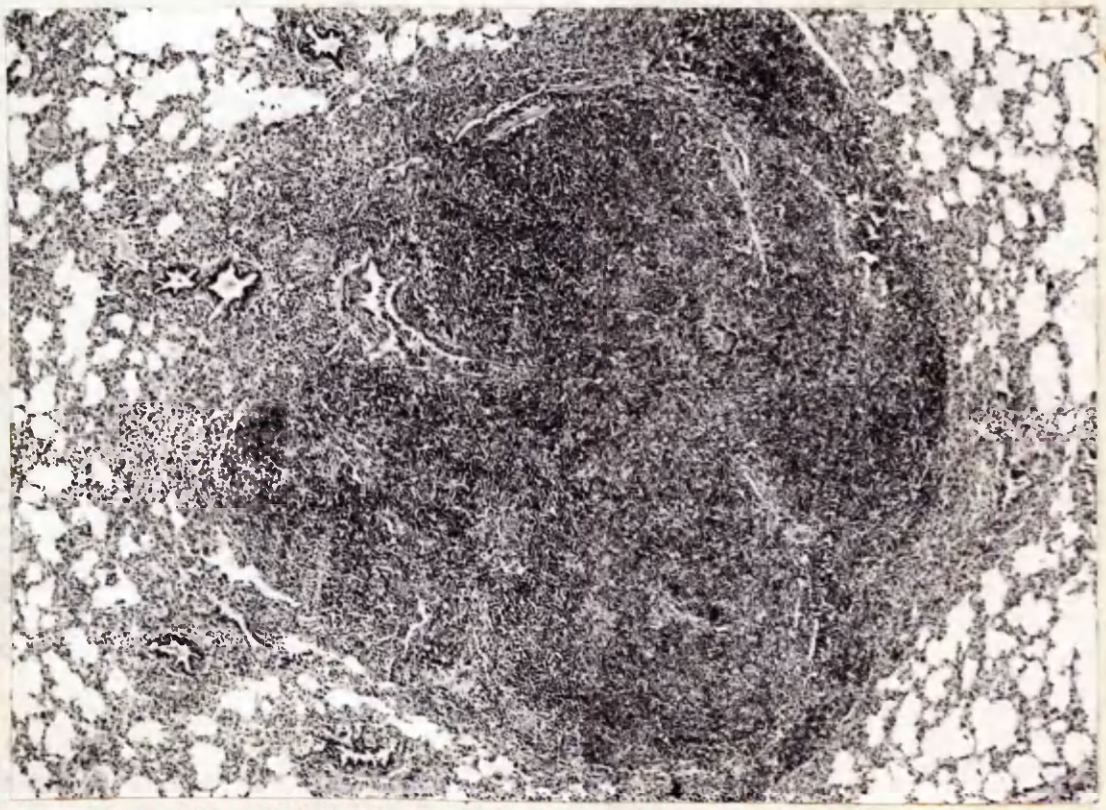


Figure 18. Lymphoid nodule showing bronchiolar lumen and dead worm (arrowed.). X30.

Figure 19. Dead worm, which has lost its normal staining affinities, surrounded by lymphoreticular tissue. X200.

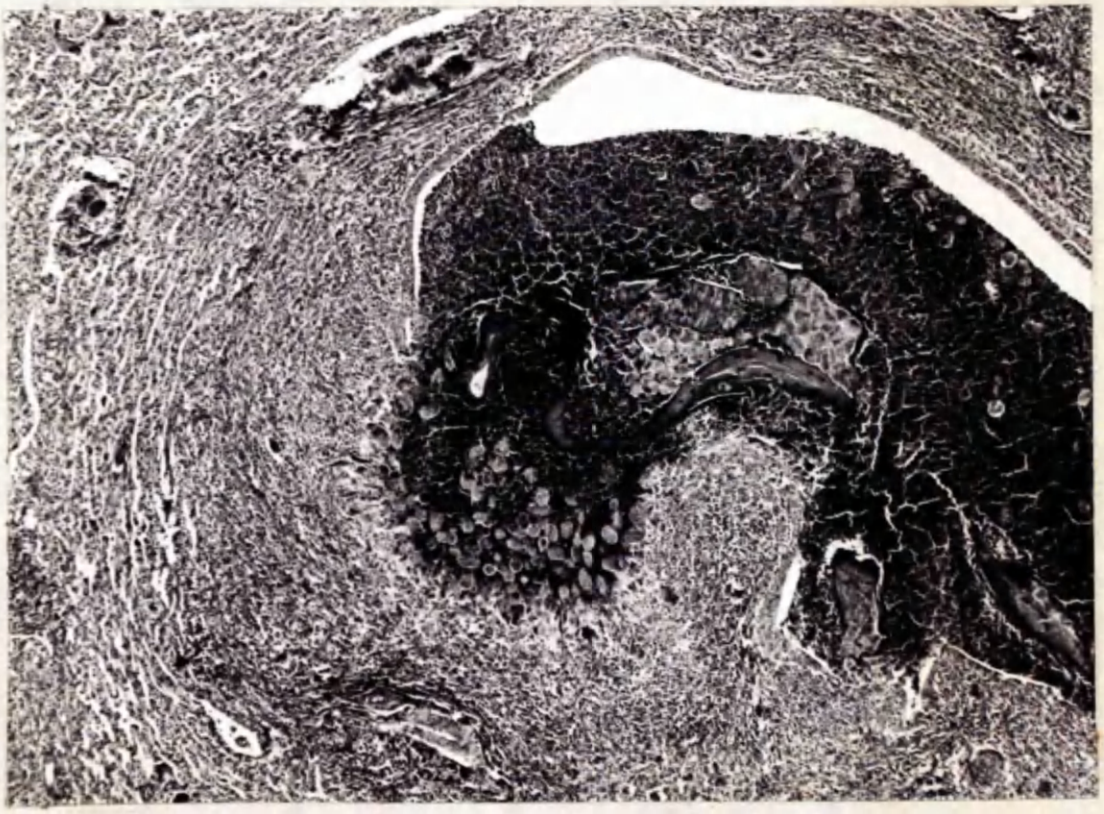


Figure 20. Disintegrating adult female in bronchus showing marked exudate of eosinophils in bronchial lumen and the reaction of the bronchial wall to the eggs. X25.

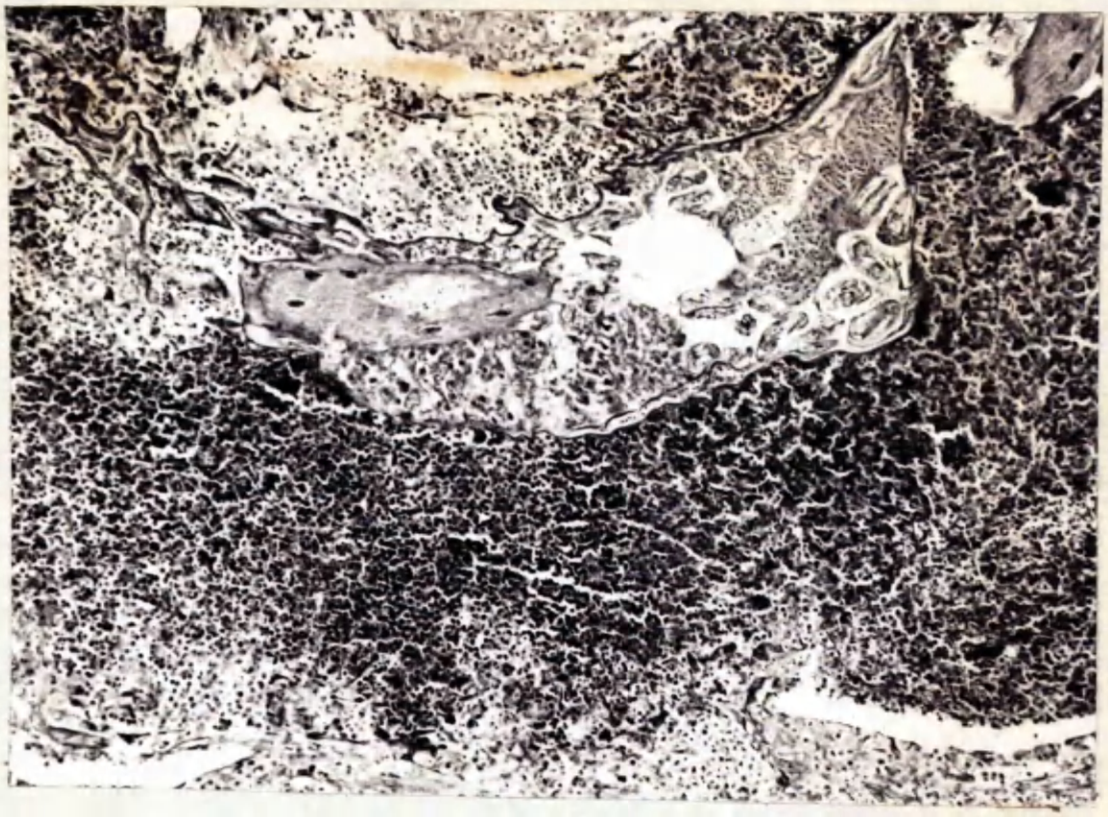


Figure 21. Disintegrating female in bronchus. X30.

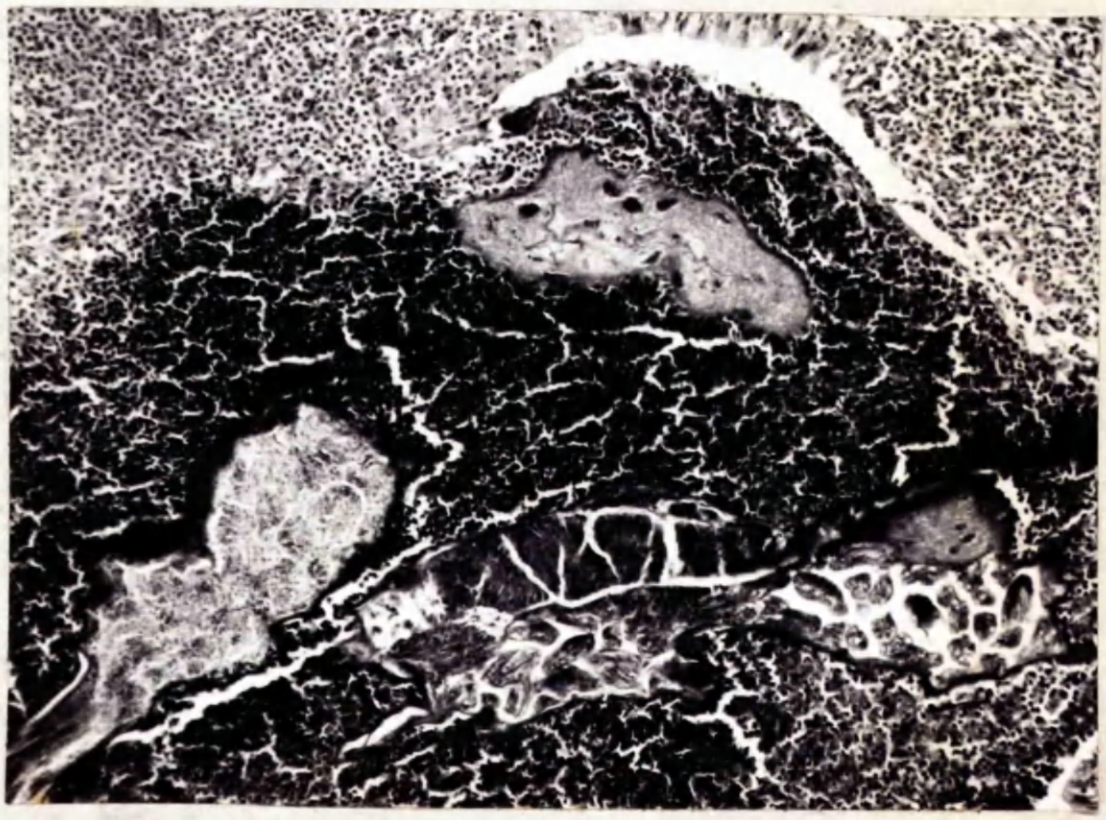


Figure 22. Ulceration of bronchial mucosa and beginning of lymphoreticular response in lamina propria. X50.

SUMMARY OF PART III

Section I.

As they are closely correlated, the three experiments described under this section carry a common discussion. They are concerned with the comparative quantitative and qualitative aspects of the course of infections of normal and irradiated D. viviparus larvae in calves.

Experiment 8. This experiment was entirely quantitative in nature. Thirteen calves each received 1,000 X-irradiated larvae and 13 calves received 1,000 normal larvae. The calves were killed in pairs, one from each group, at intervals from post-infection day 1 to day 35. Approximately a quarter of the irradiated larvae were present in the lungs 11 days after infection, but this level fell steeply until only a few stunted sterile worms with a male/female ratio of approximately 1:10 were found.

In the 'normal' group approximately 600 larvae reached the lungs by the 13th day and persisted for three to four days when there was an expulsion of immature forms via the trachea. This resulted in a final adult population of between 200 and 250 worms. This infective-dose to final-worm-burden ratio of 1:4 or 1:5 is quite familiar to everyone who has worked with D. viviparus of standardised infectivity.

Experiment 9. This was designed to investigate the host reaction to the vaccine by studying the histological reactions to irradiated larvae as they matured in the lungs, again with the use of a serial killing technique although with longer intervals

based on a probable expectation of lesion type. The pathological basis of the mild and transient clinical signs sometimes seen after vaccination is described. They are due to the temporary bronchiolar blocking effect of the eosinophil response to larvae dying in the lumen. Most of such lesions are cleared but some progress through three main stages to become fully developed lymphoreticular nodules with a lymph nodal structure and germinal centres.

Experiment 10. This experiment was performed to investigate the pulmonary immune mechanism in calves twice vaccinated with 1,000 irradiated larvae for one group and the same number of normal larvae for the other group. Both groups were heavily challenged and a serial killing procedure was followed at intervals after challenge; as before, the lungs were pathologically examined in detail. Many lymphoreticular nodules were found in both groups and the relationship of these to the clinical signs seen after heavy challenge in an immune animal and to the signs seen in an adult bovine of insufficient immunity are indicated and commented upon. The possible functions of the peribronchial and peribronchiolar lymphoreticular nodules as sites in antibody production and their influence on the interpretation of serological tests are discussed.

Section II.

Experiment 11. The effect of diethylcarbamazine (DECZ) on prepatent and patent parasitic bronchitis was tested. Eighteen calves were divided into three equal groups. All received 4,000 normal larvae of D. viviparus on day 1. Calves of one group began a three-day

dosing schedule with DECZ starting on day 15, and those of another group started a similar schedule on day 31. Calves in the third group received no drug and served as controls. All were killed on day 37.

In the calves treated in the prepatent period the disease was almost completely suppressed as judged by clinical, parasitological and pathological criteria. The only lesions produced were those of peribronchiolar and peribronchial lympho-reticular proliferation caused by intra-lumenal death of larvae. The immunogenic significance of this lesion and its similarity to that arising in other circumstances are discussed.

The calves of the late treatment group were not significantly different from the controls according to clinical, parasitological or gross pathological findings. Histologically there were severe bronchiolar and bronchial destructive lesions apparently caused by the death of adult worms in situ. The similarity between this finding and the 'lesion of insufficient immunity', which can kill partially immune cattle in the field, is discussed.

It is concluded that under the conditions of this experiment, DECZ was highly effective against the prepatent stage of the disease; the claim that it is beneficial against fully developed husk could not be substantiated and the reverse may be the case.

As a final conclusion from experiments 9, 10 and 11, it is suggested that the peribronchial or peribronchiolar lymphoid lesions are formed in response to inhibited, dying or dead parasites,

the parasitocidic or parasitostatic agent being antibodies in the case of immune animals, radiation effect in those receiving irradiated larvae or a possible opsonin or other surface effect in the case of those receiving diethylcarbamazine. The magnitude of the clinical syndrome depends on two factors:

1. If the lungworms are killed early, as fourth or early fifth stage larvae, the individual lesions tend to be smaller in size; if they are killed later, as adults or near-adults, the lesions are considerably greater.

2. The larger the number of individual worms in the lung, whatever their stage of growth, the greater will be the number of potential occlusive lesions, should the parasites be affected by any of these disabling factors.

PART IV

P A R T IV

Immunity to Haemonchus contortus in sheep.

Introduction.

After a method of vaccinating against D. viviparus had been developed, it was a natural extension of this work to investigate the possibility of applying the same general principles to gastro-intestinal parasites.

It is a reasonable supposition that the irradiation method of artificially inducing active immunity in helminth disease might be successful where there is systemic migration by the parasite; the immature larvae traverse a potential humero-cellular immunological barrier, during which it is possible for them to initiate an immune response.

However, where the parasite undergoes only limited migration, as in many gastro-intestinal infestations in which tissue penetration is minimal, the opportunity for stimulating an adequate immune response would seem to be much reduced. It was, therefore, anticipated that the present method of immunising might prove to be less effective against gastro-intestinal parasites.

Attempts by other workers to immunise sheep against H. contortus have had variable results. Stewart, (1960), using graded, spaced doses of normal infective larvae as a means of active immunisation, reported some evidence of acquired resistance, but it did not occur regularly. Using a similar immunising procedure, Lisenko (1956) also reported evidence of resistance.

Stoll (1958) produced a high degree of resistance to reinfection in nine out of ten sheep, by intraperitoneal and subcutaneous injection of large numbers of living exsheathed third-stage larvae. The sheep were nine months old at the beginning of his experiment, which is summarised below:-

VACCINATION				ORAL CHALLENGE	RESULT
Route	Number of larvae	Number of sheep getting 1 dose on day 0	Number of sheep getting 4 doses on days 0,7,14,21	Number of larvae on day 77.	
VACCINATES:					
Intra-peritoneal	3,600 - 22,000 per Kg. body weight	3	2	10,000-15,000 per Kg. body weight.	All 5 protected.
Subcut-aneous	3,100 - 4,100 per Kg. body weight.	5	-	15,000 per Kg. body weight.	4 protected. 1 death from haemonchosi
CONTROLS:					
-	-	-	-	15,000 per Kg. body weight.	2 deaths from haemonchosi and 1 severe infection.

The experiments which follow were carried out on H.contortus, a parasite with a minimal migration cycle, to investigate four main factors:-

1. Whether the parasite, in its larval form, could be partially inactivated by X-irradiation.
2. Whether it could then stimulate enough immunity in sheep to enable them to withstand large challenge doses of normal infective larvae.
3. Whether the rate of irradiation would influence the immunising properties of the vaccine larvae.
4. The level to which the numbers of larvae in each vaccine dose could be reduced without affecting a servicable level of immunity.

(Experiments 12 and 13 have been reported in two publications, respectively, Jarrett, Jennings, McIntyre, Mulligan and Sharp 1959, and Jarrett, Jennings, McIntyre, Mulligan and Sharp 1961.)

Experiment 12. Vaccination of sheep using a single dose of X-irradiated larvae of Haemonchus contortus.

Materials and Methods.

Fifty 'new born' lambs were bought and, initially, were raised in groups of three or four and fed in metal isolation units on an artificial milk diet. (Ostermilk' produced by Messrs. Glaxo Laboratories Ltd., Greenford, Middlesex.) They were later transferred to concrete pens which were cleaned daily. Their faeces were examined several times in the pre-experimental period to confirm that they were parasite-free. The lambs were seven months old when the experiment was started.

A pure strain of H. contortus was obtained (kindly supplied by Drs. Dunn and Silverman of the Moredun Research Institute, Gilmerton, Edinburgh) and was passaged through three lambs to gain a sufficient number of infective larvae to carry out the experiment. Larvae were cultured by putting 250 gm of faecal pellets in 500 ml. closed glass jars, storing at 22°C for 14 days, then they were harvested by filling the jars with water and inverting them each over a petri dish. The petri dish was half-filled with water, and the larvae were collected an hour later as they congregated in the dish. Faecal egg numbers were estimated by the McMaster method. Total worm burdens were counted by washing out the abomasal contents, adjusting the total volume to 2,000 ml. with tap water, then stirring thoroughly and counting the number of worms in three aliquots of 50 ml. each. The technique of irradiation was similar to that used for D. viviparus.

Experimental Procedure.

The 50 lambs were allotted to eight groups numbered 1 to 8. There were seven lambs in each of groups 1 to 5 and five lambs each in groups 6 to 8. On day 0, animals in groups 1 to 5 were given 10,000 infective larvae irradiated respectively with 10,000, 20,000, 40,000, 60,000 and 100,000 roentgens. These levels were arbitrarilly selected on the basis of results achieved with larvae of D. viviparus. Two lambs from each of these five groups were killed on day 37 to determine the degree of inactivation of larvae. This time was chosen as representing normal full patency of infection; it was assessed by a flattening in the egg output curve of the five control lambs. The remaining five lambs in each group were challenged on day 117 with 8,000 normal infective larvae and were killed on day 163.

The time of 117 days was used as the challenge point as this was the time taken by the two groups vaccinated with larvae irradiated at the two lower levels, 10,000 and 20,000 roentgens, to eliminate the bulk of the infection resulting from their 'vaccine' dose. This judgement was based on their egg counts becoming McMaster negative.

Each lamb of group 7 was given 10,000 normal larvae on day 0, 8,000 normal larvae on day 117 and was killed on day 163. The interval of approximately seven weeks between Challenge and Kill was chosen as being a reasonable period for the challenge infection to reach full patency, with minimal extension into the time when such an infection might naturally begin to be shed.

Group 8 was the challenge control group; each lamb received 8,000 normal larvae on day 117 and was killed on day 163.

The design of the experiment is summarised in Table 1.

Results.

Counts of faecal egg outputswere made at approximately weekly intervals beginning on day 18; these results, together with the post-mortem abomasal worm burdens, are shown in Tables 2 to 9. The final results of the experiment are summarised in Table 10.

The lambs did not show clinical signs of the disease at any time during the experiment.

Discussion.

In assessing the value of this method of making a vaccine, the first point to be decided is whether the particular parasite can be partially inactivated and, if so, what is the limiting range of radiation dosage to be applied to it. Hence two animals, from each of the five vaccine groups, were killed when faecal counts of the control lambs (group 6) showed that the normal infections had reached full patency, as shown in Table 2. Lambs in groups 1A and 2A manifested no significant reduction in 'take' over the control animals, indicating that their larvae had not been sufficiently irradiated.

The pairs of lambs forming groups 3A, 4A and 5A did have a markedly lower worm burden, which suggested that their radiation dosages probably included the optimal inactivation level. Thus H. contortus was shown to be capable of servicable inactivation by X-irradiation, at levels of 40,000 roentgens and upwards.

The second point of information essential for assessment of the vaccine is whether the inactivated parasite can confer immunity. From Table 10 it is seen that, compared to the group 8 challenge controls, the immunity was good at the first four roentgen levels; 90% to 100% protection being conferred against the challenge used. The mean of 200 worms at post-mortem of group 3 resulted almost entirely from one lamb's abomasal burden of 972 worms. The reason for this anomaly is not known; the lamb is recorded as having been correctly vaccinated and not to have shown diarrhoea at this time - which might possibly have interfered with the establishment of the immunising fraction of the irradiated larvae. Poorer immunity was shown by the 100,000 roentgen vaccine group, whose mean worm population of 442 was accounted for by three of its five individuals.

In summary so far, larvae irradiated at 10,000 and 20,000 roentgens were little inactivated, but gave good immunity; those at 40,000 and 60,000 roentgens were adequately inactivated and gave good immunity, while larvae at 100,000 roentgens were well-inactivated (probably over-inactivated) but gave poorer immunity.

Group 7, which received 10,000 non-irradiated larvae as its 'vaccinating' dose, showed some protection - a mean worm burden of 620 compared to the control mean of 2042 - but it was less than the protection conferred by the same number of irradiated larvae on groups 1B to 4B. This possibly enhanced immunogenic effect of a given number of irradiated larvae compared to a similar quantity of normal larvae has been observed in Uncinaria stenocephala and

Ancylostomum caninum (quoted in review by Urquhart, McIntyre, Mulligan, Jarrett and Sharp 1963).

Purely speculatively, the reasons for this apparent phenomenon might be that the irradiated larvae are retarded in their development and so spend longer either within or in intimate contact with the mucosa, thus possibly increasing the chances of immunogenic contact with the host.

Such contact might be twofold:

(a) a proportion of the attenuated larvae may die in situ in the mucosa.

(b) others may undergo prolonged second or third ecdyses, as a result of irradiation, and thereby expose the host to the 'moulting secretions' for longer periods. Soulsby (1959) and Soulsby and Stewart, D.F. (1960) have shown that the secretions of ecdysis are important antigens in the 'self-cure' phenomenon; they may be important in the general context of immunity to helminths, for example Stewart T.B. (1958) believes that immunity of cattle to Cooperia punctata is manifested by inhibition of the ex-sheathment of the larvae.

As the aim of the present experiment was entirely quantitative, only a few pieces of abomasum could be retained for histological examination. However, some of the sections from the pairs of lambs killed as inactivation controls (i.e. having received only irradiated larvae) showed dead larvae lying in the mucosa of the abomasum, together with the development of large lymphoid follicles.

This might indicate the site of the immune response, but this question needs a detailed pathological approach involving serial killing after both vaccination and challenge before a valid answer can be essayed.

A further point of interest in this experiment is the indication that the minimal penetration by the larval stages is sufficient to stimulate an appreciable immune response and that the presence of adults may not be necessary to produce resistance.

From earlier work on T. spiralis (Gould, Gomberg, Bethell, Villella and Hertz, 1955), Soulsby (1957) suggested that adult infections might be necessary to induce refractoriness to reinfection with gastro-intestinal nematodes. This does not appear to be the case with U. stenocephala (Dow, Jarrett, Jennings McIntyre and Mulligan), and the result of the present experiment indicates that H. contortus, a purely alimentary worm, might also fail to conform to this theory. Similarly, the experiments of Thorson (1951) on N. muris larvae and Campbell (1954) on T. spiralis larvae indicate that larval stages are capable of stimulating protective responses.

The conclusions drawn from this experiment were limited but encouraging and may be summarised as follows:

1. It was possible to partially inactivate third-stage infective H. contortus larvae by X-irradiation.
2. Within the confined experimental system used (relatively small numbers of lambs; larvae given as a single dose; subclinical levels of challenge infection; relatively short interval between

vaccination and challenge) X-irradiated larvae, particularly in the range 40,000 to 60,000 roentgens, could stimulate marked resistance to re-infection.

TABLE 1. The Roentgen and Larval Levels Used in Immunizing Doses and the Times of Administering Larvae and Killing Lambs.

Group	Roentgen dosage	Number of Lambs.	Day 0 (Number of irradiated larvae).	Day 37	Day 117 (Number of normal larvae).	Day 163
1 (A)	10,000	2	10,000	Killed	------	------
(B)	10,000	5	10,000	------	8,000	Killed
2 (A)	20,000	2	10,000	Killed	------	------
(B)	20,000	5	10,000	------	8,000	Killed
3 (A)	40,000	2	10,000	Killed	------	------
(B)	40,000	5	10,000	------	8,000	Killed
4 (A)	60,000	2	10,000	Killed	------	------
(B)	60,000	5	10,000	------	8,000	Killed
5 (A)	100,000	2	10,000	Killed	------	------
(B)	100,000	5	10,000	------	8,000	Killed
6	------	5	10,000	Killed	------	------
7	------	5	10,000	------	8,000	Killed
8	------	5	------	------	8,000	Killed

TABLE 2. Parasitological Findings in Lambs Killed on Day 37.

Group	Eggs per gram of faeces		Worm count at necropsy		
	Day 18	Day 33	Immature	Mature	Group means
1 (A)	+*	3,100	-------	1,900	
	+	1,100	-------	3,700	2,800
2 (A)	+	1,400	-------	1,850	
	+	1,500	-------	1,500	1,675
3 (A)	+	0	300	0	
	0	0	200	0	250
4 (A)	0	50	400	0	
	0	50	100	0	250
5 (A)	+	50	100	0	
	0	0	0	0	50
6	+	1,100	0	1,100	
	+	3,300	0	2,700	
	+	2,000	0	1,600	1,900
	+	1,850	0	2,400	
	+	2,050	0	1,700	

* Positive on nonquantitative zinc sulphate flotation only.

TABLE 3. Parasitological Findings in Lambs in Group 1 (B). (Vaccinated with larvae receiving 10,000 roentgens).

Eggs per gram of faeces on day:	lamb number					
	1	2	3	4	5	Mean
18	+°	0	+	+	0	-
34	300	4,400	2,500	2,000	2,450	2,200
40	1,350	700	1,200	3,000	2,100	1,670
48	2,050	1,000	4,300	1,650	0	1,800
54	2,450	500	5,800	1,000	50	1,960
61	1,850	500	3,050	1,200	0	1,280
68	1,600	0	7,000	0	0	1,720
76	800	n.s. **	2,000	200	0	750
83	500	100	2,100	50	0	550
90	0	0	3,100	0	40	630
96	0	0	3,350	50	0	680
103	0	0	2,000	0	0	400
110	0	0	1,500	0	0	300
117*	0	0	100	0	0	20
144	20	0	0	0	0	4
146	0	0	40	0	0	8
158	0	0	300	0	0	60
Number of worms at post-mortem	0	0	20	0	7	5

* Point of challenge. °Positive on nonquantitative zinc sulphate flotation only.

** No sample obtained.

TABLE 4. Parasitological Findings in Lambs of Group 2 (B).

(Vaccinated with larvae receiving 20,000 roentgens).

Eggs per gram of faeces on day:	lamb number					
	1	2	3	4	5	MEAN
18	+°	+	0	0	+	-
34	1,850	1,100	2,050	100	2,150	1,500
40	2,900	2,850	1,150	1,300	2,400	2,120
48	1,400	2,100	450	2,500	2,100	1,710
54	2,650	1,700	500	800	100	1,150
61	2,700	1,100	350	2,600	0	1,350
68	2,550	750	50	3,000	0	1,270
76	1,100	800	0	1,900	0	760
83	800	800	50	600	0	580
90	0	2,000	50	0	0	530
96	50	0	0	0	0	10
103	0	0	0	0	0	0
110	0	0	0	0	0	0
117*	0	0	0	0	0	0
144	0	0	0	0	0	0
146	0	0	0	0	0	0
158	0	0	0	0	50	10
Number of worms at post-mortem	0	0	0	0	0	0

* Point of challenge.

° Positive on nonquantitative zinc sulphate flotation only.

TABLE 5. Parasitological Findings in Lambs of Group 3 (B).

(Vaccinated with larvae receiving 40,000 roentgens).

Eggs per gram of faeces on day:	lamb number					
	1	2	3	4	5	MEAN
18	+°	0	0	0	0	-
34	150	100	0	150	0	60
40	50	50	150	0	150	80
48	0	0	100	150	n.s.**	50
54	50	0	0	50	50	30
61	0	0	40	100	0	30
68	20	20	0	0	0	8
76	50	n.s.**	0	0	0	10
83	20	0	0	0	0	5
90	0	0	n.s.**	n.s.**	n.s.**	0
96	0	0	0	0	0	0
103	0	0	0	0	0	0
110	0	0	20	0	0	4
117*	0	0	0	0	0	0
144	0	0	0	0	0	0
146	0	0	0	0	20	4
158	100	0	50	0	0	30
Number of worms at post-mortem	972	0	0	0	40	200

* Point of challenge. °Positive on nonquantitative zinc sulphate flotation only. ** No sample obtained.

TABLE 6. Parasitological Findings in Lambs of Group 4 (B) (Vaccinated with larvae receiving 60,000 roentgens).

Eggs per gram of faeces on day:	lamb number					MEAN
	1	2	3	4	5	
18	+°	+°	0	0	0	-
34	50	0	0	0	0	10
40	0	0	0	0	100	20
48	0	0	0	0	0	0
54	0	0	0	0	0	0
61	0	0	0	100	0	20
68	0	0	0	20	0	4
76	0	0	0	0	0	0
83	0	0	0	0	0	0
90	n.s. **	n.s. **	n.s. **	n.s. **	n.s.**	-
96	50	20	20	0	0	18
103	20	0	0	0	0	4
110	0	0	0	0	0	0
117*	0	0	0	0	0	0
144	0	0	0	0	0	0
146	0	0	0	0	0	0
158	0	50	0	0	0	10
Number of worms at post-mortem	0	0	0	0	0	0

* Point of challenge. ° Positive on nonquantitative zinc sulphate flotation only. ** No sample obtained.

TABLE 7. Parasitological Findings in Lambs of Group 5 (B)

(Vaccinated with larvae receiving 100,000 roentgens).

Eggs per gram of faeces on day:	lamb number					
	1	2	3	4	5	MEAN
18	0	0	+°	+°	0	-
34	50	0	0	0	0	10
40	0	0	0	0	0	0
48	0	0	0	0	0	0
54	0	0	0	0	50	10
61	0	0	0	0	0	0
68	0	0	0	20	0	4
76	0	0	0	0	0	0
83	0	0	0	0	0	0
90	n.s.**	n.s. **	n.s.**	n.s. **	n.s.**	-
96	0	0	0	20	0	4
103	0	0	0	0	0	0
110	0	0	0	0	0	0
117*	0	0	0	0	0	0
144	0	0	0	0	0	0
146	100	50	0	0	0	30
158	10,000	2,800	50	0	100	2,590
Number of worms at post-mortem	1,540	397	270	0	0	442

* Point of challenge. ° Positive on nonquantitative zinc sulphate flotation only. ** No sample obtained.

TABLE 8. Parasitological Findings in Lambs of Group 7.

(Vaccinated with normal larvae).

Eggs per gram of faeces on day:	lamb number					
	1	2	3	4	5	MEAN
18	+°	+°	0	0	0	-
34	1,000	1,000	3,850	900	2,450	1,840
40	300	2,100	2,500	1,700	4,200	2,160
48	150	450	700	1,000	250	510
54	600	800	2,050	4,200	6,450	2,820
61	150	1,550	850	3,750	7,600	2,780
68	900	300	150	4,200	4,200	1,950
76	0	0	100	2,000	1,800	780
83	0	0	350	900	300	310
90	0	0	350	0	60	82
96	0	40	0	0	150	40
103	0	0	0	0	20	4
110	0	0	0	0	0	0
117*	0	0	0	0	0	0
144	0	0	0	0	0	0
146	0	20	0	0	0	4
158	0	4,000	600	n.s.**	0	1,150
Number of worms at post-mortem	0	1,450	1,500	150	0	620

*Point of challenge. °Positive on nonquantitative zinc sulphate flotation only. ** No sample obtained.

TABLE 9 Parasitological Findings in Lambs of Group 8.

(Controls - no larvae prior to challenge).

Lamb Number	Challenge. Day 117	Eggs per gram of faeces on day: 144	146	158	Number of worms at post-mortem.
1	8,000 normal larvae	0	0	2,350	1,550
2	8,000 normal larvae	0	0	8,850	2,390
3	8,000 normal larvae	0	0	4,400	430
4	8,000 normal larvae	0	0	1,850	4,240
5	8,000 normal larvae	0	0	2,250	1,600
Mean		0	0	3,940	2,042

TABLE 10. Summary of Results of Challenge Infection.

Group	Roentgen Dose	Mean Number of worms from challenge.
1(B)	10,000	5
2(B)	20,000	0
3(B)	40,000	200
4(B)	60,000	0
5(B)	100,000	442
7	Normal larvae	620
8	Control	2,042

Experiment 13. Vaccination of sheep using two doses of X-irradiated larvae of H. contortus.

Although there is relatively little information on the quantitative aspects of the immune response to parasites, it is known (Jarrett, Jennings, McIntyre, Mulligan, Thomas and Urquhart 1959) that a typical anamnestic response occurs after reinfection of calves with D. viviparus. The much better protection conferred by two, rather than one, doses of irradiated D.viviparus vaccine was shown in experiment 5.

Since field infection of lambs can be much higher than the challenge of 8,000 larvae of the previous experiment, it was decided to apply the double dose method and to subject the lambs to much higher challenge levels.

Materials and Methods.

Twenty-two 'newborn' lambs were bought and reared worm-free. The method of feeding and the parasitological and irradiation techniques were as described in experiment 12. The lambs were eight months old at the start of the experiment.

Experimental Procedure.

The lambs were allotted to four groups numbered 1 to 4. There were five lambs each in groups 1 and 2, and six lambs each in groups 3 and 4. On day 0 the animals in groups 1 and 2 were given orally 10,000 third-stage larvae which had been irradiated the same day with 40,000 roentgens. On day 35, this was repeated. On day 64, the immunity of the lambs of groups 1 and 3 was challenged with 10,000 normal infective larvae and that of the lambs in groups 2 and 4 with 50,000 normal larvae.

All of the animals were killed on day 114, when abomasal worm counts were made. Faecal egg counts by the McMaster method were carried out at approximately weekly intervals throughout the experiment. The design of the experiment is summarised in Table I.

Results.

The faecal egg output and abomasal worm burdens of the lambs are shown in Tables 2 to 5. The group mean egg counts of the controls followed the expected pattern: the lambs challenged with 10,000 larvae reached a mean peak of 3,225 eggs per gram 28 days (day 92) after challenge; those given 50,000 larvae reached 10,975 eggs per gram on the same day; the corresponding mean counts of the vaccinates were 0 and 20, respectively. The abomasal worm burdens paralleled these results. The lower challenge-dose vaccinates (group 1) had a mean of 26 worms compared with 1,942 in the corresponding controls (group 3); the figures for the 50,000 group were a mean of 6 worms in the vaccinates as against 2,455 in the controls. These results are summarised in Table 6.

Discussion.

The results indicated that, by giving two doses of vaccine, a considerable degree of resistance to single high and low challenge doses was obtained. The challenge was divided into a high and a low component because we did not wish to overwhelm the immunity of all the lambs by using only the higher dose; the lower dose provided approximate challenge continuity with the previous experiment and provided a more sensitive test of immunity.

However, 50,000 larvae as a single dose was possibly too high from a different viewpoint. The control mean of 2,455 worms resulting from 50,000 larvae compared to 1,942 from 10,000 seemed to indicate either failure of establishment, prepatent elimination or inhibition of development of 95% of the higher dose. Immature forms were searched for but only occasionally found in the high challenge control group, which indicated one or both of the former possibilities.

The immunity produced would probably be sufficient to protect lambs against natural infections but factors still to be explored before assessing the field possibilities of such a vaccine are

(a) the duration of the immunity
(b) the effect of small, spaced doses of H. contortus, i.e., of a 'trickle challenge'
(c) the effect of vaccinating younger lambs. Ours were probably older than would be practical under farm conditions.

How long the resistance would last is at present an open question. Lisenko (1956) has claimed that the immunity conferred on sheep by doses of unaltered H. contortus can be maintained for four years by 'periodic re-infection'.

The vaccine itself is easy to make in large quantities and it appears to have good keeping qualities. This latter judgement is based solely on viability as appreciated by the appearance and motility of stored larvae. There is no information regarding potency of such larvae as immunising agents compared to recently prepared vaccine.

In general terms, X-irradiated H. contortus larvae show 90 to 95% survival after three months' storage at 6°C at a concentration of [illegible],000/ml.in aliquots of 100 ml. of tap water in 8 oz., medical flats. Some such bottles of stored larvae showed 70% viability after nine months. However, these longevity indications were incidental observations and no specific attempt was made to improve or investigate this aspect.

TABLE 1. Experimental Plan. Double vaccination of Sheep with Irradiated Haemonchus contortus larvae. Challenge with Normal Larvae.

Group of Lambs	Day 0	Day 35	Day 64	Day 114
1	10,000 irradiated larvae	10,000 irradiated larvae	10,000 normal larvae	Killed
2	10,000 irradiated larvae	10,000 irradiated larvae	50,000 normal larvae	Killed
3	---	---	10,000 normal larvae	Killed
4	---	---	50,000 normal larvae	Killed

TABLE 2. Parasitological Findings in Lambs of Group 1, Vaccinated with Irradiated Larvae. Challenge: 10,000 normal larvae of Haemonchus contortus.

Lamb number	Eggs per gram of faeces on day:							Number of worms at post-mortem.
	64*	66	78	85	92	99	106	113	
1		0	0	0	0	0	0	0	0
2		50	0	0	0	0	0	0	24
3		0	0	0	0	0	0	0	27
4		0	0	0	0	0	0	0	12
5		0	0	0	0	0	100	50	66
Mean		10	0	0	0	0	20	10	26

* Point of challenge.

TABLE 3. Parasitological Findings in Lambs of Group 2, Vaccinated with Irradiated Larvae. Challenge: 50,000 normal larvae of Haemonchus contortus.

Lamb number	Eggs per gram of faeces on day:							Number of worms at post-mortem
	64*	66	78	85	92	99	106	113	
6		0	0	0	50	50	0	0	14
7		0	100	0	0	0	0	0	11
8		0	50	0	0	0	0	0	0
9		0	50	0	0	0	0	0	0
10		0	0	0	50	50	0	20	4
Mean		0	40	0	20	20	0	4	6

* Point of challenge.

TABLE 4. Parasitological Findings in Unvaccinated Lambs of Group 3. Challenge: 10,000 normal larvae of Haemonchus contortus.

Lamb number	Eggs per gram of faeces on day:							Number of worms at post-mortem
	64*	66	78	85	92	99	106	113	
11		0	0	0	2450	1950	2350	1450	827
12		0	0	50	5400	1300	2400	500	1775
13		0	0	850	4550	6800	6900	7800	3880
14		0	0	0	2200	2300	2600	2500	2680
15		0	0	50	3350	3750	1600	350	1346
16		0	0	0	1400	1650	1200	1000	1145
Mean		0	0	158	3225	2958	2841	2267	1942

* Point of challenge.

TABLE 5. Parasitological Findings in Unvaccinated Lambs of Group 4. Challenge: 50,000 Normal Larvae of Haemonchus contortus.

Lamb number	Eggs per gram of faeces on day:							number of worms at post-mortem
	64*	66	78	85	92	99	106	113	
17		0	0	0	7050	7250	9750	12800	2740
18		0	0	600	23250	13300	6050	10600	4112
19		0	0	0	5550	5000	6650	550	1554
20		0	0	2900	12900	10350	9150	2750	1367
21		0	0	200	9900	550	200	350	630
22		0	0	650	7200	4650	7050	10350	4324
Mean		0	0	725	10975	6850	6475	6233	2455

* Point of challenge.

TABLE 6. The Peak Mean Faecal egg counts per Gram and the Group Mean Abomasal Worm Counts for Lambs Vaccinated with Irradiated *Haemonchus contortus* Larvae and Later Given a Challenge Dose of Normal Larvae.

Group		Peak Mean eggs per gram	Mean Abomasal worm counts
	1	20	26
	2	40	6
(con-trols)	3	3,225	1,842
(con-trols)	4	10,975	2,455

Experiment 14. Vaccination of sheep against Haemonchus contortus using single doses of larvae subjected to one of three regimes of X-irradiation.

This and the next experiment were performed simultaneously on the same batch of lambs, utilising larvae from common harvests. However, the experiments are described separately for convenience.

The present experiment was designed to investigate three main factors.

(a) Irradiation rate. In the previous experiments the irradiation rate had been constant between 180 to 190 roentgens per minute. This relatively slow delivery of tens of thousands of roentgens was time-consuming. However, a more powerful machine, the Siemens Stabilipan, became available, with a capability of delivering X-irradiation in the order of 600 roentgens per minute, at the same kilovoltage and with the same external filtration as before (see technical appendix). The X-ray dose of 40,000 roentgens from the Siemens was compared with 40,000 and 60,000 roentgens from the original A.E.I. GX10 machine.

(b) Vaccine pathogenicity. As H. contortus is a blood-sucking worm, it was necessary both to investigate whether the worm population which did establish itself from the irradiated larvae caused an appreciable drop in the haemoglobin of vaccinated animals, and to correlate this loss with the numbers of worms present.

(c) The effect of a high challenge (50,000 larvae) on lambs vaccinated once with 10,000 irradiated larvae. This was to complement the previous two experiments which included single vaccination with low challenge, and double vaccination with high challenge.

Materials and Methods.

Fifty lambs were bought and reared worm-free as described in experiment 12. They were nine months old at the start of the experiment.

The technique of irradiation was similar to that used for D. viviparus with the exception of the larvae irradiated by the Siemens unit, where the irradiation rate was approximately three times faster at 592 roentgens per minute compared to 186.

Parasitological techniques were as described in experiment 12 with the addition that sex ratio counts were made on worms from the four groups killed on day 30.

Haemoglobin levels were measured with a colorimeter* using a number 3625 filter.

Experimental procedure.

The lambs were allotted to ten groups numbered 1 to 10, with five lambs in each group. The allocations were based on a table of random numbers.

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* Evans Electroselenium Ltd., St. Andrews Works, Essex, England.

On day 0 the lambs were treated as follows:- Those in groups 1 and 2 received 10,000 larvae which had been given 40,000 roentgens of X-irradiation from the GX10 machine at the usual ('slow') rate of 186 per minute. Those in groups 3 and 4 received 10,000 larvae given 40,000 roentgens but at the faster rate of 592 per minute. The lambs in group 5 and 6 received larvae irradiated with 60,000 roentgens at the slower rate by the GX10. Those of group 7 received 10,000 normal larvae, from the common larval harvest, to act as vaccine larval controls. Groups 8 and 9 were designated challenge controls and received no larvae on day 0. (They also served as haemoglobin controls until the time of the inactivation kill.) Group 10 lambs served as haemoglobin controls throughout the experiment, and received no larvae at any time.

All lambs in all groups were blood-sampled every 14 days for haemoglobin and packed-cell volume estimations throughout the experiment. Over the same period, faeces samples were regularly collected from each lamb for egg counting.

On day 30, all the lambs in groups 1,3,5 and 7 were killed to determine both the degree of larval inactivation achieved and the proportion of males to females surviving the irradiation. This time interval of 30 days was chosen to approximate to the corresponding interval of experiment 12, thereby keeping the experiments in line.

On day 60, the lambs in groups 2, 6 and 9 were given a challenge infection of 50,000 normal larvae, while those of groups 4 and 8 were given 10,000 larvae.
Groups 8 and 9 thus acted as respective challenge controls.
On day 100 all lambs were slaughtered and the worms present in the abomasa were counted.

The design of the experiment is summarised in Table 1.

Results.

(a) Inactivation kill

(i) Parasitological. In Table 2 are shown the egg and worm counts of the four groups killed to assess the degree of inactivation. On a group basis, the egg counts roughly paralleled the worm counts.

Table 3 shows the results of Student's 't' test applied to the worm burdens of the four groups. The worm numbers of the irradiated groups show significant differences from the control. In addition there is a probably significant degree of difference ($p < .02$) between the two groups whose larvae received the same irradiation dose (40,000 roentgens) but given at different rates (186 and 592 roentgens per minute, respectively).

Table 4 shows the sex ratio counts and percentages of the worms from the four groups.

Table 5 contains the results of the 't' test applied to the female worm counts of each group, whereby it was confirmed that the female population of two of the irradiated groups differed significantly from that of the controls ($p < .005$, and $< .02$),

while the difference between the remaining irradiated group and the control was probably significant ($p < .05$). Also, the female population resulting from the 'fast' 40,000 roentgen group was significantly ($p < .005$) smaller than that from its 'slow' 40,000 counterpart.

(ii) Haematological. Graph 1 depicts the mean haemoglobin levels of the various comparable groups from day 0 to the time of inactivation kill, viz:- groups 8,9 and 10, which had received no larvae, served together as one larger sized group of haemoglobin controls. Against them were respectively compared the ten lambs (groups 1 and 2) dosed with larvae irradiated at 10,000 roentgens ('slow' rate), the ten lambs (groups 3 and 4) dosed with larvae irradiated at 40,000 roentgens ('fast' rate), the ten lambs (groups 5 and 6) dosed with larvae irradiated at 60,000 roentgens and the five lambs (group 7) dosed with the same number of normal larvae.

Tables 6 and 7 show the haemoglobin differences from the time of dosing to the time of inactivation kill; table 6 is concerned with groups 1 and 2, 3 and 4, and 5 and 6, - and table 7 with groups 7 and 8, and 9 and 10.

Table 8 demonstrates the application of the 't' test to the group mean haemoglobin results.
The group receiving normal larvae shows a highly significant difference ($p < .001$) from the undosed controls.

Each group receiving irradiated larvae is highly significantly different ($p < .001$) from the normal-larvae control group. Finally, the 'fast' 40,000 roentgen group and the 60,000 roentgen group do not differ significantly from the undosed controls ($p < .25$ and $< .5$ respectively), while the 'slow' 40,000 group probably ($p < .05$) differs from the undosed control group.

(b) Challenge kill.

(i) Parasitological. Table 7 shows the peak egg counts of groups 2, 6 and 9 before vaccination, after vaccination and after challenge together with the abomasal worm counts. The first was a check on the pre-experimental parasitic status, but no faeces samples were positive on Zinc-sulphate Flotation or Baermann examination prior to vaccination.

The 6X10 40,000 roentgen group had McMaster positive egg counts following vaccination, but the 6X10 60,000 roentgen group remained negative at this time. Following challenge, most lambs had McMaster positive faeces with egg counts which roughly corresponded to their final worm counts. One of the challenge controls died of haemochosis shortly before the final kill, having an abomasal burden of 13,390 H. contortus worms. On a basis of the worm counts, there is no significant difference between the 6X10 40,000 and 6X10 60,000 roentgen groups (numbers 2 and 6) and the control (group number 9), or between the two 6X10 groups themselves, (Table 11).

Table 10 shows similar data for groups 4 (Siemens 40000 roentgen) and 8 ('low' challenge control).

The Siemens group was negative for faecal eggs pre- and post-vaccination but all lambs had positive egg counts following challenge. The mean abomasal worm burden of 1030 ± 1905 of the Siemens group was significantly different ($p < .005$) from that of 3500 ± 1691 worms of control group 8. (Table 11).

(ii) Haematological.

Table 12 shows the change in haemoglobin levels of the lambs of groups 2, 6, 9 and 10 from the time of challenge to the time of kill, and Table 13 demonstrates the application of the 't' test to these group mean haemoglobin differences. Group 10 is included as a normal-haemoglobin control. There is a probably significant difference ($p < .05$) between the group (number 9) receiving normal larvae and that (number 10) receiving no larvae; the haemoglobin differences between the two vaccinated groups and the challenge control group are not significant.

The haemoglobin levels of the control lamb which died were 3.1 gms. nine days prior to death and 3.0 gms. three days before, with packed cell volume values of 12.5% and 12% respectively.

Table 14 similarly shows the haemoglobin levels of groups 4 and 8, with group 10 again included as a normal-haemoglobin control. There is a probably significant difference ($p < .05$)
(a) between the vaccinated group (number 4) and the challenge control (number 8) and
(b) between the challenge control (number 8) and the haemoglobin control (number 10).

The haematological results are illustrated in Graphs 2 and 3.

Discussion.

An assessment of the results of this experiment falls naturally into two parts:- (a) one dealing with events up to the time of the inactivation kill;

(b) the other dealing with events up to the time of the challenge kill:-

A. Inactivation.

The results obtained from the inactivation kill provided information regarding two of the three queries which formed the rationale for the experiment.

(a) The effect of increasing the rate of X-irradiation on the parasite. This component can only be taken as a pilot trial, particularly as two different X-ray machines were used, but it appeared to indicate that 40,000 roentgens of X-irradiation delivered at 592 roentgens per minute, exerted an inactivating effect on H. contortus larvae comparable to 60,000 roentgens at the rate of 186 per minute. This tentative assessment is made on a basis of: i) the total worm population resulting from the 'fast' 40,000 roentgen group differed significantly ($p < .02$) from its 'slow' 40,000 counterpart, but did not differ significantly from that of the 60,000 roentgen group.

ii) the mean number of males in the 'fast' irradiated group (0.8 ± 1.2) is nearly ten times less than that from the 'slow' group (7.2 ± 7.8), but is the same as that from the 60,000 group (0.8 ± 1.2).

However, the fast-irradiated larvae spent only 32% of the time of their 'slow' equivalents under the X-ray beam (67.5 minutes, compared to 215) and, in addition, were irradiated in a larval concentration of 12,000/ml. compared to 40,000/ml. The combined factors of shorter time and lower concentration would theoretically tend to enhance the effect of a given dosage of X-rays through providing a higher oxygen tension during irradiation.

Whether or not the 'advantageous' conditions of the faster irradiated group are quantitative to the extent of simulating an increase in the order of 20,000 roentgens, is a matter for further investigation.

The pronounced susceptibility of the male parasite to X-irradiation was again demonstrated, as in experiment 8 with D. viviparus and as reported, Jarrett, Jennings, McIntyre and Sharp 1960, with T. colubriformis. From Table 4 it can be seen that the mean percentage of males in the inactivation control group (number 7) is 49.5 ± 0.8%, compared to 3.7%, 0.8% and 0.5% of groups 1,3 and 5 respectively. This greater susceptibility of males to X-irradiation has been recorded also by Shikhobalova, Karmanova and Shekhtman (1958) with T. spiralis, Riek and Keith (1960) with O. radiatum and Ciordia and Bizzell (1960) with T. axei. In addition, Katz (1960) has recorded a similar effect on the heterogenetic generation of S. papillosus with gamma irradiation from a cobalt-60 source.

(b) The second factor which the experiment was designed to elucidate was the possibility of the vaccine itself being pathogenic through its surviving female component. A criticism of the previous experiments involving H. contortus (numbers 12 and 13) is that attention was focussed on worm numbers but not on haemoglobin levels. This omission was due to pressure of work and was rectified in the present experiment.

Reference to Table 5 shows that two of the vaccine groups - GX10 60,000 and Siemens 40,000 - were indistinguishable statistically from the untreated haemoglobin controls, while the third vaccine group - GX10 40,000 - was probably significantly ($p < .05$) affected on the same comparison. However, all three vaccinated groups showed highly significant haemoglobin differences ($p < .001$ in each case) compared to that control group which received the same number of normal larvae. In this context, the result of an experiment by Bemrack, Emerick, Shumard, Pope and Phillips (1958) is of interest. These workers infected one group of lambs with 20,000 normal H. contortus larvae; they periodically bled a second group until it approximated to the mean haemoglobin level of the infected animals; on day 55, they challenged these lambs and a third, control, group with 100,000 normal larvae; they concluded that group 2 (as well as group 1) was significantly resistant and that one of the most important factors in immunity to haemonchosis is the ability of the sheep to withstand and respond to the haemorrhagic effect of the worm. However, to deliberately induce vaccine pathogenicity would seem to be

potentially hazardous and capable of too little control for widespread use in the field.

B. Challenge.

The lambs of the remaining three vaccinate groups, numbers 2, 4 and 6 were challenged with 50,000, 10,000 and 50,000 infective larvae respectively, together with two appropriate control groups. A double level of challenge was used for two main reasons:-

(a) the 'fast' irradiation had not been used previously to produce a larval vaccine and it was not wished to risk overwhelming an unknown protective effect. Thus, a lower (10,000 larvae) challenge was used against the 'Siemens' group.

(b) in experiment 12, lambs similarly vaccinated with 10,000 larvae irradiated at 40,000 or 60,000 roentgens successfully resisted a low challenge; the present comparable groups were therefore tested against a high challenge; (50,000 larvae).

At post-mortem, the Siemens group had a mean number of 1030 $\pm$ 1905 worms, which differed significantly ($p < .005$) from the mean of 3500 $\pm$ 1691 of its challenge control group. This was not as good a level of immunity as was shown by the 40,000 or 60,000 roentgen groups of experiment 12. However, the challenge level was higher against the Siemens group, being 10,000 compared to 8,000, and producing 3500 $\pm$ 1691 worms in the present controls, compared to 2042 $\pm$ 1260 in the controls of experiment 12;

(though not significantly more, $p < 0.20$). Also, the sex ratio of 44.5% males in the abomasal count of the challenged Siemens group might indicate a persistance of irradiated females from vaccination.

Egg counts have been largely ignored in this discussion, because while they did approximately parallel the abomasal worm burdens, they are subject in general to wide and often unpredictable variation (whether measured as eggs per gram or eggs per day: Spedding, 1952) and in the particular case of this type of experiment two further factors may operate: (a) one manifestation of host immunity (Soulsby, 1958; Stewart and Gordon, 1958) may be total or partial suppression of egg production or output.

(b) in severely affected anorexic lambs, the number of eggs per gram may be artefactually boosted by a decreased faecal volume.

The mean worm burdens of the two other (GX10) vaccinate groups are harder to interpret, partly because the widely ranged worm numbers (e.g. the Standard Deviation of $\pm$ 4709 on a mean of 4410) of the 50,000 larvae control group render statistical analysis difficult compared to the tighter results of the low challenge group, (whose mean and standard deviation were 3500 $\pm$ 1691). Also, there may be a degree of competitive inhibition in a single challenge of 50,000 larvae, causing a numerical reduction in the final population through factors unconnected with the hosts' immune status. However, from table 9, it can be seen that four lambs may be considered to have been 'protected' (on a basis of 500 worms or less) in group 6 (GX10 60,000), and three in group 2 (GX10 40,000). In group 2,

a mean sex ratio of 43% males, suggests, but does not prove, that a proportion of the abomasal population was persisting from vaccination.

In summary, short answers to the main queries posed prior to the experiment are:

(a) The evidence is suggestive that the rate of X-irradiation may affect the degree of larval attenuation resulting from the same total quantity of irradiation.

(b) The vaccine larval doses used in this experiment were not pathogenic to the lambs on a basis of haemoglobin estimations.

(c) Eleven of the 15 vaccinated lambs may be considered to have been adequately protected, while eight of the 10 controls developed large worm burdens and one of them died from its challenge infection.

Table 1. Plan of Experiment 14.

The roentgen levels and rates, the larval numbers used in immunising doses, and the times of administering larvae and killing lambs.

Group	Number of lambs	Roentgen dosage	X-ray Machine	Rate of delivery in roentgens per minute.	Larvae Day 0	Day 30	Normal larvae Day 60	Day 100
1	5	40,000	GX10	186	10,000	Killed	-	-
2	5	40,000	GX10	186	10,000	-	50,000	Killed
3	5	40,000	Siemens	592	10,000	Killed	-	-
4	5	40,000	Siemens	592	10,000	-	10,000	Killed
5	5	60,000	GX10	186	10,000	Killed	-	-
6	5	60,000	GX10	186	10,000	-	50,000	Killed
7	5	-	-		10,000 normal larvae	Killed	-	-
8	5	-	-			-	10,000	Killed
9	5	-	-			-	50,000	Killed
10	5	-	-			-	-	Killed

Experiment 14.

Table 2. Inactivation kill on day 30. Abomasal worm counts and faecal egg counts, the latter being made one day previously.

Group	Roentgen dosage	X-ray Machine	Lamb Number	Number of eggs per gm. of faeces	Number of worms in abomasum
1	40,000	GX10	1	200	460
			2	50	800
			3	0	500
			4	0	1,200
			5	0	860
				Mean	764 ± 269
3	40,000	Siemens	1	50	400
			2	0	340
			3	0	120
			4	50	280
			5	0	360
				Mean	300 ± 98
5	60,000	GX10	1	0	440
			2	0	160
			3	0	260
			4	0	620
			5	50	120
				Mean	320 ± 186
7	-	-	1	1,200	3,000
			2	5,200	1,280
			3	3,750	1,660
			4	2,800	1,800
			5	10,250	1,600
				Mean	1,870 ± 591

Experiment 14.

Table 3. Results of applying Student's 't'* test to the final worm counts of the inactivation-kill groups.

Groups compared	Probability that mean worm count difference is due to chance
1 (GX'40') and 7 (control)	$p < .005$
3 (Siemens) " 7 (control)	$p < .001$
5 (GX'60') " 7 (control)	$p < .005$
1 (GX'40') " 3 (Siemens)	$p < .02$

* The Bessel correction factor for small sample bias was applied in the calculation of 't'.

Table 4. Sex ratio counts of worms from 'inactivation kill' groups.

Group		Total number of worms counted for sexing.	Number of males	Number of females	Percentage of males
1	1	177	2	175	1.1
40,000r.	2	260	5	255	1.9
GX10	3	199	22	177	11.1
	4	163	0	163	0
	5	154	7	147	4.5
	-		Mean 7.2±7.8	Mean 179± 38	3.7
	1	90	0	90	0
3	2	110	3	107	2.8
40,000r.	3	102	1	101	1
Siemens	4	45	0	45	0
	5	111	0	111	0
	-		Mean 0.8±1.2	Mean 91± 24	0.8
5	1	157	0	157	0
60,000r.	2	201	3	198	1.5
GX10	3	104	1	103	1.0
	4	47	0	47	0
	5	68	0	68	0
	-		Mean 0.8±1.2	Mean 115± 56	0.5
7	1	934	458	476	48.9
CONTROL	2	451	225	226	49.9
	3	479	232	247	48.4
	4	665	338	327	50.8
	5	415	205	210	49.4
	-		Mean 292±95	Mean 297± 98	Mean 49.5± .83

Table 5. Results of applying Student's 't'* test to the total female counts of the inact--ivation-kill groups.

Groups compared	Probability that inter-group difference is due to chance.
1 (GX'40') and 7 (Control)	$p < .05$
3 (Siemens) " 7 (Control)	$p < .005$
5 (GX'60') " 7 (Control)	$p < .02$
1 (GX'40') " 3 (Siemens)	$p < .005$

* The Bessel correction figure for small sample bias was applied in the calculation of 't'.

Table 6. Individual and mean differences in haemoglobin levels of vaccinated groups 1 and 2, 3 and 4, and 5 and 6 from the third day following vaccination to the day before the inactivation kill.

Group number and description	Lamb Number	Haemoglobin level in gms/100 ml. on Day 3	Haemoglobin level in gms/100 ml. on Day 29.	Difference
1.40,000r. GX10	1	11.1	10.6	-0.5
	2	11.1	9.9	-1.2
	3	11.5	10.2	-1.3
	4	11.4	9.9	-1.5
	5	9.9	10.0	+0.1
2.40,000r. GX10	1	10.9	9.6	-1.3
	2	11.4	10.2	-1.2
	3	11.6	9.0	-2.6
	4	12.5	10.5	-2.0
	5	11.6	10.3	-1.3
	Mean	11.30	Mean 10.02	Mean 1.28±0.70
3.40,000r.Siemens	1	12.4	9.9	-2.5
	2	10.6	9.9	-0.7
	3	10.3	9.6	-0.7
	4	10.0	9.8	-o.2
	5	9.8	10.2	+0.4
4.40,000r.Siemens	1	10.9	10.3	-0.6
	2	11.5	9.9	-1.6
	3	11.1	9.9	-1.2
	4	11.4	12.0	+0.6
	5	10.3	10.0	-0.3
	Mean	10.83	Mean 10.15	Mean -0.68 ± 0.87
5.60,000r.GX10	1	10.6	10.5	-0.1
	2	12.2	11.1	-1.1
	3	12.6	10.8	-1.8
	4	11.1	10.8	-0.3
	5	12.0	11.6	-0.4
6. 60,000r.GX10	1	11.5	10.6	-0.9
	2	10.2	10.2	0.0
	3	12.2	11.1	-1.1
	4	11.2	9.8	-1.4
	5	11.2	10.6	-0.6
	Mean	11.48	10.71	Mean-0.77±0.60

Table 7. Individual and mean differences in haemoglobin levels of undosed control groups 8,9 and 10 and larval control group 7 from the third day after dosing group 7 to the day before the inactivation kill.

Group number and description.	Lamb Number	Haemoglobin level in gms/100 ml. on Day 3	Haemoglobin level in gms/100 ml. on Day 29	Difference
8 undosed control	1	12.0	12.0	0.0
	2	12.5	12.0	-0.5
	3	12.3	11.2	-1.1
	4	10.9	11.2	+0.3
	5	10.9	11.2	+0.3
9 undosed control	1	11.5	10.3	-1.2
	2	14.0	12.6	-1.4
	3	11.9	11.2	-0.7
	4	12.4	10.6	-1.8
	5	10.9	10.9	0.0
10 undosed control	1	11.1	11.2	+0.1
	2	11.2	11.5	+0.3
	3	10.4	10.9	+0.5
	4	11.8	10.5	-1.3
	5	13.5	11.9	-1.6
	Mean	11.82	Mean 11.28	Mean -0.54±0.77
7 control dosed with 10,000 normal larvae.	1	12.5	8.7	-3.8
	2	13.5	9.9	-3.6
	3	12.3	9.5	-2.8
	4	12.3	9.8	-2.5
	5	12.0	8.8	-3.2
	Mean	12.52	Mean 9.34	Mean -3.18±0.46

Table 8. Results of applying Student's 't'* test to the group mean haemoglobin-level differences of tables 6 and 7.

Groups compared	Probability that mean difference is due to chance.
7 (normal-larvae controls) and 8-9-10 (undosed controls)	$p < .001$
1-2 (40,000r.GX10) and 8-9-10 (Undosed controls)	$p < .05$
3-4 (40,000r.Siemens) and 8-9-10 (undosed controls)	$p < .25$
5-6 (60,000r. GX10) and 8-9-10 (undosed controls)	$p < .5$
1-2 (40,000r. GX10) and 7 (normal-larvae controls)	$p < .001$
3-4 (40,000r. Siemens) and 7 (normal-larvae controls)	$p < .001$
5-6 (60,000r. GX10) and 7 (normal-larvae controls).	$p < .001$

* The Bessel correction factor for small sample bias was applied in the calculation of 't'.

Table 9. Results of faecal egg counts before vaccination and before and after challenge with 50,000 larvae, together with the abomasal worm burdens, of groups 2, 6 and 9.

Group no. and description.		Max. $ZNSO_4$ flotation egg count before vaccination.	Peak McMaster egg count before challenge.	McMaster egg count after challenge.	Abomasal worm count.
2.	1	-ve	50	-ve	0
40,000r.	2	-ve	50	50	0
GX10	3	-ve	-ve	-ve	500
	4	-ve	50	3,000	1,480
	5	-ve	-ve	34,250	14,860
				Mean	3,370± 5,771
6.	1	-ve	-ve	-ve	0
60,000r.	2	-ve	-ve	250	40
GX10	3	-ve	-ve	-ve	60
	4	-ve	-ve	1,150	460
	5	-ve	-ve	1,100	1640
				Mean	640± 654
9. 'High	1	-	-ve	100	0
Challenge	2	-	-ve	9,450	1,580
controls'	3	-	-ve	17,250	2,740
	4	-	-ve	2,750	4,340
	5	-	-ve	12,650	13,390
				Mean	4,410± 4,709

Table 10. Results of faecal egg counts before vaccination and before and after challenge with 10,000 larvae, together with abomasal worm burdens, of groups 4 and 8.

Group no. and description		Max. $ZNSO_4$ flotation egg count before vaccination.	Peak McMaster egg count before challenge	Peak McMaster egg count after challenge	Abomasal worm counts.
4.	1	-ve	-ve	2,750	100
40,000r.	2	-ve	-ve	50	160
Siemens	3	-ve	-ve	9,350	520
	4	-ve	-ve	4,600	600
	5	-ve	-ve	4,100	3,760
					Mean 1,030± 1,905
8.	1	-	-ve	3,450	820
'Low chall-	2	-	-ve	12,500	2,680
-enge'	3	-	-ve	7,200	3,400
controls	4	-	-ve	34,650	5,140
	5	-	-ve	14,350	5,440
					Mean 3,500± 1,691

Table 11. Results of applying Student's 't'* test to the group abomasal worm counts of tables 9 and 10.

Groups compared	Probability that mean difference is due to chance.
2. (40,000r.GX10) and 9. (High challenge controls).	p .8
6. (60,000r. GX10) and 9. (High challenge controls)	p .2
2. (40,000r. GX10) and 6. (60,000r. GX10)	p .4
4. (40,000r.Siemens) and 8. (Low challenge controls)	p .005

*The Bessel correction for small sample bias was applied in the calculation of 't'.

Table 12. Individual and mean differences in haemoglobin levels of vaccinate groups 2 and 6, challenge control group 9 and undosed haemoglobin control group 10, from the time of challenge to the time of kill.

Group no. and description		Haemoglobin level in gms/ 100 ml. on Day 57	Haemoglobin level in gms/ 100 ml. on Day 99	Difference
2. (40,000r. GX10)	1	11.5	12.1	+0.6
	2	10.9	11.8	+0.9
	3	12.3	11.5	-0.8
	4	10.9	6.4	-4.5
	5	11.0	11.9	+0.9
		Mean 11.32	Mean 10.74	Mean -0.5 ± 2.01
6. (60,000r. GX10)	1	11.0	10.6	-0.4
	2	11.2	11.5	+0.3
	3	11.5	10.9	-0.6
	4	10.0	10.5	+0.5
	5	11.8	11.1	-0.7
		Mean 11.10	Mean 10.92	Mean -0.18 ± 0.49
9. ('High challenge controls')	1	10.3	5.4	-4.9
	2	12.6	12.0	-0.6
	3	11.2	3.0*	-8.2
	4	10.6	9.4	-1.2
	5	10.9	10.8	-0.1
		Mean 11.12	Mean 8.12	Mean-3.0 ± 2.99
10. (Undosed haemoglobin controls).	1	11.2	11.8	+0.6
	2	11.5	11.6	+0.1
	3	10.9	11.5	+0.6
	4	10.5	11.5	+1.0
	5	11.9	11.7	-0.2
		Mean 11.20	Mean 11.62	Mean +0.42 ± 0.42

*Died later in day 99.

Table 13. Results of applying Student's 't' test to the group mean haemoglobin differences of table 12.

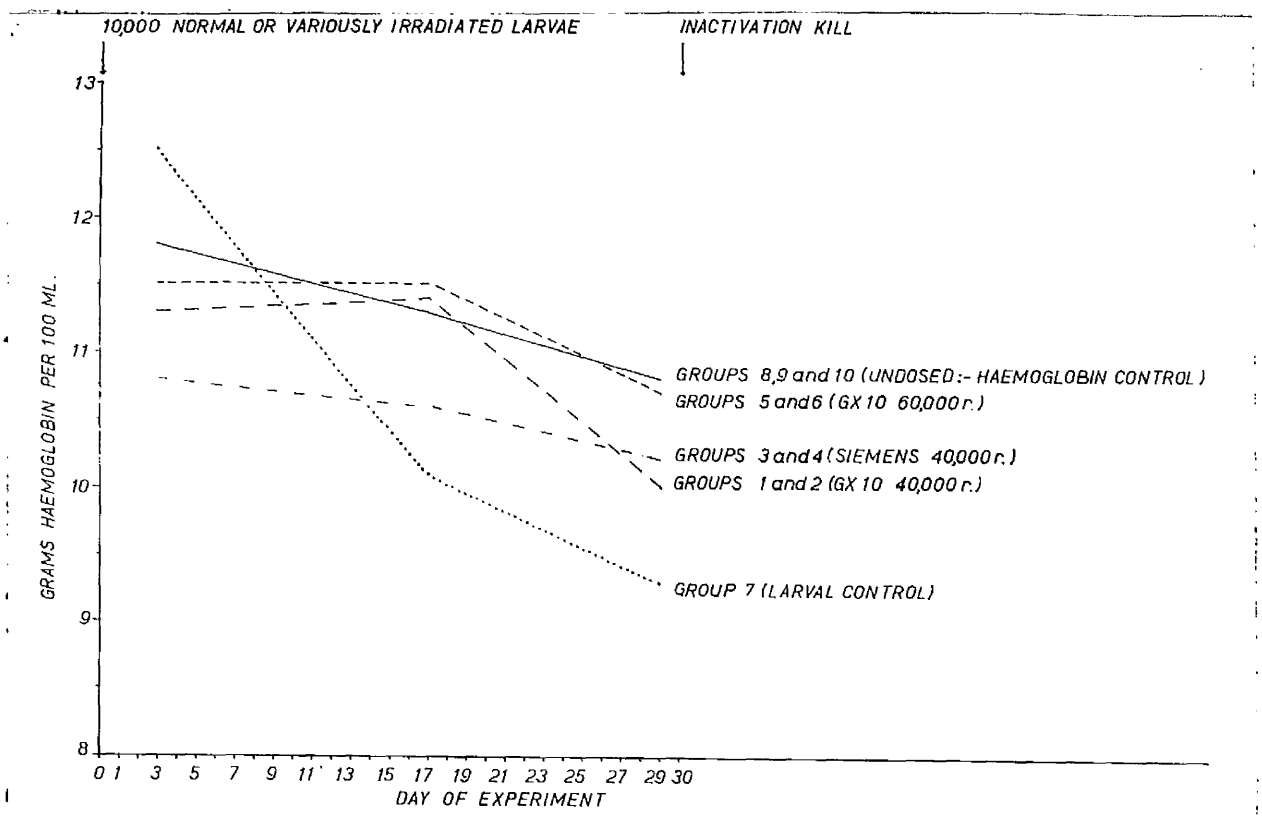
Groups compared	Probability that mean difference is due to chance.
9. (High challenge controls) and 10. (Undosed haemoglobin controls).	$p < .05$
9. (High challenge controls) and 2. (40,000r. GX10)	$p < .24$
9. (High challenge controls) and 6. (60,000r. GX10)	$p < .10$
10. (undosed haemoglobin control) and 2. (40,000r.GX10)	$p < .40$
10. (undosed haemoglobin controls) and 6. (60,000r. GX10)	$p < .10$

*The Bessel correction for small sample bias was applied in the calculation of 't'.

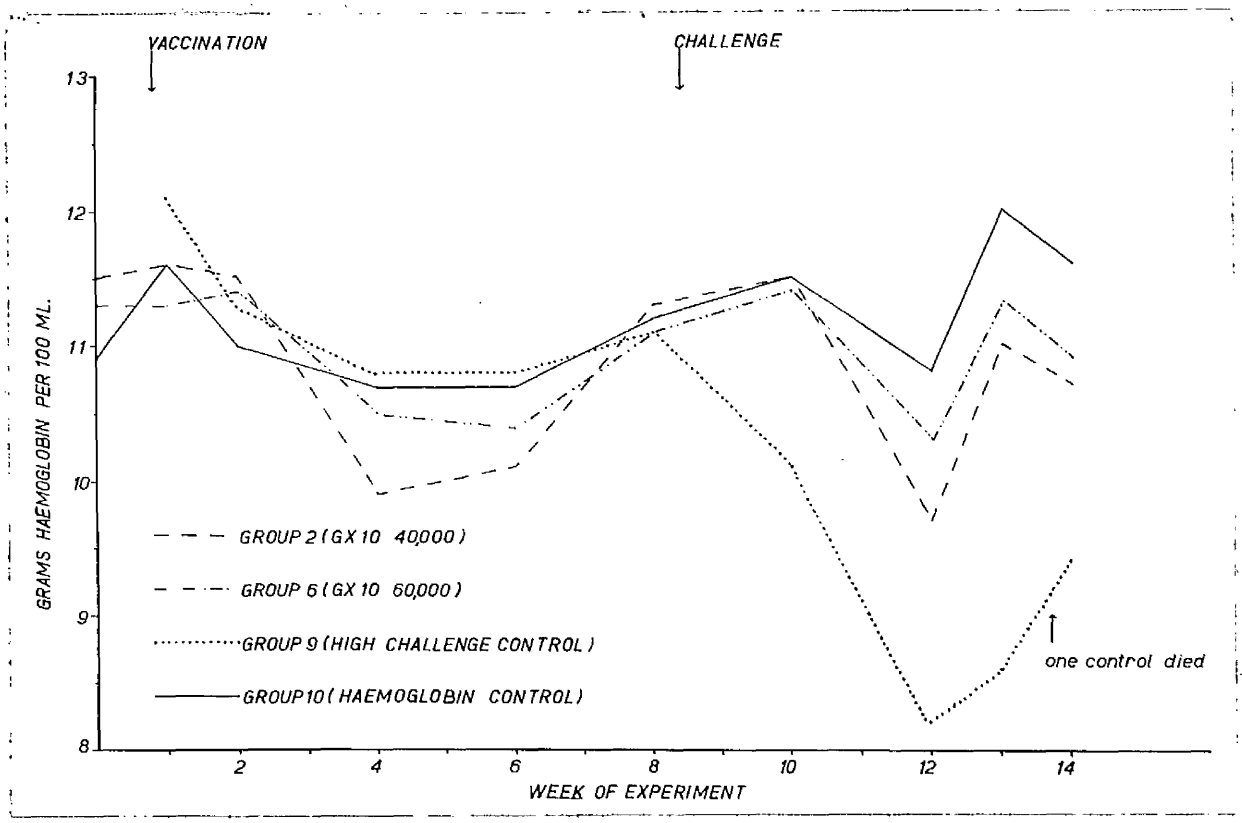
Table 14. Individual and mean differences in haemoglobin levels of vaccine group 4, challenge group 8 and undosed haemoglobin control group 10, from the time of challenge (Day 60) to the time of kill, (Day 100).

Group No. and description		Haemoglobin level in gms/100 ml. on Day 57		Haemoglobin level in gms/100 ml. on Day 99	Difference
4* (40,000r. Siemens)	1	10.8		10.5	-0.3
	2	10.6		11.3	+0.7
	3	10.3		10.8	+0.5
	4	10.9		12.2	+1.3
	5	9.9		9.0	-0.9
	Mean	10.50	Mean	10.76	Mean+0.26 ± 0.84
8* (low challenge controls)	1	12.0		9.1	-2.9
	2	12.0		9.0	-3.0
	3	11.2		10.6	-0.6
	4	11.2		5.1	-6.1
	5	11.2		9.9	-1.3
	Mean	11.52	Mean	8.74	Mean 2.78 ± 2.38
10* (Undosed haemoglobin controls)	1	11.2		11.8	+0.6
	2	11.5		11.6	+0.1
	3	10.9		11.5	+0.6
	4	10.5		11.5	+1.0
	5	11.9		11.7	-0.2
	Mean	11.20	Mean	11.62	Mean +0.42 ± 0.42

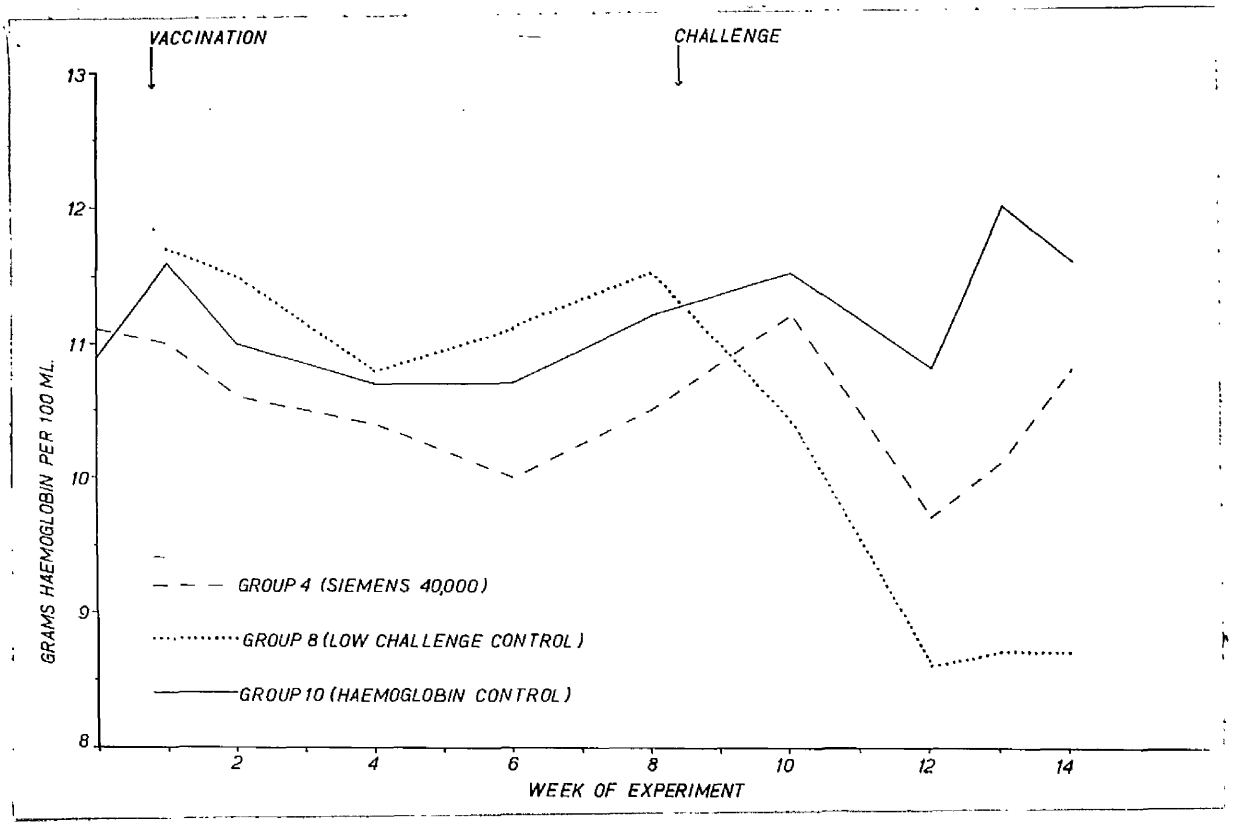
* $p < .05$ for the 't' test comparison of the mean differences in haemoglobin levels of groups 4 and 8, and similarly $p < .05$ for groups 8 and 10.



Graph 1. Haemoglobin levels in Gms. per 100 ml. of groups 8-9-10 compared to groups 1-2, 3-4, 5-6 and 7, from the time of vaccination to the inactivation kill.



Graph 2. Haemoglobin levels in Gms. per 100 ml. of groups 2, 6, 9 and 10 over the whole experiment.



Graph 3. Haemoglobin levels in Gms. per 100 ml. of groups 4, 8 and 10 over the whole experiment.

Experiment 15. Single and double vaccination of sheep, with two grades of larval number per vaccine dose, against a low (10,000 larvae) and a high (50,000 larvae) challenge of Haemonchus contortus.

In experiment 13, double vaccination of sheep conferred a high degree of resistance to each of the two challenge levels of 10,000 and 50,000 larvae respectively. The number of irradiated larvae per vaccine dose had been chosen as 10,000. It was now thought desirable to investigate whether adequate protection could be produced with smaller numbers of larvae since, should such a vaccine become a commercial possibility, it would be important to avoid larval wastage if half or a quarter of the original number would suffice. Hence an experiment was set up to test the variables of number of larvae per dose, one and two doses, and low and high (10,000 and 50,000) larval challenge; i.e. this experiment was designed to dovetail experiment 13, therefore close continuity was observed particularly regarding age of lambs, vaccine - challenge - kill intervals, irradiation dosage and rate, and both challenge levels.

Materials and Methods.

As stated above, this and the previous experiment ran simultaneously on worm-free lambs from a common batch, with larvae from a common harvest, irradiated where appropriate at the same time. The same groups, numbers 8,9 and 10, served as 10,000 larvae challenge controls, 50,000 challenge controls and untreated haemoglobin controls, respectively, for the two experiments.

The parasitological and haematological techniques were the same as before, as was the method of irradiation, except that only one level - 40,000 roentgens - was used, this being applied at the usual rate of 186 roentgens per minute, using the GX10 machine.

Experimental procedure.

The lambs to be vaccinated were allotted to eight groups of five each, numbered 11 to 18. On day 0 the lambs in groups 11 to 14 received 2,500 X-irradiated larvae and those in groups 15 to 18 received 5,000 X-irradiated larvae. (The larval controls for this batch of vaccine were the lambs of group 7, described in the previous experiment.)

On day 30, four of the groups were re-vaccinated as follows:- Groups 13 and 14 received a second dose of 2,500 X-irradiated larvae, and groups 17 and 18 a second dose of 5,000. This batch of vaccine was not strictly controlled by dosing and killing a group of same-harvest normal larval controls, but five culture lambs, dosed with normal larvae from this batch, produced the expected total faecal egg output.

On day 60, all the vaccinates were challenged with normal infective larvae; lambs in groups 11, 13, 15 and 17 received 10,000 each and those in groups 12, 14, 16 and 18 each received 50,000 larvae. As stated, this and the previous experiment shared common control groups, two of which - numbers 8 and 9 - received on day 60 10,000 and 50,000 normal larvae, respectively, for them to act as controls for the two levels of the challenge infection.

A third group, number 10, received no larvae throughout the experiment and served to control the haemoglobin results.

All sheep were faeces-sampled for egg-counting regularly throughout the experiment. All sheep were blood-sampled on days -6, 1 and 8 (to establish a baseline) and thereafter at intervals of two weeks, the last samples being obtained on the day prior to the final kill.

All vaccinated and control groups were killed on day 100 and the worms present in their abomasa were counted.

The design of the experiment is summarised in Table 1.

Results.

Parasitological. Tables 2 to 5 show the peak egg counts before and after vaccination, and after challenge, together with the abomasal worm burdens. None of the lambs had positive faecal egg counts before vaccination; after vaccination six groups contained McMaster positive individuals but none with counts above 50 eggs per gram; the post-challenge egg counts mostly approximated to the worm counts. The main characteristic of the vaccinated groups was the marked variation in worm counts within each group; this tendency seemed to be more exaggerated in the high challenge groups (including the control). This intra-group disparity, together with the small numbers of lambs in the groups, precludes the effectiveness of analysing the worm counts statistically.

Haematological. Tables 6 to 9 list the drop in haemoglobin levels of the various groups over the 40 days between challenge and final kill.

The Tables also include the worm counts for reference. Tables 10 and 11 list the results of applying Student's 't' test to the group results. Graphs 1 to 4 illustrate the mean haemoglobin levels over the whole experiment.

DISCUSSION.

Three methods of evaluating the results of this experiment are:-

(a) Statistical comparison of the group mean worm counts with the appropriate controls.

(b) Similar comparisons of the group mean drop in haemoglobin values between the times of challenge and kill.

(c) Semi-objective assessment of the 'numbers protected' within each group on the basis of, for example, final worm counts; i.e. having set a somewhat arbitrary worm count as the level below which lambs may be considered 'protected'.

Method (a) can be discarded due to the disparity in worm numbers stated above, and method (b) is invalid largely for the same reason.

The histogram (fig. 1) indicates that the challenged vaccinates may be considered to be a bi-modal population and this increases the validity of attribute sampling on the basis of the lambs' being 'protected' or 'not protected'. From fig. 1, it seems reasonable to estimate that the 'protected' level lies in the range 1,000 to 2,000 worms; accordingly, 1,000 worms was chosen as the level above which lambs were not considered to have gained protection by use of the vaccine.

(N.B. It was, of course, considered that the 'bi-modality' might merely have been a reflection of the use of 2 challenge levels. Therefore, in fig. 1, the higher challenged individuals are represented by the cross-hatched areas; it still appears reasonable to suppose bi-modality for each challenge level.)

Tables 12 and 13 list the numbers of lambs in each group which gave evidence of protection; in Table 12, the lambs are considered on a single or double vaccine basis and in Table 13, the basis of comparison is the challenge level - i.e. whether it was 50,000 or 10,000 larvae. It is evident that double vaccination has conveyed no advantage over single vaccination in the experimental system used, and there appears to be little difference between the two challenge levels. 10,000 larvae is an easier challenge to evaluate, having a standard deviation of $\pm$ 1691 on a mean of 3,500, compared to $\pm$ 4709 on a mean of 4410 for the 50,000 controls. It is possible that the higher challenge was partially inhibiting itself through factors not directly connected with the immune status of the hosts, although inhibited larvae were not recorded during the worm counts on high-challenge lambs.

However, of the 40 lambs variously vaccinated and challenged, 26 can be considered 'protected' (tables 12 and 13) compared to the 10 controls, 7 of which had worm burdens over 2,500 and one of which died from its challenge infection. Thus, the vaccine may be considered to have conferred a protection which was real, but quantitatively marginal and it would appear that 2 doses of 10,000 irradiated larvae, as tested in experiment 13, do form the minimum basis for adequate immunisation against H. contortus.

It seems valid to compare Experiment 13 with the present one (15) as the latter was designed to complement it as closely as possible, as was mentioned above. Reasonable parity of challenge was achieved between Experiments 13 and 15 and statistically there is no significant difference between either the two 10,000 challenge control groups or the two 50,000 challenge controls ($p < 0.1$ and $p < 0.4$ respectively).

There was also no statistical difference in worm counts or haemoglobin drops between the inactivation controls (group 7) of Experiment 14 and thecomparable challenge controls(group 8) of the present experiment, ($p < 0.2$ for worm counts and $p < 0.8$ for haemoglobin drops).

Regarding the haemoglobin results, the question of possible pathogenic effects of the vaccine was specifically investigated in experiment 14. In the present experiment, the mean haemoglobin levels of all the vaccinate groups dropped by approximately 1 to 2 gms. in the first three weeks after vaccination (Graphs 1 to 4). The control haemoglobins also dropped over this period, though to a lesser degree, and it is probable that the vaccine was responsible for a proportion of the drop among the former. As can be seen from graphs 1 to 4, only one vaccinated group - the 5,000 larvae single dose - dropped below 10 grams of haemoglobin before challenge, on one occasion when it reached 9.9 grams four weeks after vaccination.

The results of applying the 't' test to the compared mean haemoglobin values after challenge of the vaccinated groups and their appropriate controls are shown in Tables 10 and 11.

It can be seen that, although both challenge controls differed 'probably significantly' ($p < .05$) from the non-parasitised haemoglobin controls, the majority of the vaccinated groups, despite all having final means approximately 2 gms. higher than the challenge controls, nevertheless did not differ significantly from them (or from the haemoglobin controls).

Table 1. Plan of experiment 15.

Group	No. of lambs	Roentgen dosage	No. of larvae per vaccine dose	No. of times vaccin--ated	DAY 0	DAY 30	DAY 60	DAY 100
7	5	-	-	-	10,000n. larvae	killed		
8	5	-	-	-	-	-	10,000n. larvae	kille
9	5	-	-	-	-	-	50,000n. larvae	kille
10	5	-	-	-	-	-	-	-
11	5	40,000	2,500	1	1st dose vaccine	-	10,000n. larvae	kille
12	5	40,000	2,500	1	1st dose vaccine	-	50,000n. larvae	kille
13	5	40,000	2,500	2	1st dose vaccine	2nd dose vaccine	10,000n. larvae	kille
14	5	40,000	2,500	2	1st dose vaccine	2nd dose vaccine	50,000n. larvae	kille
15	5	40,000	5,000	1	1st dose vaccine	-	10,000n. larvae	kille
16	5	40,000	5,000	1	1st dose vaccine	-	50,000n. larvae	kille
17	5	40,000	5,000	2	1st dose vaccine	2nd dose vaccine	10,000n. larvae	kille
18	5	40,000	5,000	2	1st dose vaccine	2nd dose vaccine	50,000n. larvae	kille

Table 2. Results of faecal egg counts before vaccination, and before and after challenge with 10,000 larvae, together with abomasal worm burdens, of groups 11, 13 and 8.

Group No. and description		Max. $ZnSO_4$ flotation egg count before vaccinat^{n}	Peak McMaster egg count before challenge	McMaster egg count after challenge	Abomasal worm count.
11. Single 2,500	1	-ve	-ve	50	0
	2	-ve	-ve	-ve	0
	3	-ve	20	100	0
	4	-ve	-ve	1,600	600
	5	-ve	-ve	600	680
13. Double 2,500	1	-ve	-ve	-ve	20
	2	-ve	-ve	150	100
	3	-ve	-ve	1,800	440
	4	-ve	-ve	5,100	1,900
	5	-ve	-ve	14,150	2,440
8. 'Low challenge' control.	1	-	-	3,450	820
	2	-	-	12,500	2,680
	3	-	-	7,200	3,400
	4	-	-	34,650	5,140
	5	-	-	14,350	5,440

Table 3. Results of faecal egg counts before vaccination, and before and after challenge with 50,000 larvae, together with abomasal worm burdens, of groups 12, 14 and 9.

Group No. and description		Max. $ZNSO_4$ flotation egg count before vaccinat.n	Peak McMaster egg count before challenge	McMaster egg count after challenge	Abomasal worm count
12. Single 2,500	1	-ve	-ve	-ve	20
	2	-ve	50	-ve	60
	3	-ve	-ve	950	160
	4	-ve	-ve	-ve	960
	5	-ve	-ve	12,600	7,360
14. Double 2,500	1	-ve	-ve	-ve	0
	2	-ve	-ve	50	0
	3	-ve	-ve	2,350	0
	4	-ve	-ve	10,100	3,000
	5	-ve	-ve	16,500	7,380
9. 'High challenge' control	1	-	-ve	100	0
	2	-	-ve	9,450	1,580
	3	-	-ve	17,250	2,740
	4	-	-ve	2,750	4,340
	5	-	-ve	12,650	13,390

Table 4. Results of faecal egg counts before vaccination, and before and after challenge with 10,000 larvae, together with abomasal worm burdens, of groups 15, 17 and 8.

Group No. and description		Max. $ZNSO_4$ flotation egg count before vaccinat^n.	Peak McMaster egg count before challenge	McMaster egg count after challenge	Abomasal worm count.
15. Single 5,000	1	-ve	50	200	440
	2	-ve	-ve	750	1,000
	3	-ve	-ve	5,000	1,840
	4	-ve	-ve	100	2,000
	5	-ve	-ve	22,700	6,000
17. Double 5,000	1	-ve	50	300	40
	2	-ve	20	100	60
	3	-ve	-ve	250	300
	4	-ve	-ve	4,900	1,300
	5	-ve	50	9,700	3,720
8. 'Low challenge' control	1	-	-ve	3,450	820
	2	-	-ve	12,500	2,680
	3	-	-ve	7,200	3,400
	4	-	-ve	34,650	5,140
	5	-	-ve	14,350	5,440

Table 5. Results of faecal egg counts before vaccination, and before and after challenge with 50,000 larvae, together with abomasal worm burdens of groups 16, 18 and 9.

Group No. and description		Max. $ZnSO_4$ flotation egg count before vaccination.	Peak McMaster egg count before challenge	McMaster egg count after challenge	Abomasal worm count
16. Single 5,000	1	-ve	-ve	-ve	40
	2	-ve	50	-ve	500
	3	-ve	-ve	600	860
	4	-ve	-ve	14,350	4,920
	5	-ve	-ve	11,750	5,600
18. Double 5,000	1	-ve	50	-ve	0
	2	-ve	-ve	50	0
	3	-ve	20	20	40
	4	-ve	-ve	1,550	560
	5	-ve	-ve	5,000	2,180
9. 'High challenge' control	1	-	-ve	100	0
	2	-	-ve	9,450	1,580
	3	-	-ve	17,250	2,740
	4	-	-ve	2,750	4,340
	5	-	-ve	12,650	13,390

Table 6. Individual and mean differences in haemoglobin levels of groups 10,11,13 and 8 from the day (59) before challenge with 10,000 larvae to the day (99) before final kill; together with abomasal worm burdens.

Group No. and description		Haemoglobin level in gms/100 ml. on Day 59	Haemoglobin level in gms/100 ml. on Day 99.	Difference	Abomasal worm burden.
10. Undosed 'haemoglobin control'	1	11.2	11.8	+0.6	
	2	11.5	11.6	+0.1	
	3	10.9	11.5	+0.6	
	4	10.5	11.5	+1.0	
	5	11.9	11.7	-0.2	
	Mean	11.20	11.62	+0.42± 0.42	
11. Single 2,500	1	10.5	11.5	+0.1	0
	2	11.8	11.8	0.0	0
	3	11.6	12.1	+0.5	0
	4	10.3	10.4	+0.1	600
	5	9.6	10.9	+1.3	680
	Mean	10.76	11.34	+0.58± 0.51	256
13. Double 2,500	1	11.5	13.5	+2.0	20
	2	10.9	10.6	-0.3	100
	3	11.1	11.2	+0.1	440
	4	11.5	10.6	-0.9	1,900
	5	10.5	10.4	-0.1	2,440
	Mean	11.10	11.26	+0.16± 0.98	980
8. 'Low challenge' control	1	11.2	10.6	-0.6	820
	2	12.0	9.1	-2.9	2,680
	3	11.2	9.9	-1.3	3,400
	4	11.2	5.1	-6.1	5,140
	5	12.0	9.0	-3.0	5,440
	Mean	11.52	8.74	-2.78± 2.40	3,500

Table 7. Individual and mean differences in haemoglobin levels of groups 10, 12, 14 and 9 from the day (59) before challenge with 50,000 larvae to the day (99) before final kill, together with abomasal worm burdens.

Group No. and description		Haemoglobin level in gms/100 ml. on Day 59	Haemoglobin level in gms/100 ml. on Day 99	Difference	Abomasal worm burden
10. Undosed 'Haemoglobin control'	1	11.2	11.8	+0.6	
	2	11.5	11.6	+0.1	
	3	10.9	11.5	+0.6	
	4	10.5	11.5	+1.0	
	5	11.9	11.7	-0.2	
	Mean	11.20	11.62	+0.42± 0.42	
12. Single 2,500	1	10.0	10.5	+0.5	20
	2	11.1	12.3	+1.2	60
	3	10.2	10.9	+0.7	160
	4	11.1	12.1	+1.0	960
	5	9.3	8.7	-0.6	7,360
	Mean	10.34	10.90	+0.56± 0.45	1,712
14. Double 2,500	1	11.4	11.7	+0.3	0
	2	10.6	12.6	+2.0	0
	3	9.9	10.6	+0.7	0
	4	10.2	8.4	-1.8	3,000
	5	11.6	9.2	-2.4	7,380
	Mean	10.74	10.50	-0.24± 1.31	2,076
9. 'High challenge' control	1	10.9	10.8	-0.1	0
	2	12.6	12.0	-0.6	1,580
	3	10.3	5.4	-4.9	2,740
	4	10.6	9.4	-1.2	4,340
	5	11.2	3.0	-8.2	13,390
	Mean	11.12	8.12	-3.0± 2.99	4,410

Table 8. Individual and mean differences in haemoglobin levels of groups 10, 15, 17 and 8 from the day (59) before challenge with 10,000 larvae to the day (99) of final kill, together with abomasal worm burdens.

Group No. and description		Haemoglobin level in gms/100 ml. on Day 59	Haemoglobin level in gms/100 ml. on Day 99	Difference	Abomasal worm burden
10. Undosed 'Haemoglobin control'	1	11.2	11.8	+0.6	
	2	11.5	11.6	+0.1	
	3	10.9	11.5	+0.6	
	4	10.5	11.5	+0.1	
	5	11.9	11.7	-0.2	
	Mean	11.20	11.62	+0.42± 0.42**	
15. Single 5,000	1	10.8	11.5	+0.7	440
	2	10.5	10.9	+0.4	1,000
	3	9.8	10.2	+0.4	1,840
	4	10.8	11.5	+0.7	2,000
	5	9.8	6.8	-3.0	6,000
	Mean	10.34	10.18	-0.16± 1.42**	2,256
17. Double 5,000	1	10.2	11.2	+1.0	40
	2	11.2	12.2	+1.0	60
	3	10.5	11.4	+0.9	300
	4	10.2	10.5	+0.3	1, 300
	5	12.8	10.8	-2.0	3, 720
	Mean	10.98	11.22	+0.24 ± 1.54	1, 084
8. 'Low challenge' control	1	11.2	10.6	-0.6	820
	2	12.0	9.1	-2.9	2,680
	3	11.2	9.9	-1.3	3,400
	4	11.2	5.1	-6.1	5,140
	5	12.0	9.0	-3.0	5,440
	Mean	11.52	8.74	-2.78± 2.40	3,500

Table 9. Individual and mean differences in haemoglobin levels of groups 10, 16, 18 and 9 from the day (59) before challenge with 50,000 larvae to the day (99) of final kill, together with abomasal worm burdens.

Group No. and description		Haemoglobin level in gms/100 ml. on Day 59	Haemoglobin level in gms/100 ml. on Day 99	Difference	Abomasal worm burden
10. Undosed 'Haemoglobin control'	1	11.2	11.8	+0.6	
	2	11.5	11.6	+0.1	
	3	10.9	11.5	+0.6	
	4	10.5	11.5	+1.0	
	5	11.9	11.7	-0.2	
	Mean	11.20	11.62	+0.42± 0.42	
16. Single 5,000	1	12.1	10.9	-1.2	40
	2	10.6	11.1	+0.5	500
	3	11.4	11.4	0.0	860
	4	10.3	8.3	-2.0	4,920
	5	11.2	9.1	-2.1	5,600
	Mean	11.12	10.16	-0.96± 1.05	2,384
18. Double 5,000	1	11.5	11.7	+0.2	0
	2	10.2	10.8	+0.6	0
	3	10.3	11.5	+1.2	40
	4	11.1	11.6	+0.5	560
	5	10.0	10.0	0.0	2,180
	Mean	10.62	11.12	+0.5± 0.36	556
9. 'High challenge' control	1	10.9	10.8	-0.1	0
	2	12.6	12.0	+0.6	1,580
	3	10.3	5.4	-4.9	2,740
	4	10.6	9.4	-1.2	4,340
	5	11.2	3.0	-8.2	13,390
	Mean	11.12	8.12	-3.0± 2.99	4,410

Table 10. Results of applying Student's 't' test to the group mean haemoglobin differences listed in tables 6 and 8

Groups compared	Probability that the haemoglobin difference between groups is due to chance.
10.(undosed 'haemoglobin' control) and 8.('low challenge' control)	$p < .05$
11.(single 2,500) and 8.('low challenge' control)	$p < .05$
13.(double 2,500) and 8.('low challenge' control)	$p < .10$
15.(single 5,000) and 8.(low challenge control)	$p < .10$
17. (double 5,000) and 8. (low challenge' control)	$p < .10$

Table 11. Results of applying Student's 't' test to the group mean haemoglobin differences listed in tables 7 and 9

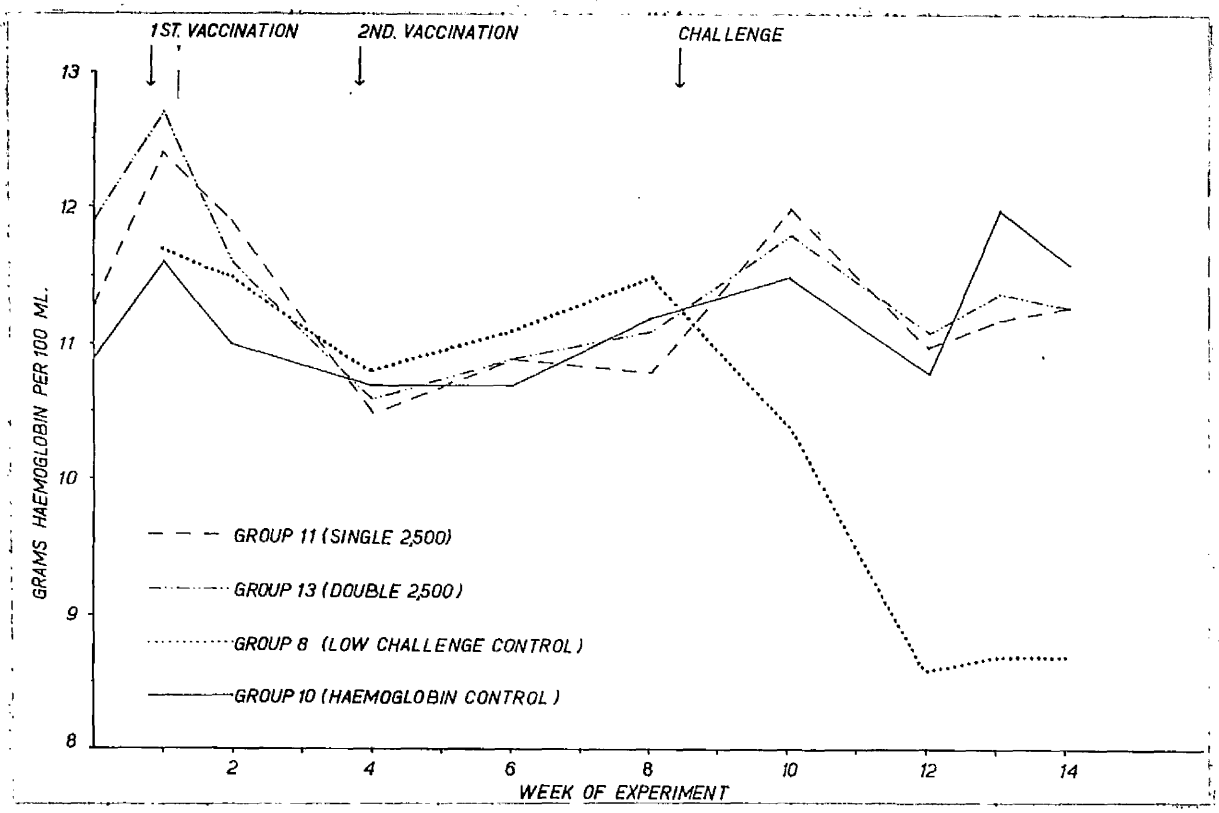
Groups compared	Probability that the haemoglobin difference between groups is due to chance.
10. (undosed haemoglobin control) and 9. ('High challenge' control)	$p < .05$
12. (single 2,500) and 9. ('High challenge' control)	$p < .05$
14. (double 2,500) and 9. ('High challenge' control)	$p < .20$
16. (single 5,00) and 9. ('High challenge' control)	$p < .25$
18. (double 5,000) and 9. ('High challenge' control).	$p < .05$

Table 12. Comparison between single and double vaccination on an attribute basis of the number of lambs 'protected' or 'not protected'.

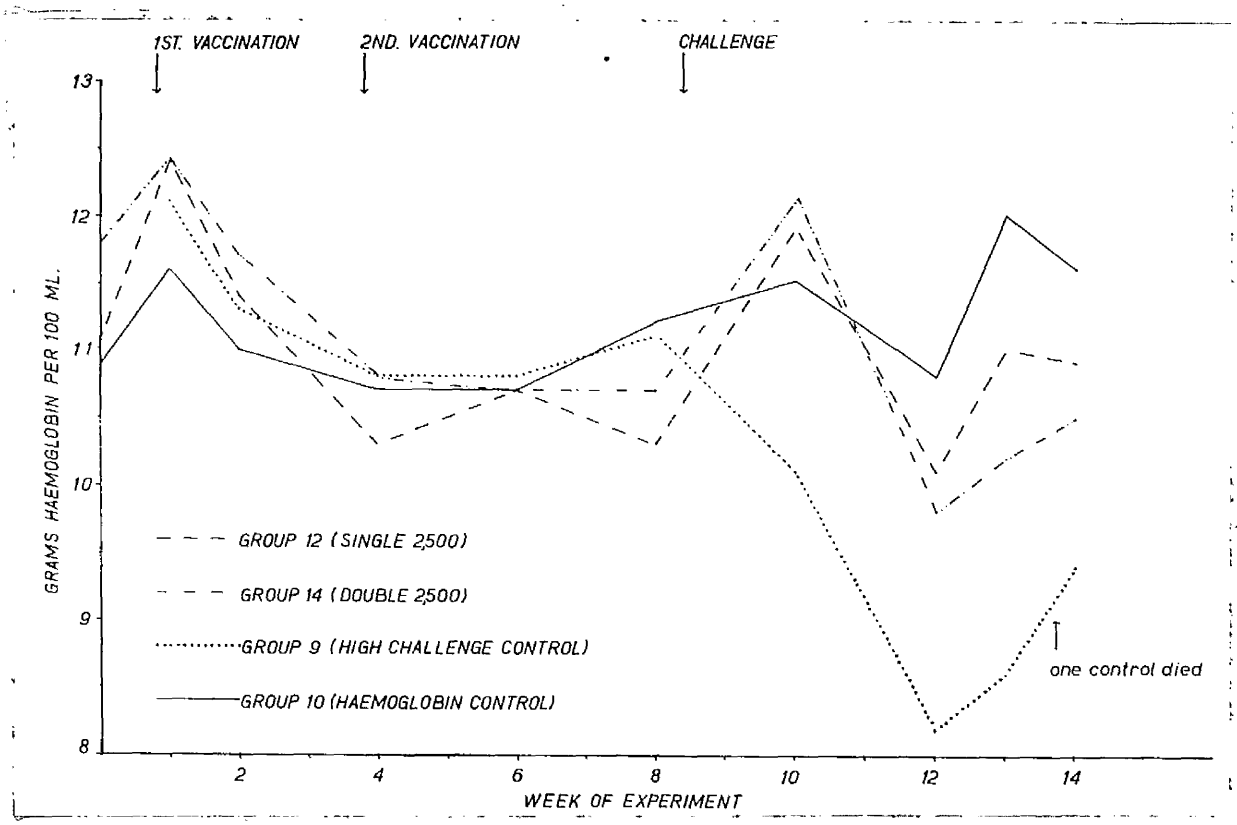
Experimental treatment	Group No. and description	'PROTECTED' 0-99 worms	'PROTECTED' 100-999 worms	'NOT PROTECTED' 1,000 and more worms	Ratio o: protecte not- protecte
single vaccination and challenge	11. single 2,500	3	2	0	
	12. single 2,500	2	2	1	13:7
	15. single 2,500	0	1	4	
	16. single 5,000	1	2	2	
double vaccination and challenge	13. double 2,500	1	2	2	
	14. double 2,500	3	0	2	13:7
	17. double 5,000	2	1	2	
	18. double 5,000	3	1	1	
normal larvae	8. 'low challenge' control	0	1	4	
	9. 'High challenge' control.	1	0	4	2:8

Table 13. Comparison between low (10,000) and high (50,000) levels of challenge on an attribute basis of the number of lambs 'protected' or 'not protected'

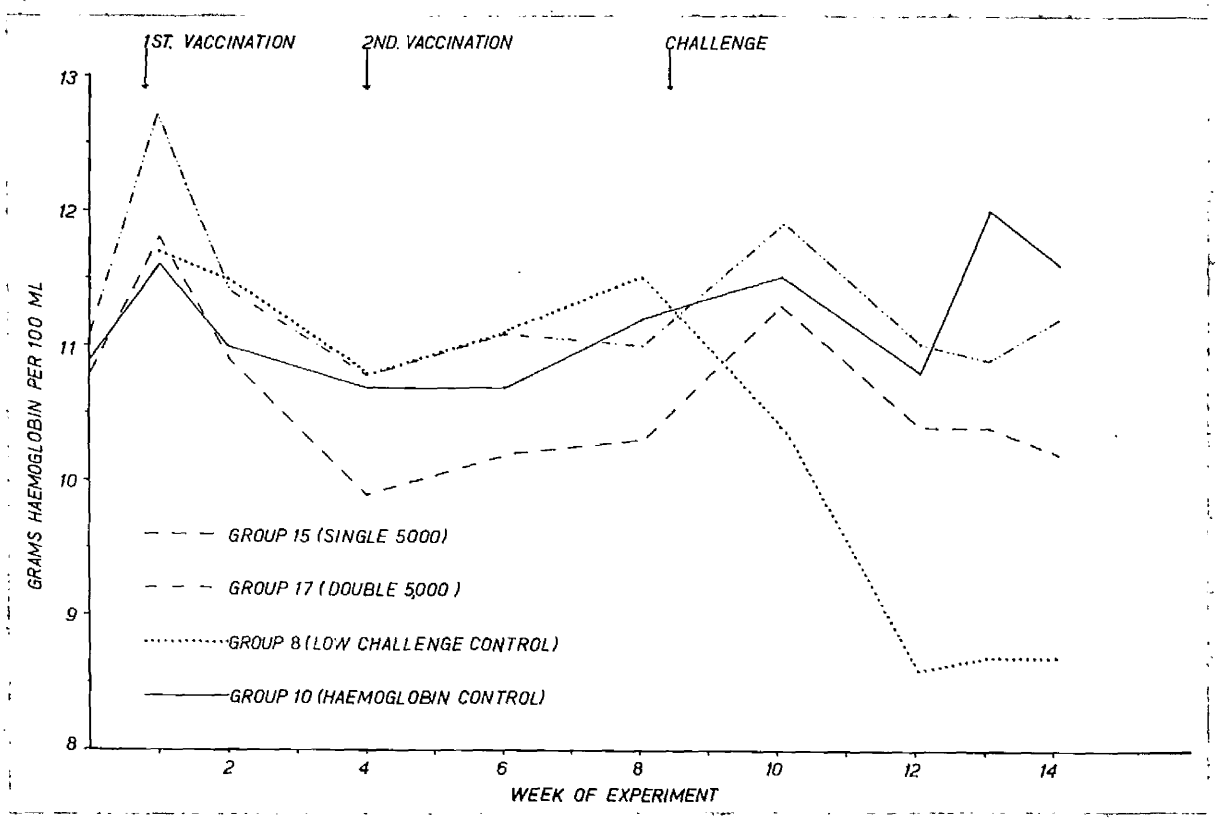
Experimental treatment	Group No. and description	'PROTECTED' 0-99 worms	'PROTECTED' 100-999 worms	'NOT PROTECTED' 1,000 and more worms	Ratio of protected: not-protect
single or double vaccination; Low challenge	11. single 2,500	3	2	0	12:8
	13. double 2,500	1	2	2	
	15. single 5,000	0	1	4	
	17. double 5,000	2	1	2	
Low challenge	8. control	0	1	4	1:4
single or double vaccination; High challenge	12. single 2,500	2	2	1	14:6
	14. double 2,500	3	0	2	
	16. single 5,000	1	2	2	
	18. double 5,000	3	1	1	
High challenge	9. control	1	0	4	1:4



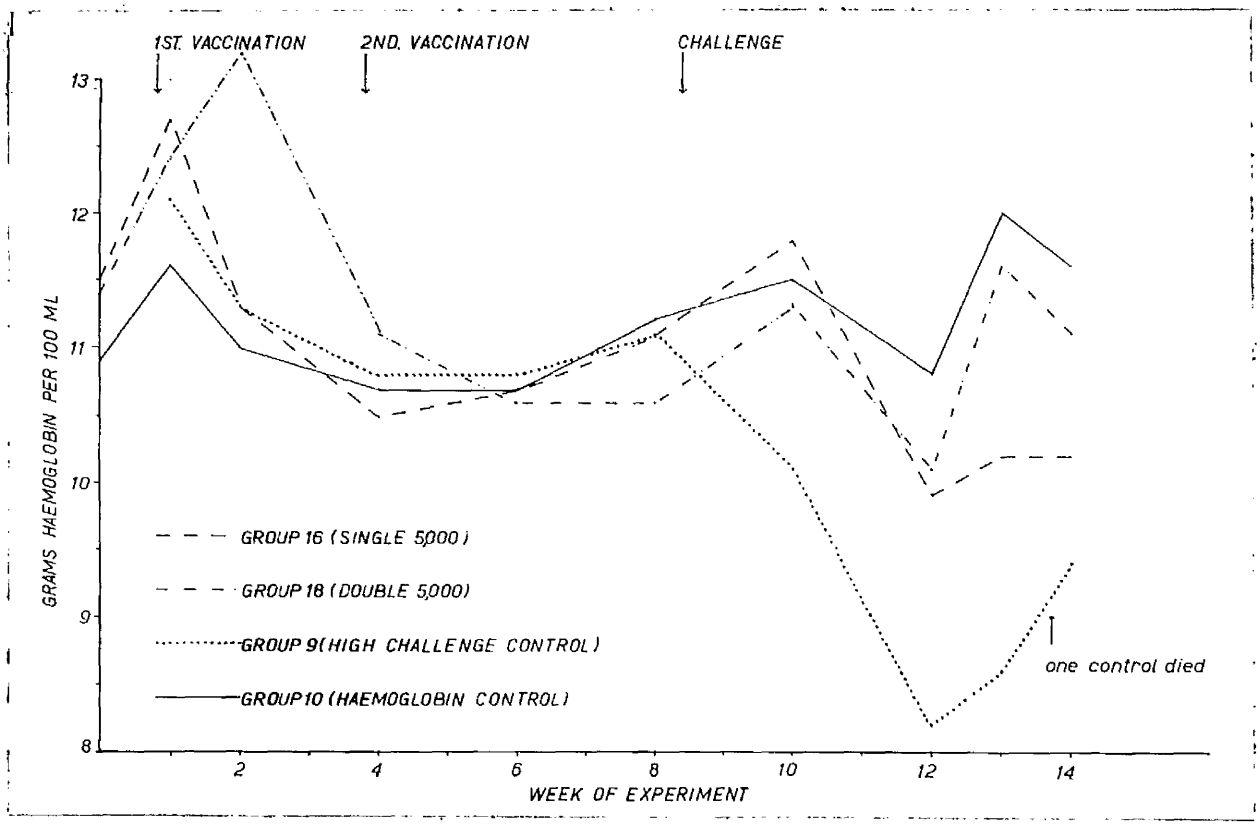
Graph 1. Haemoglobin levels, in Gms. per 100 ml., of groups 8, 10, 11 and 13 over the whole experiment.



Graph 2. Haemoglobin levels, in Gms. per 100 ml., of groups 9, 10, 12 and 14 over the whole experiment.



Graph 3. Haemoglobin levels, in Gms.per 100 ml., of groups 8, 10, 15 and 17 over the whole experiment.



Graph 4. Haemoglobin levels, in Gms. per 100 ml., of groups 9, 10, 16 and 18 over the whole experiment.

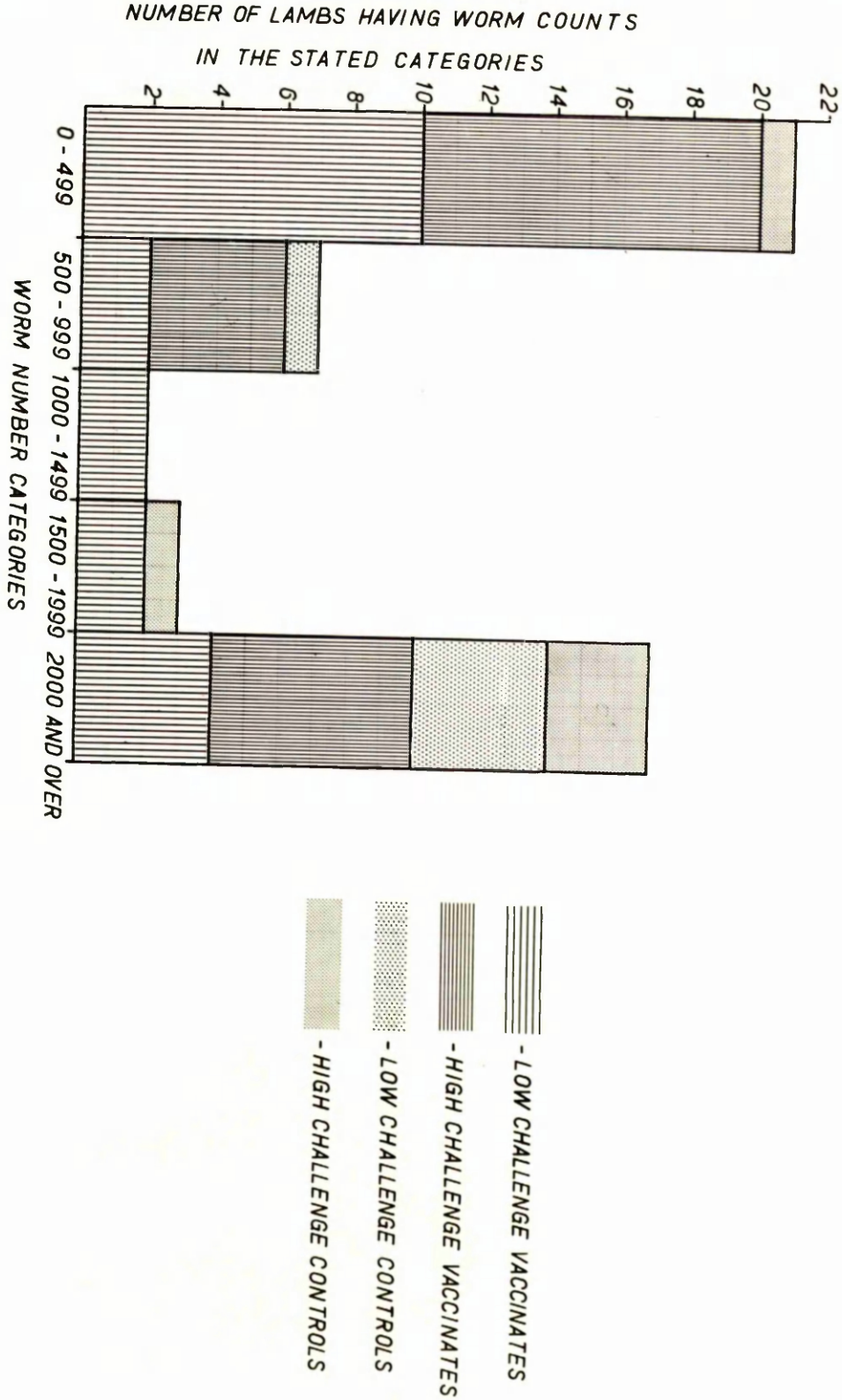


Figure 1. Histogram showing numbers of lambs distributed according to five categories of worm population magnitude.

SUMMARY OF PART IV

The irradiation method of producing an anti-parasitic vaccine was evolved on D. viviparus, a nematode with a systemic migratory cycle. In Part IV are described experiments in sheep in which a similar type of vaccinating procedure is applied to H. contortus, which undergoes only a limited local migration.

Experiment 12. The main object of this experiment was to calibrate the amount of X-irradiation required to appropriately inactivate the larvae. Five levels of irradiation were used, ranging from 10,000 to 100,000 roentgens. Each lamb was vaccinated once with 10,000 larvae. One month later, lambs from each group were killed to determine the degree of inactivation. It was found that, as applied in the present experiment, 10,000 and 20,000 roentgens provided too little inactivation, and 100,000 too much. The remaining vaccinates were challenged, together with controls, and all were killed 46 days later for worm counting. The serviceable level of irradiation, to provide both inactivation and immunisation, lay in the range 40,000 to 60,000 roentgens.

Experiment 13. In this trial, two groups of lambs were vaccinated twice, (10,000 larvae, 40,000 roentgens). Two groups were used in order to accommodate two challenge levels, a lower one of 10,000 larvae to provide continuity with experiment 12, and a higher one of 50,000 larvae to subject the vaccine to a more rigorous test. Within the limitations of the experimental system used, a high degree of resistance was obtained.

Experiment 14. Three main factors were investigated. First, a batch of vaccine was irradiated with 40,000 roentgens, but at three times the rate previously used. For inactivation, it appeared to correspond to 60,000 roentgens at the slower rate. Second, vaccinated lambs were investigated haematologically for possible pathogenic effects of the vaccine. Such effects were not significant in the 'slow' 60,000 and 'fast' 40,000 groups, and probably significant, but marginal, in the 'slow' 40,000 group. Third, a high challenge, (50,000 larvae), was given to two groups of lambs vaccinated once with 10,000 larvae (irradiated with 40,000 and 60,000 'slow' roentgens). This was to complement experiments 12 and 13, which did not include single vaccination against high challenge. Seven of the ten lambs so challenged were considered to have been protected. The basis of this assessment is discussed.

Experiment 15. In the three previous experiments, the vaccinating number of H. contortus larvae was constant at 10,000 per dose. The present experiment was set up to investigate the effects of reducing this number to 5,000 or 2,500, with single or double dosing, against a low or high challenge. Of the 40 vaccinates under trial, 26 were considered to have been protected, whereas seven of ten controls, one of which died, were severely infected. However, it was concluded that none of these vaccinating regimes were suitable and that a minimum basis for adequate protection would be two doses of 10,000 irradiated larvae.

TECHNICAL APPENDIX.

Technical Appendix.

Baermann. The method used was the standard one of spreading approximately 100 gms. of faeces onto a filter paper and inverting this over a nine-inch circle of copper gauze (of 20 meshes per linear centimetre). The gauze rested on the water surface of a Baermann funnel. The apparatus was then placed in a heated room, at approximately 24°C., for 12 hours, after which the bottom 30 ml. of water were collected and examined for sedimented larvae.

As no reference could be readily found to the specific sensitivity of the Baermann method towards larvae of D. viviparus, the following brief test was run. Approximately 600 gms. of faeces were collected from a culture calf and thoroughly mixed. Fifty separate McMaster estimations were made on it in an attempt to provide a count of reasonable accuracy. The mean of these, 250 ± 45 larvae per gram, was taken as the larval content per gram. The remaining faeces were mixed with autoclaved faeces from a non-infected calf, for 'ballast' purposes, in the proportions given in Table 1, to form a series of Baermanns.

Table 1. Proportions of 'larval' to 'ballast' faeces, with the number of larvae estimated to be present in each Baermann funnel, together with the percentage recovered.

Larval faeces	'Ballast'	Estimated number of larvae	Number recovered	Percentage recovered
100G	-	25,000	13,500	54.2
50G	50G	12,500	6,450	51.6
25G	75G	6,250	3.900	62.4
12.5G	87.5G	3,125	1,150	36.7
6.25G	93.75G	1,560	850	54.4
3.0G	97.0G	750	225	30.0
1.5G	98.5G	375	100	26.6
.75G	99.25G	190	75	39.4
.5G	99.5G	125	50	40.0
.25G	99.75G	60	21	35.0
.1G	99.9G	25	6	24.0

The results listed in the table are for Baermanns which were set up with 'cold' water as it came from the tap at approximately 12°C. However, at each faecal weight a duplicate Baermann was set up, similar except that the initial water temperature was 37°C., in case the higher temperature stimulated larval movement and possibly increased the collection. In fact, this had no apparent effect on the percentage recovery.

In another series of Baermanns, the addition of a non-ionic detergent to the water made no appreciable difference, although this has been reported to increase larval yields from Baermannised clover and grass (Rohrbacher, 1957).

In this Baermann sensitivity test, it was felt better to use faeces already containing larvae, than to add known numbers of harvested larvae to sterile faeces. The latter method would have been more accurate, but such larvae would have been third-stage forms and might have had different migratory tendencies from first-stage larvae, which is the stage found in rectal faeces samples examined within 24 hours.

It was concluded that Baermann examination of faeces for larvae of D. viviparus was a test of adequate sensitivity for the experiments in which it was to be used.

Zinc-sulphate flotation. Three grams of faeces were shaken vigorously with 42 ml. of water, and glass beads, in a 100 ml. screw-topped jar, then sieved through a 20-mesh copper gauze, Thirty ml. of the filtrate were centrifuged in two flat-bottomed tubes at 1,000G for three minutes in an MSE * Super Minor centrifuge. The supernatant was gently poured off. The sediment was then agitated and both tubes were filled with saturated zinc-sulphate. A cover-slip was placed on the meniscus of each of the tubes, which were centrifuged at approximately 45G for one minute. The cover-slips were removed and examined on glass slides. A similar procedure was then followed with a second coverslip from each tube.

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* Measuring and Scientific Equipment Ltd., Crawley, Sussex, England.

McMaster. In the case of examinations for larvae of D. viviparus, the standard method was modified because concentrated saline is rapidly lethal to them. Three grams of faeces were shaken vigorously with 42 ml. of water, and glass beads, in a 100 ml. screw-topped jar, then sieved through a 20-mesh copper gauze. Fifteen ml. of the filtrate were centrifuged at 1,000G for three minutes. The supernatant was poured off and the numbered tubes were placed in a rack. Immediately prior to examination the sediment was agitated, and the tube was filled with saturated saline and mixed by inversion. A sample was pipetted from the tube to a double chambered McMaster slide. In the case of there being no larvae under the ruled square, the surrounding area was searched.

Bronchial worm count. The lungs were washed externally and stripped of heart, great vessels and oesophagus. The trachea and bronchi were opened with scissors. The secondary and tertiary bronchi were opened with fine probe-pointed scissors. All worms were carefully lifted into petri dishes filled with normal saline. All worms were counted on being transferred to further dishes of saline. Where desired, the opened lungs were subjected to Baermann examination.

Culturing D. viviparus larvae. Culture-calf faeces were collected thrice daily from the rectum or, if freshly voided, from the straw. The faeces were cultured by spreading aliquots of approximately 30 grams in open glass pomades, to a depth of about four cms., and with a ridged surface.

Ten to 12 such dishes were placed in a three-gallon enamel bucket. The bucket contained three cms. of water in the bottom and it was covered on top with a double sheet of newspaper and a sheet of plate glass. The buckets were placed in natural light at a temperature of approximately 15°C.

Under these conditions, third-stage larvae were ready for harvesting in eight days. To do this, a fine spray of water was directed onto the faecal surface and around the inside of the dish - and on its outside bottom surface to account for migrating larvae. The resulting larval-rich fluid was suitably collected in beakers and, if necessary, it was cleaned by filtration through a 20-mesh copper gauze.

This method produces a larval yield of between 45 and 55 per cent, and it also provides larvae of constant infectivity - a factor of extreme importance in parasitological work.

Two contaminants were encountered in the course of culturing D. viviparus:-

1) Fungal. Two types were noted.

(a) An erect, spore-bearing fungus which seemed not to affect significantly the larval yield. This was almost always present to a small or moderate extent.

(b) A species which covered the faecal surface with a smooth, white skin. This fungus, which was much less common than the first, did have a very adverse effect on larval yield, but whether this was through preventing the larvae from 'surfacing', or through a more specific larvicidal effect, was not investigated.

2) Larval. Various species of soil nematodes were occasionally encountered where faeces had been collected from the floor of the metal huts. They did not seem to affect the larval yield, but such contaminated larval solutions were not used for experiment.

Counting larvae in suspension. Except when required for irradiating, larval-harvest suspensions were diluted, following a pilot count, to a concentration of approximately 100 to 200 per ml. The suspension was thoroughly mixed by being poured from one beaker to another for a few minutes. Then ten separate 0.1 ml. samples were removed, the suspension being mixed between each. The samples were placed on separate glass slides, with coverslips, and counted under the microscope.

Rearing worm-free calves. All the calves used in experiments 3, 5, 8, 9, 10 and 11, and all 'culture' calves, were bought in the local cattle market as 'day-olds', being bull-calf discards from the dairy herds. Their feeding and management is described on page 123. All calves were from either the Ayrshire or Friesian breeds.

'Culture calves'. For the purpose of producing large numbers of D. viviparus larvae, calves of over 12 weeks of age were preferred. Above this age, the faecal consistency was more suitable for the culture method, and the calves themselves could fairly easily withstand a bronchial worm burden large enough to produce between 300 and 900 larvae per gram of faeces over the patent period of approximately four weeks.

Each calf was dosed with 4,000 infective larvae, and patency usually began on the 24th day. Faecal collection was started on the 26th day. Approximately five per cent of calves died during patency. It is of interest to note that approximately 20 per cent of 'ex-culture' calves died up to six months later, as a result of the epithelialising pneumonia characteristic of post-patent parasitic bronchitis (see Part 1).

Dosing calves with larvae. Since many of the experiments described here are quantitative, careful attention was paid that calves ingested the entire dose of normal or irradiated larvae. Such doses were made up either in 50 ml. of water in a six-ounce medical flat or, from experiment 6 onwards, in 30 ml. of water in a 30 ml. vial.

For dosing, the calf was held from the right with the left arm across the back of its poll, holding its head close to the left side of one's body. When the calf had quietened, the bottle was taken with the right hand, shaken, and the bottleneck put into the mouth just in front of the right commisure. The dose was then given in two or three swallows, without any forcing. The bottle was removed, refilled with water and shaken and its contents similarly dosed to the calf. Then the ear number was read and recorded. Where practicable, dosing was carried out midway between morning and evening feeds. Many of the bottles were checked for remaining larvae, and the mean remainder from 150 vaccine doses of 1,000 was eight $\pm$ six larvae.

Clinical examination of calves. (1) Those in isolation huts. The method was to enter the hut and stand quietly for a minute. The respiratory rate was counted first, by watching the respiratory movement. Then the calf was loosely restrained against the pen wall and auscultated on each side of its thorax with a phonendoscope. The character of the respiratory sound was noted under headings in increasing order of harshness, viz:-
vesicular inspiration, harsh vesicular inspiration, broncho-vesicular inspiration, broncho-vesicular breathing and bronchial breathing. Rales, ronchi and emphysematous crackling were also recorded if present. Coughing during examination was noted, otherwise an attempt was made to elicit a cough by pinching the trachea, twice. Finally, a rectal faeces sample was taken, and a blood sample if required, and the ear number was noted and checked against the experimental list. This clinical routine and the classification of respiratory sounds was devised by Professor McIntyre.

(2) Calves in hospital paddock. These were gathered into a pen 30 to 60 minutes before examination. Otherwise the method was similar except that, on completion, each calf was marked for ease of identification.

(3) In the case of calves on the farm, we asked the farmers to bring them into the byre and tie them some time before our expected arrival. The examination was similar.

Pulmonary 'lesion score' method. This was a method evolved by Dr. Jarrett to quantitate, approximately, the amount of consolidation present in a given pair of lungs at post-mortem. As 60 to 70 per cent consolidation was frequently noted to be incompatible with life, this was taken as the upper score of ten, in the range one, two, four, six, eight and ten. A score of one was given in the case of a few scattered lobular areas of consolidation and the more widespread lesions were graded accordingly.

Preparation of larvae for irradiation.

(a) D. viviparus. Only eight to ten-day larvae were used in the experiments. These were harvested, as cleanly as possible, on the morning of the irradiation day. The suspension was filtered through a 20-mesh copper gauze to remove gross faecal debris and fungal spores, if any. A pilot count was done. The suspension was then concentrated by several rounds of centrifugation at 1,500 G. Finally, the original larvae were present in a volume of between five and seven ml.

The concentrated larvae were taken immediately to the X-ray machine and transferred to the irradiation dish. Care was taken to keep the larvae for as little time as possible at the high concentration (from 20,000 to 80,000 per ml.) before and after irradiation. All pipettes and glassware involved in the handling of the larvae immediately prior to irradiation were collected in a marked wire basket, to prevent any possibility of later contamination of the irradiated larvae with normal larvae.

(b) H. contortus. For the purpose of other experiments, not recorded here, it was found that larvae of Trichostrongylus colubriformis could be concentrated for irradiation by the same technique of centrifugation as could D. viviparus. However, considerable difficulty was experienced in trying to concentrate larvae of H. contortus by centrifugation in water. Even at 3,000 G. for ten minutes, only approximately 50 per cent of larvae sedimented. This difficulty was circumvented by harvesting the larvae late on the night before irradiation, putting them into a three-litre measuring cylinder and placing this overnight in a cold-room at $+4^{\circ}C$. Early the following morning, the supernatant was decanted by suction, and if this was done with care, one could obtain approximately 95 per cent of the larvae in a volume of six to seven ml.

Due to the different culture and harvesting techniques, (described in experiment 12), H. contortus larvae could always be obtained in a very clean suspension, compared to D. viviparus larvae.

Irradiation. In the present series of experiments, two X-ray machines were used as described in the text - a Newton Victor GX 10 (figure 1) and a Siemens Stabilipan (figure 2). The main difference between them was that the latter was a more powerful machine, as indicated in Experiment 14. The quality of irradiation from each was similar. In both, the external filtration consisted of 0.25 mm. copper and 1.0 mm. aluminium.

The Newton Victor GX 10 was operated at 140 kV and 5mA and gave an output of between 180 to 190 roentgens per minute over the series of experiments. The Siemens Stabilipan was operated at 140 kV and 20 mA, with a minute output in the order of 590 roentgens.

Calibration of the machines was performed by members of the Western Regional Hospitals Physics department, using a Baldwin-Farmer sub-standard dosimeter.

Table 2 shows two specimen calibrations and illustrates two main points. The first is the importance of measurement when positioning the dish for larval irradiation. The points of measurement used in the experiments described were
(a) The under-surface of the filter, and,
(b) A point corresponding to either the water surface of the larval suspension (older method; experiments 3,4,5,7,8,9 and 10), or to the internal base of the dish (present method; experiments 12, 13, 14 and 15). The Table shows a calibration based on the older method, between filter and water surface.

The second feature from Table 2 is the fall-off in output of the X-ray machine as the number of filament-hours of use increased. This made recalibration necessary, if successive irradiations were more than one month apart.

Throughout the experiments with D. viviparus described in this thesis, the technique of calibration involved measuring the X-ray dose delivered to a point in air corresponding to the surface of the larval suspension. Furthermore, the larvae were irradiated each time in the same glass petri dish, of 4.4 cm. internal diameter. Glass is not an ideal medium for irradiation, as its contaminant heavy metals produce back-scatter. However, the original irradiation of D. viviparus had been done with a glass dish, so this factor was kept constant with this parasite.

During the use of the glass dish for irradiation, two factors modified the amount of irradiation received by the larvae.

(a) Back-scatter. The back-scatter factor was estimated to be approximately 1.15 (V.G. Cottrel, 1959, personal communication).

(b) For a dish of 4.4 cm. internal diameter, 10 ml. of suspension forms a layer of 0.7 cm. deep. This depth of water would permit approximately 80 to 85 per cent of the dose rate at the surface to be applied to the larvae lying on the bottom. (V.G. Cottrel, 1959, personal communication).

These factors are worth recording, although they probably did not significantly affect the results because (a) they were constant throughout experiments 3,4,5,7,8,9 and 10.

(b) the calibration of X-ray dose was ultimately a biological one, obtained during the

search for the level of irradiation most suitable for larval inactivation consistent with adequate immunisation.

The beginning of the experiments involving H. contortus (and T. colubriformis) was a convenient time to modify the irradiation technique as far as the dish and the point of calibration were concerned. All irradiation of these two parasites was done in a dish constructed from 'perspex'. The internal dimensions were made to closely correspond to the original glass dish, being 4.5 cms. in internal diameter, by 1.5 cms. deep (figure 3).

One such perspex dish was adapted as a calibration 'phantom' to contain the ionisation chamber of the dosimeter by having a hole drilled through the side and continuing as a groove of the same diameter along the base, (figure 4). (This enabled the axis of the ionisation chamber to correspond to the level of the internal base of the irradiation dish proper, so the dose measured was that delivered to the larval mass resting on the base.) An amount of tissue-equivalent bolus was then put into the dish to correspond to the volume of water and larvae which would be present in an actual irradiation. As before, the dish was then placed in a square of tissue-equivalent pressed wood, having an appropriate circular recess and also with a horizontal hole through the side to accommodate the lead to the ionisation chamber. The space between the side of the dish and of the recess was also filled with bolus.

Finally, the prepared phantom was placed on two larger blocks of pressed wood, each 15 cm. deep.

This arrangement for calibration is illustrated diagramatically in figure 5.

The procedure for larval irradiation was as follows: The larvae were transferred from the tube to the irradiation dish in a pasteur pipette. Tube and pipette were then washed out in whatever quantity of water was required to bring the final volume to ten ml., usually three to four ml. The appropriate blocks of pressed wood were laid in position on the treatment table. On top of these was placed the smaller piece of recessed pressed wood. The dish was placed in the recess and the surrounding space was filled with tissue equivalent bolus. The machine head was swung into approximate position and centred over the dish. Its height above the dish (water surface or base according to the calibration) was accurately set by means of a piece of stick cut to size. This arrangement for irradiation is shown in figure 6.
Irradiation was then begun.

For the GX 10, the maximum length of run was 55 minutes. At the end of each 55 minute period, ten minutes were allowed for the machine to cool.

The Siemens Stabilipan required restarting every 15 minutes, but it did not require a cooling period.

Figures 1 and 2 show the 'perspex' dishes approximately in position (not yet placed in the small recessed pressed-wood block) before the start of irradiation, by each X-ray machine, of H. contortus larvae for experiment 14.

When a range of different levels of irradiation was being applied, the appropriate fraction of the suspension was removed from the dish and replaced with water as each irradiation level was reached. (This probably resulted in some variability in the effectiveness of the irradiation dose, due to the oxygenating effect of the added water - and possibly to the lowered number of larvae. However, due to pressure on the time of the therapeutic X-ray units, this was the only method practicable).

At the end of the total irradiation period, D. viviparus larvae were removed from the dish into a Roux bottle containing approximately 500 ml. of tap water, brought from our laboratory. The dish was flushed several times to ensure that all larvae were recovered.

In the case of H. contortus, the larvae were diluted to a concentration of approximately 100 per ml. of tap water immediately following irradiation, to prevent larval clumping which was frequently noted if this parasite was kept for any length of time at intermediate dilutions, i.e. in the order of 1,000 per ml. Fortunately it did not occur at the high concentrations upwards of 10,000 per ml. - required for irradiation.

Such clumps consisted of hundreds of larvae and made counting very inaccurate. It was not discovered whether such larvae were able to infect normally, as suspensions containing clumps were not used.

Irradiated larvae were immediately taken back to the Veterinary Hospital where they were counted and made into doses. These were administered to the appropriate experimental animals on the same day. Unused irradiated larvae were retained at $+4^{o}C.$ for morphological viability checking. Such checks were only of a pilot nature, and did not include the infectivity testing necessary for a critical evaluation.

Other techniques used in this thesis are either standard pathological or parasitological procedure, or are described or referred to in the appropriate part of the text.

Table 2. Two specimen calibrations of the Newton Victor GX 10 showing:- (a) variation of X-ray dose with distance, in the beam axis; (b) fall-off in output of X-ray machine with increasing number of filament-hours of use.

Distance between bottom of filter and surface of water in dish.	Output in roentgens per minute on March 12th.	Output in roentgens per minute on August 11th.
1.0 cms.	213	205
1.5 cms.	190	184
2.0 cms.	171	169

(Machine operated at 140 kV and 5 mA; external filtration 0.25 mm. Cu. and 1.0 mm. Al.; half-value layer - 0.44 mm. Cu.: Baldwin-Farmer sub-standard dosimeter; calibrations performed by V.G. Cottrel).

TECHNICAL APPENDIX. Illustrations for irradiation techniques.

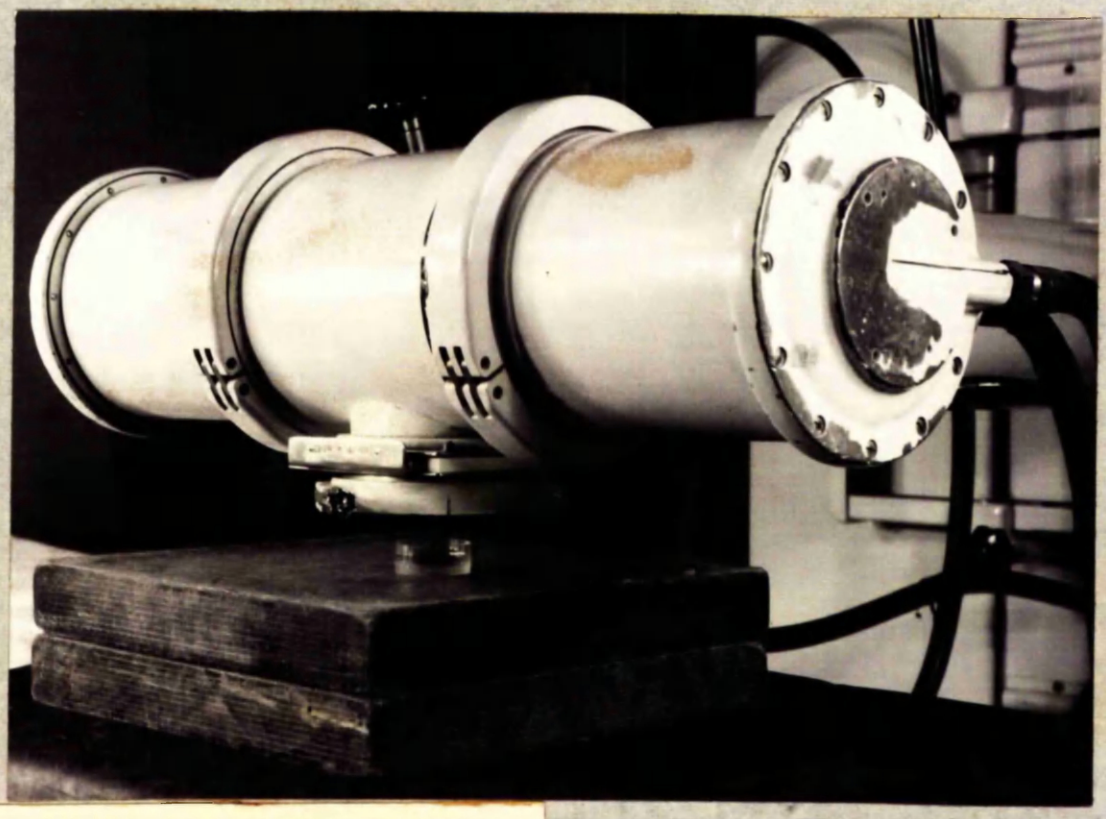

Figure 2. The Siemens Stabilipan therapeutic X-ray machine. A 'perspex' dish containing larval suspension is resting on two large blocks of pressed wood.

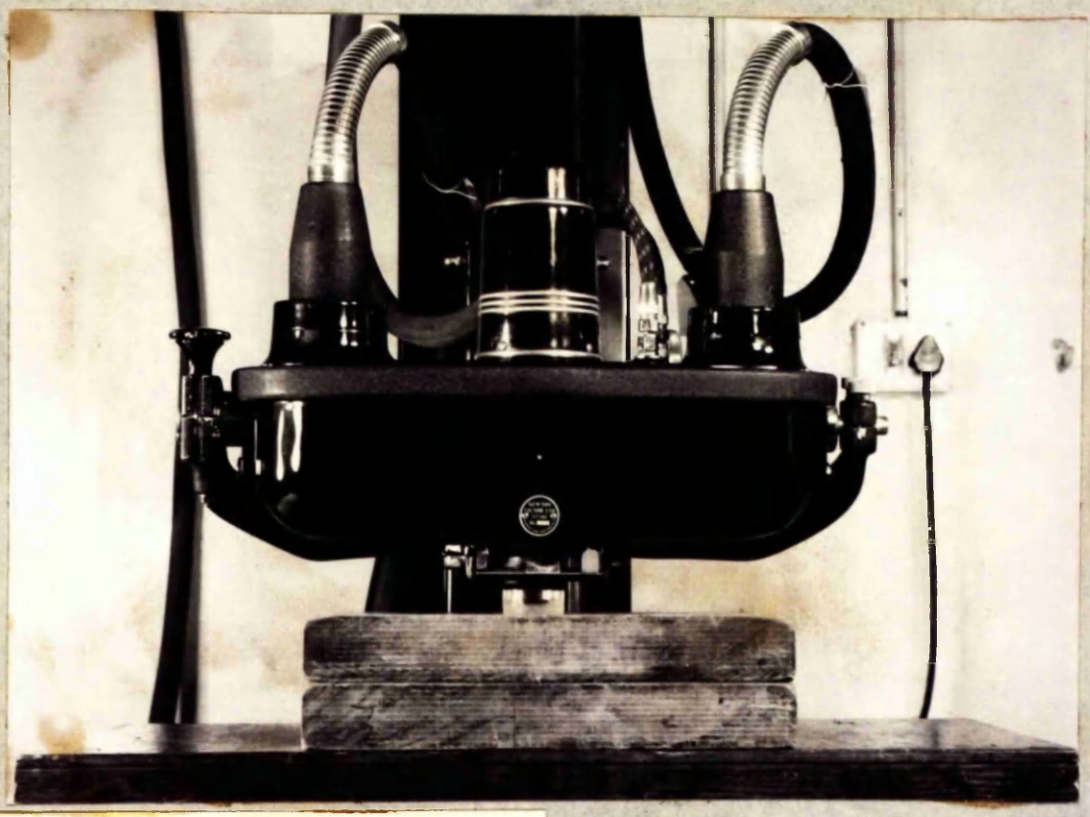

Figure 1. The Newton Victor GX 10 therapeutic X-ray machine, with 'perspex' dish and pressed wood in approximate position for irradiation.

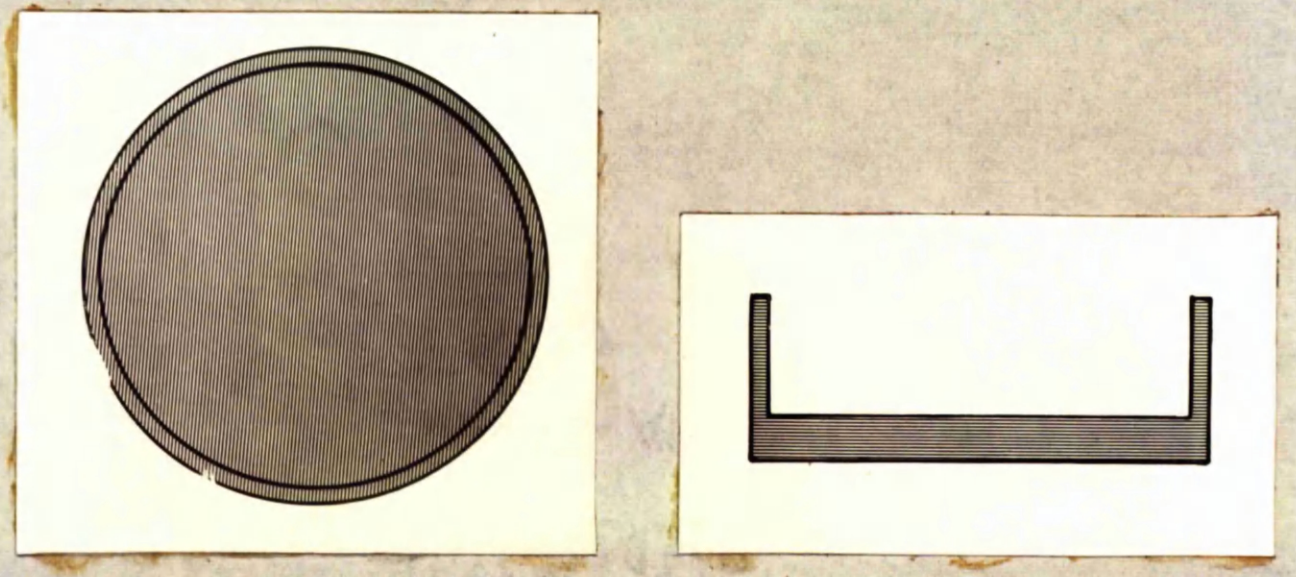

Figure 3. Diagram of 'perspex' irradiation dish.

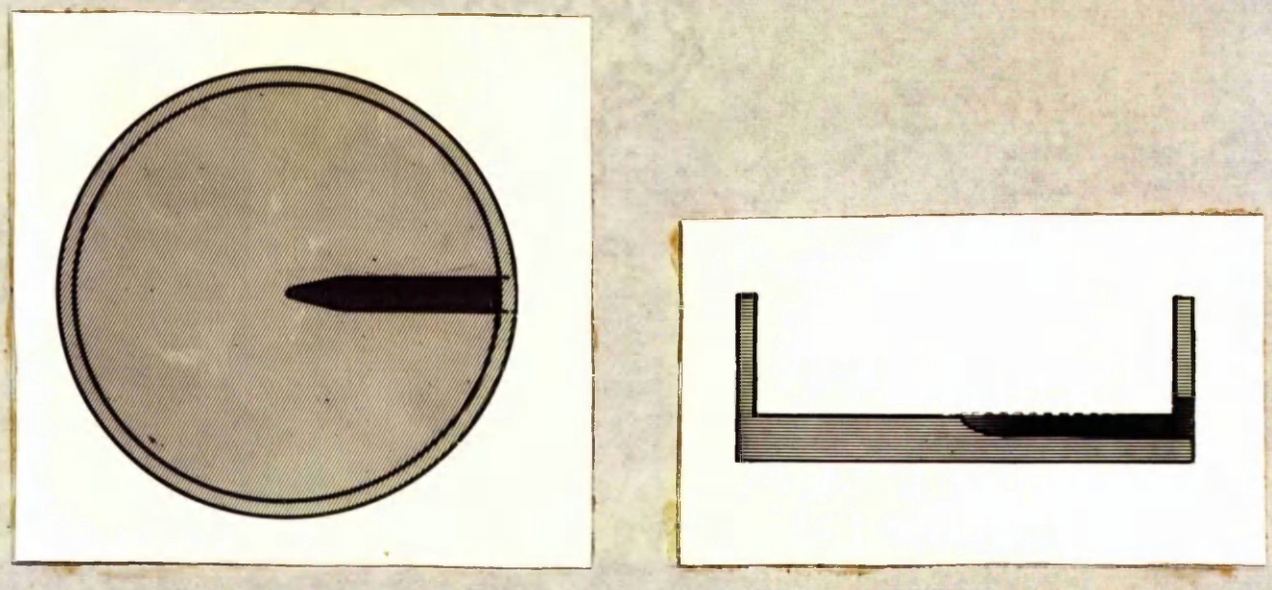

Figure 4. Plan and elevation diagram of 'perspex' calibration 'phantom' with recess to accommodate the dosimeter's ionisation chamber.

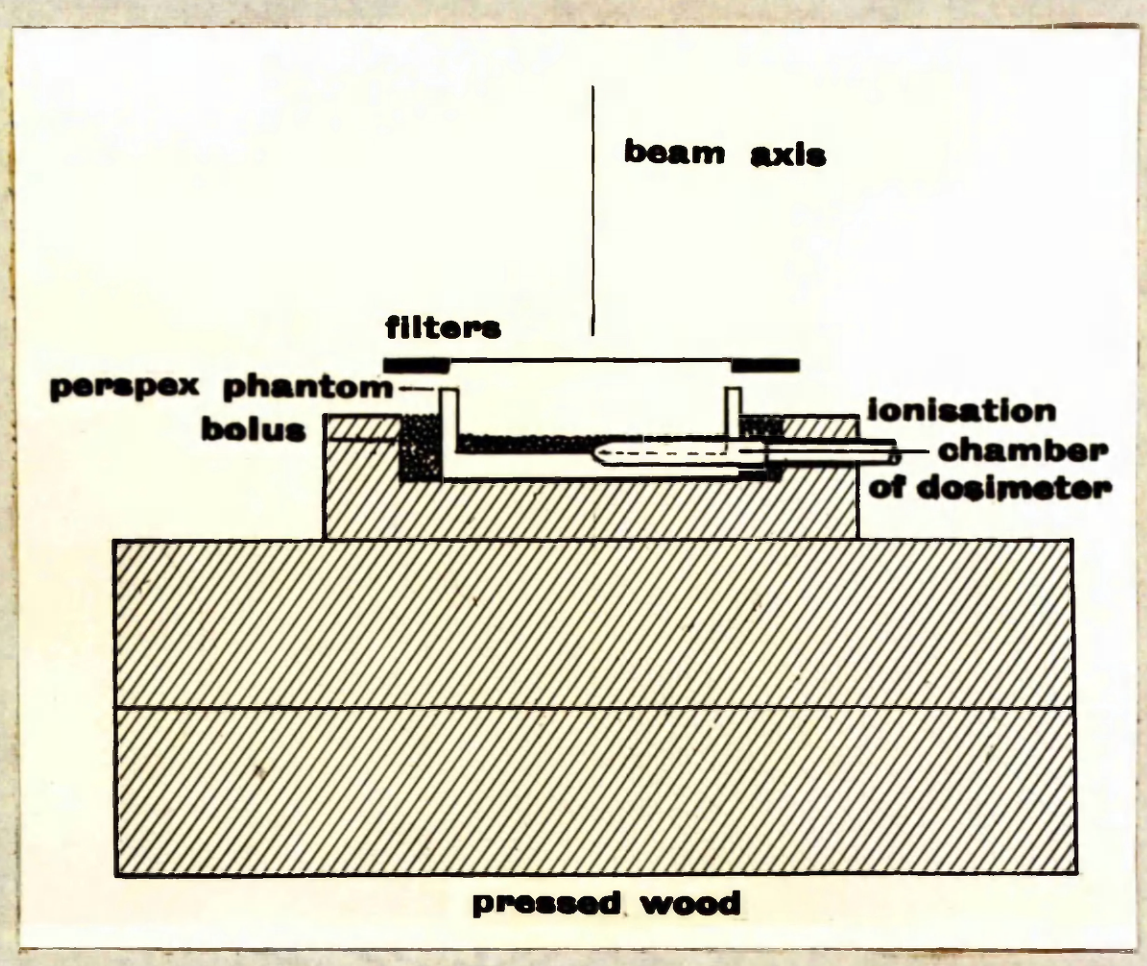

Figure 5. Cross-sectional diagram illustrating the arrangement of the components during calibration.

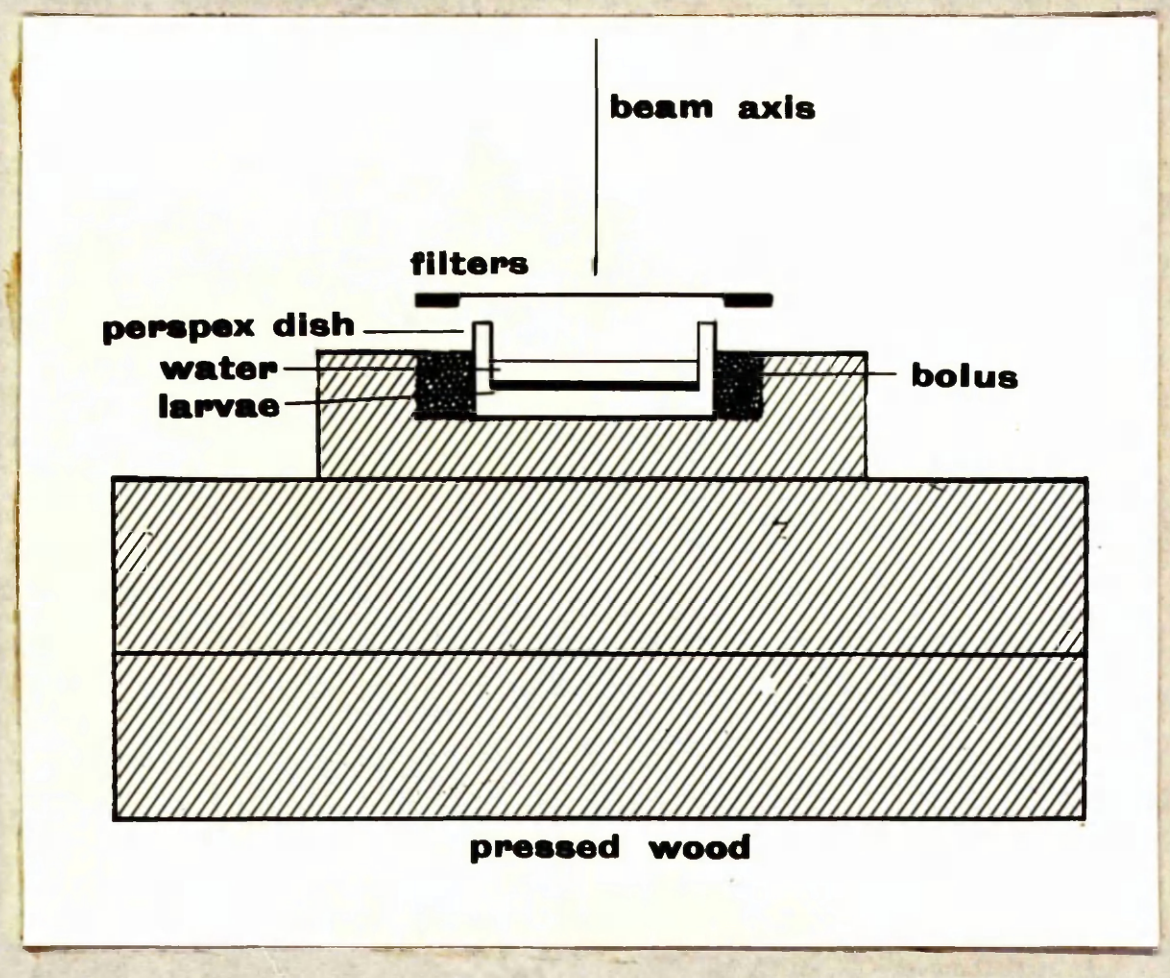

Figure 6. Cross-sectional diagram illustrating the arrangement of the components during an irradiation run.

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p r o t o c o l s

f o r

Experiment 3.

Weights* of vaccinated and control calves from Day 83 (10 days before challenge) to Day 125 (day before final kill).

Calf number	83	90	93	97	105	112	120	125	126
	- Day of Experiment -								
	3/3	10/3	13/3	17/3	25/3	1/4	9/4	14/4	15/4
9513	305	300		304	315	330	349	354	
9719	278	272		288	303	303	318	324	K
9720	280	285		293	302	311	334	334	I
9721	290	290	CHAL-	286	296	300	315	322	L
9722	255	255		262	286	292	307	314	L
9723	293	299		320	328	332	354	357	E
9718	240	246	-LENGE.	254	258	270	294	291	D
9725	225	235		246	256	248	284	282	
9726	252	255		272	288	290	300	310	
9727	284	283		288	300	308	341	339	
Group 2. (1,000 : 2,000 irradiated larvae).									
9728	274	276		292	301	312	337	332	
9729	268	276		292	298	310	332	325	K
9730	252	261		257	278	280	302	290	I
9731	241	251		267	277	285	297	290	L
9732	260	272	CHALL-	284	286	293	322	315	L
9733	250	258		276	282	289	308	304	E
9734	230	236	-ENGE.	264	259	260	275	285	D
9512	310	301		315	334	346	381	374	
9736	254	247		257	266	277	293	292	
9737	230	254		261	274	286	300	303	

* in pounds

Group 3. (1,000 : 1,000 irradiated larvae).

Calf number	- Day of Experiment - 83	90	93	97	105	112	120	125	126
	- Date - 3/3	10/3	13/3	17/3	25/3	1/4	9/4	14/4	15/4
9738	248	253		266	278	281	293	305	
9739	239	256		258	267	280	299	302	
9740	268	276		274	280	285	302	209	
9741	244	250		258	282	290	302	305	
9802	268	273		275	289	296	309	316	
9743	310	314		312	336	340	361	375	
9744	240	240		240	254	264	273	278	
9746	281	290		292	298	309	334	332	
9839	228	240		252	269	269	297	301	
9843	243	244		251	252	259	272	284	
	Group 4. (1,000:— irradiated larvae).								
9836	234	252		257	247	251	274	272	
9842	281	278		258	270	271	289	296	
9834	228	223		239	214	210	214	210	
9833	259	265		292	278	276	270	282	
9763	250	257		277	256	258	241	244	
9832	190	197		198	198	194	203	204	
9832	270	278		295	300	309	327	330	
9830	260	258		274	282	281	281	282	
9829	219	225		239	239	242	260	256	
9828	234	234		236	249	257	268	268	

CHALLENGE

KILLED

Group 5A. (10,000 challenge controls).

Calf number	- Day of Experiment - 83	90	93	97	105	112	120	125	126	
	-Date - 3/3	10/3	13/3	17/3	25/3	1/4	9/4	14/4	15/4	
9912	267	272		270	260	254	254	253		K
9913	312	297		314	312	290	296	306		I
9821	239	241		239	241	250	258	264		L
9514	286	294		314	307	304	306	316		L
9819	238	250		233	252	233	225	240		E D
			C H A L L E N G E							
Group 5B. (5,000 challenge controls).										
9911	310	311		317	320	327	324	323		K
9914	314	268		278	286	288	286	300		I
9916	200	210		220	221	220	230	232		L
9817	234	244		250	260	267	281	276		L E D
9818	213	220		241	239	245	236	228		
			C H A L L E N G E							
GROUP MEAN WEIGHTS										
Group number										
1.	270	272		280	293	298	320	323		
2.	257	263		277	286	294	315	311		K
3.	257	264		268	281	287	304	309		I
4.	243	246		257	253	255	263	264		L
5A+B*	261	261		269	270	268	270	274		L E D
			C H A L L E N G E							

* The two control groups are considered together as the weight difference between them is not significant.

Faecal larval levels (larvae per gram:McMaster)

GROUP	CALF NUMBER	Day of Experiment 24	27	29	31	34	36	38	41	43
		Date 16/8	19/8	21/8	23/8	26/8	28/8	30/8	2/9	4/9
VACCINATES	8623	0	0	100	20	0	0	50	0	20
	8630	50	0	20	40	100	50	100	50	0
	8631	0	0	0	0	100	0	20	0	0
	8632	0	0	40	100	50	100	0	0	50
	8633	0	50	50	50	250	0	50	0	0
	8634	0	0	300	20	400	1600	1100	350	250
	8635	0	100	0	100	40	50	0	N.S.	50
	8636	0	0	0	0	0	0	0	100	0
	8638	0	20	200	700	250	400	500	N.S.	500
	8675	0	250	50	150	D I E D 26/8				
	8677	0	0	50	40	50	100	250	0	0
	8678	0	0	20	150	0	0	0	0	0
	8679	0	0	100	0	50	50	20	40	50
	8680	0	50	350	100	600	650	200	0	400
	8684	0	20	100	50	200	100	200	800	100
CONTROLS	GL13	0	200	100	40	1650	1000	750	750	400
	GL14	50	100	D I E D 21/8						
	G46	0	1600	1900	D I E D 22/8					
	G47	0	0	0	50	50	100	200	150	150
	8621	0	0	50	100	20	0	50	N.S.	50
	8594	0	0	0	20	50	50	0	50	100
	8690	50	150	450	200	1150	1000	1900	800	1100
	8691	0	0	50	0	100	50	700	40	50
	8698	0	50	50	50	0	150	20	50	450
	8699	0	200	250	D I E D 21/8					
	8700	0	250	350	300	1300	600	1400	950	2650
	8614	300	450	D I E D 19/8						

N.S. - No sample obtained.

of vaccinates and controls

45	48	50	52	55	57	59	62	64	66	73	80	87
6/9	9/9	11/9	13/9	16/9	18/9	20/9	23/9	25/9	27/9	4/10	11/10	18/10
0	50	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	50	N.S.	0	0	0	D I E D 3/10		
700	150	150	100	400	50	0	50	0	50	50	0	0
150	250	50	100	50	50	0	N.S.	0	50	0	0	0
50	0	150	0	0	0	0	0	0	0	0	0	0
1550	650	850	D I E D 12/9									
0	100	50	40	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	200	0	N.S.	0	0	0	0	0	0	0	0
1250	D I E D	9/9										
40	450	0	550	20	700	100	40	50	0	0	0	0
600	150	1850	450	D I E D	14/9							
250	200	200	400	200	450	1150	400	300	1100	D I E D 4/10		
200	100	250	600	100	450	150	300	550	1050	D I E D 4/10		
N.S.	80	0	0	120	120	0	0	N.S.	0	0	0	0
1300	2200	D I E D	9/9									
0	0	50	N.S.	0	100	0	0	0	50	20	0	0
400	550	0	D I E D 12/9									
2650	950	2750	450	D I E D	15/9							

KEY TO SYMBOLS USED IN THE FOLLOWING TABLE.

a) Respiratory sounds (in order of increasing harshness).

VI - vesicular inspiration.
PVI - prolonged vesicular inspiration.
HVI - harsh vesicular inspiration.
BVI - broncho-vesicular inspiration.
BVB - broncho-vesicular breathing.
BI - bronchial inspiration.
BB - bronchial breathing.

b) Adventitious sounds.

E - localised emphysema.
GE - generalised emphysema.
R - other adventitious sounds.

c) Character of respiration.

D - Dyspnoea.

- presence of cough is not recorded here -

Rate and Type of respiration

GROUP	CALF NUMBER	Day of Experiment: 24	27	29	31	34	36	38	41	43	45
		Date: 16/8	19/8	21/8	23/8	26/8	28/8	30/8	2/9	4/9	6/9
VACCINATES	8623	40 BI	25 BVI	25 BVI	30 VI	30 HVI	30 HVI	20 HVI	30 HVI	24 BVI	20 HVI
	8630	60 BI	40 BI	60 BB+R	65 BVI	35 HVI	50 BI	40 HVI	50 HVI+E	40 BVI	60 BVI
	8631	40 BI	20 BVI	36 BI	40 HVI+E	25 HVI	30 VI	40 BVI+R	40 VI	32 BVI	48 BVI
	8632	60 BI	25 BVI	45 BI	45 BVI	60 BVI	40 HVI	40 HVI	40 HVI	20 HVI	48 BVI
	8633	60 BI	40 BI	50 BB	55 VI	40 HVI+R	60 BVI+R	50 BVI	60 BVI	44 BVI	60 BVI
	8634	-	50 BI	80 BI+E	80 BVI+E	40 HVI+E	60 BVI+E	50 BVI+E	75 HVI+E	46 BVI	48 BVI
	8635	40 BI	40 BI	70 BI+R	40 BVI	30 VI	50 BVI	40 HVI	60 HVI+E	48 BI	60 BVI
	8636	40 BI	20 BVI	25 BI	20 BI	20 VI	20 VI	20 VI	20 VI	16 HVI	16 VI
	8638	40 BI	20 BVI	35 BV+R	60 BVI	65 BVI+E	50 BVI	40 BVI	70 BVI+E	40 BVI	60 BVI
	8675	30 BVI	50 BVI	84 BI+E	60 BVI+E	D I E D 26/8					
	8677	50 BVI	40 BVI	60 BVI+R	60 BVI	50 BVI+R	70 BVI+R	50 BVI	80 BVI+R	46 BVI	80 BI
	8678	-	45 BVI	70 BVB	60 BVI	60 BVI	80 BVI	60 BVI	60 BVI	64 BVI	60 BVI
	8679	50 BI	50 BI	30 VI	50 BVI	30 VI	50 HVI	40 BVI	50 BVI	32 HVI	40 BVI+E
	8680	70 BI	70 BI	70 BI+E	80 BI	75 BB+GE	90 BI+E	80 BB+E	70 BVI+E	72 BVI	60D. BI
	8684	50 BI	30 BI	50 BI	60 BVI	65 BI+R	60 BVI+R	50 BVI	110 BB+E	52 BVI	60 BVI
CONTROLS	GL13	70 BI+E	65 BI	80 BI+E	80 BVB+E	60 BVI+E	80 BVI+E	80 BB	90 BB+E	60 BI+E	120 BI+E
	GL14	120 BI	130 BI+E	100 BI+E	D I E D		21/8				
	G46	60 BB+E	40D. BB+E	66 BB+GE	D I E D		22/8				
	G47	70 BVI	45 BI	90 BI+E	60 BVI	60 BVB+R	80 BVI+R	50 BVI	70 BVI+E	60 BVI	64 BVI
	8621	70 BI	60 BI	110 BB	110 BB	70 BI+E	100 BVI+E	80 BB+E	110 BVI+E	76 BI	96 BI
	8594	40 BI	50 BI+E	50 BI+R	60 BVI	60 BVI+R	70 BVI+R	50 BVI	80 BB	65 BVI	60 BVI
	8690	50 BVI	50 BI+R	55 BVB+E	60 BVI+E	70 BVI+E	90 BI+E	60 BVI	80 BVI+E	40 BVI+E	48 BVI
	8691	30 PVI	30 BVI	40 BVI	45 BVI	20 HVI+R	30 HVI+R	40 HVI	40 VI	24 BVI	40 BVI
	8698	70 BI	30 BVI	80 BI+R	60 BVI+E	70 BVI+R	90 BI+E	70 BI+E	75 BVI+E	56 BI	64 BVI
	8699	80 BI+E	80 BI+E	110 BB+E	DIED	21/8					
	8700	70 BI	40 BI	68 BI+E	80 BVI+E	65 BVI+E	60 BVI	50 BVI	80 BI+GE	48 BVI	72 BVI
	8614	130 BI	120D.D I E D 19/8 BI+E								

48	50	52	55	57	59	64	66	73	80	87
9/9	11/9	13/9	16/9	20/9	23/9	25/9	27/9	4/10	11/10	18/10
36	38	44	32	28	28	36	36	40	48	44
HVI	HVI	BVI	BVI	HVI	BVI	BVI	HVI	BVI	BVI	BVI
44	48	44	52	40	36	40	52	48	56	40
BVI	BVI	BVI	BVI	BVI	BVI+E	NVI+E	BVI+E	HVI	BVI	HVI
40	44	40	48	32	40	36	36	28	48	30
BVI	BVI	BVI	BVI	HVI+E	BVI	BVI	BVI	HVI	BVI	HVI+E
52	48	64	52	28	20	36	32	36	44	28
BVI	BVI	BVI	BVI	BVI	BVI+E	HVI	BVI	HVI	HVI	HVI
40	56	64	48	48	36	60	48	D I E D		3/10
BVI	BVI	BVI	HVI	BVI	BVI	BVI	BVI+E			
40	68	76	68	56	48	48	48	40	40	40
BVI	BVI	BVI	BVI	BVI	HVI	BVI	BVI	BVI+E	HVI	HVI
76	60	52	44	72	36	24	56	40	44	60
BVI	BVI	BVI	BVI	BI+E	BVI	BVI+E	BVI	BVI	HVI	BVI
16	24	32	24	24	16	28	20	24	48	24
HVI	BVI	HVI	HVI	HVI	HVI	HVI	HVI	HVI	BVI	HVI
48	40D.									
BVI	BVI	D I E D	12/9							
80	72	60	64	60	56	80	60	60	60	60
BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI
52	64	68	36	60	52	56	48	32	56	42
BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	HVI	HVI
40	28	28	24	32	40	48	40	44	40	44
HVI	BVI	HVI	HVI	HVI	BVI+E	BVI	BVI	BVI+E	BVI	HVI
D I E D	9/9									
56	56	56	60	56	40	56	56	48	48	60
BI	BI	VBI	BVI+E	BVI+E	BVI	BVI+E	BVI+E	BVI+E	BVI	BVI
84	80	96								
BI	BI	BI	D I E D	14/9						
80	64	104	80	72	48	72	80	D I E D		4/10
BI	BVI+E	BVI	BI+E	BI+E	BVI+E	BVI	BVI+E			
80D.	52D.	52	48	36	64D.	60D.	60D.	D I E D		4/10
BI	BI+E	BI	BI+E	BI	BI	BI	BI+E			
48	68	68	68	52	40	52	48	56	45	52
BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI	BVI+E
56										
BI+E	D I E D	9/9								
36	44	56	48	60	28	44	48	76	40	52
BVI	BI	BVI	BVI	BVI	BVI	BVI+E	BVI	BVI	BVI	BVI
60	48D.									
BI	BI+E	D I E D	12/9							
56	60	56								
BVI	BI	BI+E	D I E D	15/9						

p r o t o c o l s

for

Experiment 5.

Calf number		- Day of Experiment - 0	0	2	4	7	9	11	14	17	19	22	24
		- Date - 9/12	9/12	11/12	13/12	16/12	18/12	20/12	23/12	26/12	28/12	31/12	2/1
Group 1. (1,000 : 4,000 irradiated larvae).													
9513	1st.	30	40	35	30	40	35	40	30	25	35	30	
9719		25	30	30	35	30	30	50	85	70	50	40	
9720	V	30	30	40	35	60	40	70	70	65	50	45	
9721	A	30	30	30	25	35	40	50	55	30	25	45	
9722	C	35	30	30	35	30	30	50	50	40	40	35	
9723	C	35	25	30	30	35	35	45	50	35	40	35	
9718	I	35	30	30	30	30	45	60	85	60	50	40	
9725	N	35	30	35	25	30	35	40	60	60	40	40	
9726	E	30	40	40	40	50	40	55	50	55	45	50	
9727		30	30	30	30	35	30	60	80	55	40	50	
Mean		31	32	33	32	37	36	52	62	50	42	41	
Group 2. (1,000 : 2,000 irradiated larvae).													
9728	1st.	35	40	35	30	35	30	35	40	30	35	30	
9729		25	30	30	25	25	40	35	35	30	30	30	
9730	V	20	30	25	25	40	35	60	40	30	30	40	
9731	A	35	30	35	25	30	35	45	70	60	55	55	
9732	C	35	30	30	30	30	30	35	30	30	35	30	
9733	C	25	30	30	30	30	35	35	50	40	40	45	
9734	I	45	30	30	30	40	35	50	50	35	40	35	
9512	N	50	60	40	40	50	45	60	55	40	45	55	
9736	E	35	35	30	30	50	35	35	60	40	35	40	
9737		35	35	35	30	40	30	40	40	35	30	35	
Mean		34	35	32	30	37	35	43	47	37	38	40	
Group 3. (1,000 : 1,000 irradiated larvae).													
9738	1st.	20	30	25	30	30	30	30	45	25	30	30	
9739		30	35	30	30	30	30	40	45	40	40	35	
9740	V	25	25	30	30	30	35	40	50	50	45	50	
9741	A	25	50	35	40	50	35	50	55	40	45	45	
9802	C	35	35	35	35	35	35	35	45	40	40	45	
9743	C	30	30	30	30	45	35	35	50	35	30	40	
9744	I	30	30	40	35	35	40	50	45	50	40	40	
9746	N	30	35	30	30	30	35	50	60	50	40	30	
9839	E	35	50	35	40	65	40	50	50	40	45	50	
9843		30	40	30	30	40	30	40	50	50	50	50	
Mean		29	36	32	33	39	35	42	50	42	41	42	

26	28	30	32	35	37	39	42	46	49	51	54
4/1	6/1	8/1	10/1	13/1	15/1	17/1	20/1	24/1	27/1	29/1	2/2
Group 1. (1,000 : 4,000 irradiated larvae).											
35	30	30	35	30	40	40	2nd.	45	50	40	50
35	40	45	40	35	40	40		50	55	40	45
40	40	45	40	35	40	30	V	40	55	45	50
30	40	45	45	40	40	35	A	35	40	40	50
30	40	35	30	35	35	35	C	40	55	40	40
40	50	50	40	40	45	45	C	45	60	65	60
40	45	50	40	40	45	35	I	35	40	45	45
45	35	40	35	45	55	50	N	55	45	55	60
40	40	40	45	40	45	45	E	50	45	45	50
50	40	35	40	35	35	30		40	45	40	45
39	40	42	40	38	42	39		44	49	46	50
Group 2. (1,000 : 2,000 irradiated larvae).											
35	40	35	35	45	40	40	2nd.	40	45	30	40
20	25	12	30	30	35	30		30	35	30	35
30	40	30	30	35	40	35	V	35	35	40	45
45	45	30	35	40	40	35	A	35	35	40	40
30	25	30	30	35	40	40	C	40	40	40	45
30	40	35	30	40	40	40	C	45	45	35	40
40	40	40	30	35	40	35	I	40	45	50	50
45	50	50	40	45	40	35	N	35	45	35	50
35	40	45	40	45	45	40	E	40	45	40	40
30	40	40	40	45	40	35		40	40	60	45
34	39	35	34	40	40	37		38	41	39	43
Group 3. (1,000 : 1,000 irradiated larvae).											
35	30	50	40	30	35	30	2nd.	35	35	30	40
30	30	40	35	40	45	50		45	45	40	40
40	50	45	45	45	45	40	V	40	35	40	40
25	30	35	35	40	50	40	A	40	50	50	50
50	50	55	45	55	60	45	C	35	35	35	45
25	35	30	30	40	45	40	C	45	40	40	35
35	35	40	40	50	50	45	I	50	70	35	45
25	45	30	30	40	45	30	N	40	50	35	45
60	40	45	45	50	60	60	E	60	60	45	50
45	55	40	40	45	50	45		45	50	50	45
36	40	41	39	44	49	43		44	47	40	44

57	59	63	66	70	74	77	80	83	87	90
5/2	7/2	11/2	14/2	18/2	22/2	25/2	28/2	3/3	7/3	10/3
55	50	60	60	60	45	35	40	35	35	30
55	45	45	65	50	40	50	45	45	45	40
55	50	45	60	50	45	50	50	45	50	45
60	55	60	55	45	50	40	55	45	60	50
45	45	60	50	45	50	50	55	55	60	50
60	60	60	70	50	50	65	65	60	60	50
50	55	85	70	45	70	50	70	60	55	45
80	60	80	80	50	70	70	60	55	45	40
50	50	70	50	50	45	50	60	65	55	50
50	45	50	75	50	45	60	45	50	40	45
56	52	62	64	50	51	52	55	52	51	45
50	50	60	50	45	50	55	70	60	70	50
45	35	40	50	45	40	55	55	60	50	45
45	45	50	60	35	45	35	45	50	30	35
50	45	40	45	45	45	30	45	50	60	50
45	45	45	45	50	40	40	55	60	40	40
45	50	60	70	40	45	45	45	55	50	45
45	45	45	70	60	50	40	50	50	45	50
60	55	55	45	55	40	35	50	40	55	50
40	40	40	45	40	40	40	65	50	50	45
45	45	50	50	50	60	45	50	55	50	50
47	46	49	53	47	46	42	53	53	50	46
45	30	40	50	40	45	35	40	30	45	35
45	45	45	60	45	50	50	50	50	40	40
45	55	45	65	40	40	45	35	45	45	45
55	60	40	50	55	35	30	50	40	40	35
50	30	55	50	45	30	50	50	45	40	35
55	40	60	50	45	45	60	45	45	40	40
40	30	40	40	50	50	40	40	45	40	40
40	45	45	60	50	50	45	60	60	50	40
60	50	55	95	110	75	65	60	55	65	50
40	45	50	75	55	60	45	60	45	40	45
48	43	48	60	54	48	47	49	46	45	41

Individual and group mean

92	93	94	97	101	105	108	112	114	116	119
12/3	13/3	14/3	17/3	21/3	25/3	28/3	1/4	3/4	5/4	8/4
Group 1. (1,000 : 4,000 irradiated larvae).										
25	C	30	40	30	35	25	25	30	35	30
40	H	40	40	30	35	35	30	30	35	35
40	A	40	45	50	45	35	40	40	30	35
40	L	40	40	45	40	30	40	50	30	30
50	L	50	55	45	60	40	40	45	35	40
40	E	50	55	60	60	50	45	50	45	45
40	N	45	50	50	50	35	45	45	40	35
35	G	40	40	45	50	40	45	45	35	30
40	E	45	50	45	45	40	40	30	45	30
55		45	40	45	45	40	35	40	40	30
41		43	46	45	47	37	39	41	37	34
Group 2. (1,000 : 2,000 irradiated larvae).										
35	C	35	40	40	45	40	50	55	30	35
45	H	40	40	45	-	25	40	50	35	30
40	A	35	30	40	-	35	40	45	45	30
45	L	45	40	40	-	40	40	40	45	30
40	L	40	45	50	-	50	45	50	35	35
40	E	40	45	45	-	40	40	45	35	30
50	N	45	40	45	-	50	50	45	30	35
35	G	40	40	40	-	25	30	35	40	30
40	E	45	50	45	40	40	40	45	35	30
40		50	50	45	45	40	40	40	35	35
41		42	42	44	-	39	42	45	37	32
Group 3. (1,000 : 1,000 irradiated larvae).										
30	C	40	45	45	45	35	40	40	45	45
40	H	45	50	40	35	45	40	35	35	35
40	A	40	40	40	40	35	30	30	40	40
35	L	35	35	35	35	30	35	40	30	40
30	L	30	35	35	35	35	35	30	35	30
50	E	45	45	50	60	45	45	50	40	40
35	N	45	50	40	35	30	40	55	30	45
40	G	45	50	45	40	25	30	25	40	30
40	E	40	60	55	50	55	50	50	40	35
50		40	45	45	45	30	30	30	30	30
39		41	46	43	42	37	38	39	37	37

respiratory rates (continued).

121	123	125	126
10/4	12/4	14/4	15/4
35	35	40	
35	40	40	
45	40	45	K
45	35	40	I
55	35	40	L
50	35	40	L
50	45	50	E
45	50	45	D
50	50	45	
50	50	45	
46	42	43	
50	40	45	
40	40	35	
35	35	30	K
35	40	35	I
40	40	30	L
45	40	45	L
40	35	40	E
40	25	30	D
40	50	35	
45	45	40	
41	39	37	
45	40	40	
45	35	35	
40	35	40	K
50	35	50	I
40	30	30	L
45	40	45	L
50	50	50	E
40	50	40	D
50	60	50	
35	35	35	
44	41	42	

Individual and group mean respiratory rates.

Calf number		- Day of Experiment - 0	0	2	4	7	9	11	14	17	19
		- Date - 9/12	9/12	11/12	13/12	16/12	18/12	20/12	23/12	26/12	28/12
Group 4. (1,000:—— irradiated larvae).											
9836	VACCINE		30	35	30	35	35	30	35	50	40
9842			30	30	30	35	35	35	35	35	40
9834			25	25	25	30	25	25	25	45	30
9833			30	25	25	25	35	30	30	40	35
9763			35	20	25	25	30	35	30	30	30
9831			25	30	25	30	30	30	35	45	35
9832			25	30	30	30	30	35	40	45	35
9830			30	30	30	30	40	30	40	60	30
9829			30	30	30	35	45	35	50	50	35
9828			30	25	35	35	45	30	40	55	60
Mean			29	29	29	31	35	32	36	45	37
Group 5A. (10,000 normal larvae challenge controls).											
9912	-		25	25	30	35	25	35	30	35	30
9913	-		35	35	35	30	35	35	40	40	40
9514	-		30	30	35	35	30	30	35	40	40
9821	-		35	30	40	30	30	35	35	40	35
9819	-		25	30	25	35	30	30	35	35	30
Mean	-		30	30	32	32	30	32	35	37	35
Group 5B. (5,000 normal larvae challenge controls).											
9911	-		25	-	25	25	20	25	25	30	35
9818	-		35	-	30	25	25	25	35	35	35
9817	-		30	-	30	30	30	30	25	35	50
9916	-		25	-	25	25	20	25	30	35	35
9914	-		35	-	30	30	30	30	35	40	45
Mean	-		30	-	28	27	25	27	30	35	40

Individual and Group Mean

Calf number	- Day of Experiment - 22	24	26	28	30	32	35	37	39	46
	-Date- 31/12	2/1	4/1	6/1	8/1	10/1	13/1	15/1	17/1	24/1
Group 4. (1,000 : —— irradiated larvae).										
9836	40	40	40	30	30	30	40	40	35	40
9842	40	45	40	40	45	45	45	50	55	50
9834	35	35	30	35	30	30	35	40	30	40
9833	30	25	25	40	40	40	45	65	55	40
9763	30	30	30	30	35	30	40	40	35	25
9831	30	35	30	40	35	35	40	60	55	50
9832	40	35	40	45	40	45	40	45	40	45
9830	35	35	30	30	40	40	40	40	30	30
9829	50	110	55	45	35	35	40	60	45	50
9828	50	50	55	40	50	55	45	60	60	55
Mean	38	44	37	37	38	39	41	50	44	43
Group 5A. (10,000 normal larvae challenge controls).										
9912	40	40	35	35	-	40	40	35	45	40
9913	35	35	40	45	-	45	45	35	40	45
9514	40	40	45	40	-	50	50	45	45	45
9821	40	35	35	40	-	40	50	40	55	55
9819	35	40	35	40	-	35	40	35	30	30
Mean	38	38	38	40	-	45	45	38	43	43
Group 5B. (5,000 normal larvae challenge controls).										
9911	35	35	30	35	35	35	45	40	45	45
9818	35	40	35	40	40	40	45	40	40	40
9817	45	40	45	35	40	40	50	45	45	40
9916	35	40	45	45	55	45	50	50	60	65
9914	40	45	45	45	45	50	50	50	50	50
Mean	38	40	38	40	43	42	48	45	48	48

Respiratory Rates (continued).

49	51	54	57	59	63	66	70	74	77
27/1	29/1	2/2	5/2	7/2	11/2	14/2	18/2	22/2	25/2
40	40	40	45	40	40	60	55	45	40
55	40	40	35	30	45	60	40	35	45
40	30	35	40	30	45	45	40	35	40
35	35	40	40	35	60	50	35	45	40
25	25	30	35	35	45	40	35	40	35
55	50	45	40	45	45	40	50	45	45
45	40	40	40	45	60	45	45	40	45
35	35	40	40	35	45	35	40	40	45
60	40	40	40	40	45	40	40	45	45
55	45	45	40	40	50	55	45	45	45
45	38	40	40	38	48	47	43	42	43
35	40	45	50	45	40	40	40	40	40
40	40	50	50	50	45	50	45	40	45
45	45	60	60	60	40	40	45	30	35
45	40	50	55	50	50	50	45	50	50
35	45	55	60	35	40	45	40	40	40
40	42	52	55	48	43	45	43	40	42
45	40	45	50	40	45	50	45	40	45
35	45	50	55	45	45	45	40	45	45
40	40	45	45	45	40	50	45	45	50
60	40	45	50	45	55	70	50	45	50
45	45	50	50	50	50	60	45	50	50
45	42	47	50	45	47	55	45	45	48

Individual and Group Mean

	-Day of Experiment -									
	80	83	87	90	92	93	94	97	101	105
	-Date -									
	28/2	3/3	7/3	10/3	12/3	13/3	14/3	17/3	21/3	25/3
Group 4. (1,000 : —— irradiated larvae).										
	45	40	40	35	30		30	40	50	80
	50	40	40	40	40	C	40	40	45	50
	40	40	30	35	40	H	40	40	40	45
	40	40	45	40	35	A	35	35	40	45
	30	30	40	30	25	L	25	25	30	45
	50	50	45	35	30	L	30	35	40	55
	50	40	45	40	35	E	35	40	40	45
	45	45	45	40	35	N	35	30	35	40
	45	45	40	40	40	G	35	30	35	50
	50	45	50	40	35	E	35	40	45	50
Mean	45	42	42	38	35		34	36	40	51
Group 5A. (10,000 normal larvae challenge controls).										
	45	45	35	35	40	C	35	50	50	60
	40	40	30	30	35	H	30	40	45	60
	40	40	30	30	35	A	25	30	35	65
	45	45	40	40	45	L	45	50	50	55
	40	40	25	30	30	L	30	30	30	65
Mean	42	42	32	33	37	E N G E	33	40	42	61
Group 5B. (5,000 normal larvae challenge controls).										
	55	45	45	45	45	C	45	40	45	60
	45	45	40	45	40	H	45	40	45	55
	45	30	45	40	45	A	40	30	40	65
	60	45	40	45	40	L	45	40	40	65
	45	35	40	40	40	L	40	25	30	50
Mean	50	40	42	43	42	E N G E	43	35	40	59

Respiratory Rates (continued).

108	112	114	116	119	121	123	125	126
28/3	1/4	3/4	5/4	8/4	10/4	12/4	14/4	15/4
55	55	60	50	45	80	65	80	
55	55	50	35	30	60	75	60	
50	60	70	100	70	75	80	65	K
50	70	90	70	100	80	80	90	I
40	55	75	70	100	60	65	60	L
70	75	70	80	90	75	85	70	L
50	50	50	70	60	70	60	50	E
40	45	50	60	50	75	60	55	D
65	60	55	55	55	80	65	65	
40	50	50	60	50	70	70	70	
52	58	62	65	65	73	71	67	
60	60	60	70	70	50	55	60	K
70	85	90	110	100	80	80	90	I
70	65	70	70	80	75	70	70	L
50	75	80	100	90	60	75	70	L
80	85	90	110	110	90	90	70	E
66	74	78	92	90	71	68	72	D
55	70	80	120	110	90	90	85	K
50	50	60	80	100	75	65	70	I
50	50	55	80	65	70	65	65	L
70	80	80	90	80	75	70	70	L
50	70	80	110	110	95	90	80	E
55	64	71	96	93	81	76	74	D

Individual and group

Calf number	Day of Experiment - 0	0	2	4	7	9	11	14	17
	Date - 9/12	9/12	11/12	13/12	16/12	18/12	20/12	23/12	26/12
	Group 6. (larval controls for vaccine used on Day 0).								
51		25	30	25	30	35	35	35	40
52	Dosed	25	40	30	30	30	30	35	40
53	4,000	35	30	20	30	45	35	35	60
54	Normal	35	35	35	35	25	35	30	55
55	Larvae	35	30	25	25	25	25	25	35
Mean		31	33	27	30	32	32	32	46
	Group 7. (larval controls for vaccine used on Day 42).								
59	-	35	30	30	25	25	25	35	35
60	-	30	25	30	30	30	30	25	35
61	-	25	25	25	25	20	25	30	35
62	-	25	30	25	25	30	30	25	40
Mean		29	28	28	26	26	28	29	36

mean respiratory rates.

19	22	24	26	28	30	32	35	37	39	42	46	49
28/12	31/12	2/1	4/1	6/1	8/1	10/1	13/1	15/1	17/1	20/1	24/1	27/1
40	40	40	40	60	90	70	70	60	65	-	60	70
40	50	45	45	50	50	60	70	70	80	-	70	70
60	50	55	60	55	70	65	70	70	60	-	60	70
55	50	45	45	40	50	55	65	100	90	-	50	40
50	45	40	40	30	35	35	35	55	60	-	60	55
49	47	45	46	47	59	57	62	71	71		62	61
35	40	40	35	35	30	30	40	40	35	Dosed	35	40
50	45	40	35	40	40	45	45	40	40	4,000	40	45
35	35	40	40	40	35	35	40	35	35	Normal	35	40
40	35	35	30	35	30	35	30	30	30	Larvae	35	40
40	39	39	35	38	34	36	39	36	35		36	41

Individual and group

51	54	57	59	63	66	70	74	77	80	83
29/1	2/2	5/2	7/2	11/2	14/2	18/2	22/2	25/2	28/2	3/3

Group 6. (larval controls for vaccine used on Day 0).

45	60	60	60	K
55	60	65	60	I
70	60	65	80	L
-	65	65	60	L
45	60	70	80	E
54	61	65	68	D

Group 7. (larval controls for vaccine used on Day 42).

35	35	40	35	45	50	50	60	80	100	75
45	45	40	40	45	50	50	55	80	65	70
45	35	40	40	45	70	80	80	90	80	75
40	35	35	40	40	50	70	80	110	110	95
41	38	39	39	44	55	63	69	90	89	79

mean respiratory rates (continued).

87	90	92	94	97	101	101
7/3	10/3	12/3	14/3	17/3	21/3	21/3
65	70	70	75	65	60	KILLED
65	65	70	65	60	50	
70	70	60	65	70	70	
90	80	80	70	65	80	
73	71	70	69	65	65	

p r o t o c o l s

f o r

Experiment 7.

Calf Weights

Day of Experiment	1	72		100	
Date	30/8	10/11	Individual Gains on Previous Weight.	8/12	Individual Gains on Previous Weight
VACCINATES					
6	128	206	+ 78	217	+ 11
7	128	166	+ 38	183	+ 17
8	112	176	+ 64	193	+ 17
9	119	170	+ 51	190	+ 20
10	148	204	+ 56	222	+ 18
	Group gain on previous weight		+ 287		+ 83
			Total Group Gain		+370
1	131	171	+ 40	128	- 43
2	161	230	+ 69	262	+ 32
3	173	229	+ 56	238	+ 9
4	173	(188) Dead 7/11			
5	134	190	+ 56	198	+ 8
	Group gain on previous weight		+ 221		+ 6
			Total Group Gain		+227

in pounds.

Group Percentage Weight Gains

Group weight on Day 1 = 635 lbs

Group weight on Day 72 = 922: % gain from Day 1-72 = 45.2%

Group weight on Day 100= 1005: % gain from Day 72-100 = 9.0%

% Group weight gain over the 100 days = 58.2%

Group (4 survivors) weight on Day 1 = 599 lbs.

Group (4 survivors) weight on Day 72 = 820 lbs.
% gain from Day 1 to Day 72 = 36.7%

Group (4 survivors) weight on Day 100 = 826 lbs.
% gain from Day 72 to Day 100 = 0.7%

% Group (4 survivors) weight gain over the 100 days = 37.9%

Faecal Larval Outputs

Day of Experiment	1	24	31	31	51	51	55
Date	30/8	23/9	30/9	30/9	20/10	20/10	24/10
VACCINATES							
6	1st	-ve	2nd	-ve	ALL	-ve	-ve
7		-ve		-ve	CALVES	-ve	-ve
8	DOSE	-ve	DOSE	-ve	TURNED	-ve	-ve
9		-ve		-ve	OUT	-ve	-ve
10	VACCINE	-ve	VACCINE	-ve	ONTO	-ve	-ve
CONTROLS					LUNG-WORM IN-FESTED PADDOCK		
1		-ve		-ve		-ve	-ve
2		-ve		-ve		-ve	-ve
3		-ve		-ve		-ve	-ve
4		-ve		-ve		-ve	-ve
5		-ve		-ve		-ve	-ve

and Total Worm Counts

81	86	88	91	95	100	Number and type of worm.	Pulmonary lesion score.
19/11	24/11	26/11	29/11	3/12	8/12		
-ve	-ve	-ve	-ve	-ve	ALL	0	1
-ve	-ve	-ve	-ve	-ve	CALVES	70 immature	5
-ve	-ve	-ve	-ve	-ve		0	2
-ve	-ve	-ve	-ve	-ve		0	0
-ve	-ve	-ve	-ve	-ve		55 immature	3
200/g.	650/g.	550/g	800/g.	1600/g.	KILLED	1250 Fully mature	8
-ve	-ve	-ve	-ve	-ve		0	2
-ve	100/g.	200/g.	250/g.	350/g.		367 Fully mature	10
450/g.	350/g.	-ve	100/g.	50/g.		150 Fully mature	3

Died 7/11 265 Immature worms and many early 5th stage in lungs.

RESPIRATORY RATES

Day of Experiment	1	19	24	31	31	39	41	45
Date	30/8	18/9	23/9	30/9	30/9	8/10	10/10	14/10
VACCINATES								
6	1st DOSE VACCINE	45	35	2nd DOSE VACCINE	35	55	55	50
7		60	70		60	55	50	50
8		25	30		30	50	50	65
9		40	60		50	60	70	70
10		25	40		40	70	45	65
CONTROLS								
1		30	30		35	35	30	35
2		35	30		35	55	40	45
3		40	30		35	45	50	55
4		35	40		40	40	35	35
5		60	50		50	50	50	60
Vaccinates		39	47		43	58	54	60
Controls		40	36		39	45	41	46

RESPIRATORY RATES

47	51	51	55	60	63	66	70	75	80
16/10	20/10	20/10	24/10	29/10	1/11	4/11	8/11	11/11	18/11
VACCINATES									
55	All	50	40	35	30	25	30	40	35
70	calves	60	50	50	35	110	80	60	60
80	turned	65	50	35	70	70	40	40	45
50	out	40	45	40	30	45	50	40	30
55	onto	50	55	50	50	70	85	70	60
CONTROLS									
40	Lung-	35	35	25	30	60	70	60	50
35	worm	30	30	35	45	60	60	40	60
40	Infested	40	35	35	55	90	85	60	55
30	Paddock	30	35	30	65	70	----------		
50		45	50	45	40	50	55	40	60
			- Group Mean Respiratory Rates -						
62		53	48	42	43	64	57	50	46
39		36	37	34	47	66	68	50	56

RESPIRATORY RATES

84	87	91	95	98
22/11	25/11	29/11	3/12	6/12
30	45	45	40	50
55	85	60	60	60
30	25	30	25	35
40	30	35	35	45
60	60	70	55	35
60	70	70	90	95
70	55	50	50	70
70	90	90	80	95
--Died 7/11 ----------				
50	45	50	60	70
43	49	48	43	45
63	65	65	70	83

p r o t o c o l

for

Experiment 8

Comparative measurements of normal and irradiated female D. viviparus larvae recovered from the lungs of four respective calves at nine and 17 days after infection with 1,000 larvae.

NORMAL			X - IRRADIATED		
Age	Total length in millimetres	Length of oesophagus in millimetres.	Age	Total length in millimetres	Length of oesophagus in millimetres.
9 days	1.48 1.64 1.62 1.50 1.90 1.54 1.86 1.64 1.60 1.70		9 days	1.40 0.98 1.48 1.12 1.30 0.92 0.84 0.92 1.48 1.20	
Mean 1.65 ± 0.13			Mean 1.16 ± 0.23		
17 days	16.0 15.0 15.0 10.5 14.0 13.5 10.5 14.5	0.58 0.56 0.55 0.48 0.56 0.48 0.60 0.58	17 days	4.84 3.94 4.30 4.0 4.90 3.90 3.90 4.64	0.40 0.44 0.38 0.34 0.38 0.36 0.38 0.40
Mean 13.6 ± 1.95		0.55 ± 0.04	Mean 4.30 ± 1.14		0.39 ± 0.08

p r o t o c o l s *

for

Experiment 11.

*Commencing 'Table 3'; Tables 1 and 2 are incorporated in the text.

TABLE 3 - Respiratory Rates per Minute of the Calves in Group 2

Day	Calf Number						Mean	S.D. ±
	58	48	61	46	50	59		
1	35	40	25	20	30	40	32	8.17
2	25	45	35	25	35	40	34	8.01
3	40	40	35	35	35	30	36	3.76
4	35	35	35	25	30	40	33	5.17
5	45	40	30	25	30	40	35	7.74
6	35	35	35	30	30	35	33	2.60
7	50	45	50	35	40	50	45	6.32
8	50	30	40	35	35	45	39	7.36
9	50	40	35	30	35	50	40	8.36
10	35	35	40	50	30	50	40	8.36
11	50	35	40	50	40	40	43	6.14
12	50	40	50	40	30	40	42	7.53
13	40	60	60	40	40	50	48	9.83
14	45	70	60	60	45	70	58	11.26
15	90	50	60	75	50	70	66	15.53
16	100	75	60	80	60	70	74	18.85
17	90	55	50	70	60	70	66	14.29
18	85	70	50	90	55	110	77	22.73
19	90	70	50	100	55	105	78	23.37
20	100	80	60	110	50	130	88	22.77
21	90	70	60	100	55	120	83	25.25
22	90	90	60	90	55	130	86	26.91
23	105	100	70	110	50	115	92	25.82
24	105	80	80	100	60	140	93	30.28
25	100	90	80	90	60	130	92	23.14
26	90	85	70	120	65	130	93	26.39
27	100	110	80	90	70	130	97	21.6
28	100	130	80	90	80	130	102	23.17
29	110	110	65	100	90	115	98	21.13
30	90	90	90	90	90	130	97	16.33
31	90	85	100	70	100	100	91	12.01
32	120	110	70	100	80	110	98	19.41
33	90	115	100	100	75	120	100	16.55
34	110	100	80	80	65	100	89	16.86
35	110	100	80	100	75	110	96	14.97
36	90	120	105	75	65	110	94	21.31

TABLE 4 - Respiratory Rates per Minute of the Calves in Group 3

Day	Calf Number						Mean	S.D. ±
	62	53	52	49	51	47		
1	45	30	25	30	30	50	35	10.0
2	40	25	20	40	30	40	33	8.82
3	40	30	25	35	45	35	35	7.07
4	35	30	25	30	50	35	24	8.61
5	40	30	20	40	50	30	35	10.49
6	45	40	30	35	40	35	38	5.27
7	55	30	35	40	45	35	40	8.94
8	35	30	35	40	40	30	35	4.47
9	35	30	50	35	40	50	40	8.36
10	50	40	30	40	40	40	40	6.32
11	50	35	35	45	55	55	46	9.17
12	55	30	30	50	40	50	43	10.85
13	60	50	50	60	50	60	55	5.47
14	60	50	45	80	55	90	63	17.80
15	90	50	50	110	70	80	75	23.45
16	70	60	45	105	80	80	73	20.41
17	80	50	50	110	70	100	77	25.03
18	75	60	40	120	80	100	79	28.36
19	85	70	60	120	80	120	89	25.32
20	120	70	60	140	105	110	101	30.73
21	95	65	70	70	100	110	85	18.97
22	90	55	80	120	100	130	96	15.3
23	100	65	90	100	120	130	101	22.9
24	100	80	50	140	120	130	103	33.9
25	100	65	70	110	130	110	98	23.0
26	110	65	70	110	120	120	99	24.9
27	110	70	100	130	130	130	112	24.02
28	90	75	100	130	130	120	108	23.5
29	90	80	90	120	120	130	105	20.7
30	110	80	80	120	110	120	105	20.7
31	110	70	65	120	110	130	101	26.9
32	110	80	80	125	110	130	106	21.5
33	120	75	90	130	130	130	113	24.0
34	120	130	90	130	120	130	120	15.49
35	130	90	80	130	130	130	115	23.4
36	125	70	70	140	100	120	104	29.4

TABLE 5 - Body Weight of Calves in Group 1.

Day	Calf Number						Mean	S.D. ±
	57	55	56	45	54	60		
1	111	134	122	103	122	88	113	16.32
4	116	140	125	110	133	92	119	17.28
8	119	144	128	112	135	94	122	16.79
11	127	150	132	115	140	95	126	19.45
15	127	154	137	128	144	98	131	19.23
18	122	150	140	124	146	96	130	20.05
22	135	159	148	127	151	102	137	20.64
25	134	162	150	136	156	106	141	20.32
29	131	172	154	152	166	111	148	27.31
32	136	176	159	147	169	112	150	23.53
36	130	175	161	154	167	117	151	22.75
Wt. Increase	29	45	51	39	41	19	37	11.53

TABLE 6 - Body Weight of Calves in Group 2.

Day	Calf Number						Mean	S.D. ±
	58	48	61	46	50	59		
1	136	136	100	114	94	126	118	18.04
4	144	145	106	125	100	125	124	18.67
8	150	146	110	124	104	134	128	18.76
11	154	150	114	125	105	132	130	19.42
15	156	154	111	125	108	134	131	20.69
18	152	153	120	123	110	136	132	15.66
22	157	155	125	118	120	128	134	17.54
25	162	155	125	126	120	117	134	19.23
29	168	163	127	126	122	120	138	21.77
32	164	157	129	128	119	124	137	18.79
36	168	169	132	130	121	132	142	20.92
Wt. Increase	32	33	32	16	27	6	24	11

TABLE 7 - Body Weight of Calves in Group 3

Day	Calf Number						Mean	S.D. ±
	62	53	52	49	51	47		
1	130	114	110	113	100	103	112	10.56
4	131	120	117	124	115	102	118	9.74
8	132	124	117	128	109	105	119	10.73
11	141	126	128	134	115	108	125	12.14
15	142	131	128	132	118	116	128	9.64
18	144	126	132	122	120	110	126	11.55
22	138	129	138	118	118	115	126	10.45
25	141	136	142	157	116	114	134	16.55
29	148	144	142	128	118	114	132	14.40
32	145	142	148	125	123	112	133	14.52
36	146	139	159	130	124	121	137	10.98
Wt. Increase	16	25	49	17	24	18	25	12.41

TABLE 8 - The Larval Output in Faeces of Calves in Group 2

Day	Calf Number						Mean	S.D. ±
	58	48	61	46	50	59		
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	50	8	20
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	100	17	40
27	0	100	0	0	100	0	33	51
28	50	250	200	50	50	800	233	290
29	50	0	100	250	0	900	217	347
30	50	350	150	0	0	1350	317	523
31	0	450	250	550	100	1750	517	634
32	0	1100	0	800	0	1000	483	435
33	0	500	0	600	0	800	317	359
34	0	100	150	600	50	550	242	240
35	0	0	100	650	250	850	308	359
36	0	50	50	600	50	400	192	247

TABLE 9 - The Larval Output in Faeces of Calves in Group 3

Day	Calf Number						Mean	S.D. ±
	62	53	52	49	51	47		
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	50	0	0	100	25	42
25	0	0	0	0	100	50	25	42
26	0	0	0	0	0	0	0	0
27	0	0	0	50	0	150	33.3	60
28	100	0	350	25	100	300	145.8	145
29	200	100	100	100	0	500	166.6	175
30	200	150	0	100	0	500	168	185
31	300	200	150	900	200	300	341.6	280
32	150	0	50	550	300	850	316.6	328
33	50	0	0	100	0	300	75	117
34	850	0	100	250	250	150	266.6	299
35	100	500	50	300	300	450	283	180
36	100	50	0	650	350	750	317	321

TABLE 10 - Haemoglobin Values in gm/100 ml. of the calves in Group 1

Day	Calf Number						Mean	S.D. ±
	60	54	45	56	55	57		
4	8.4	9.45	9.3	9.3	10.2	9.0	9.27	0.59
8	7.4	8.0	9.1	8.6	8.3	8.0	8.23	0.58
11	7.4	9.6	9.4	10.0	9.7	9.0	9.18	0.93
15	8.4	8.85	9.3	9.0	10.65	9.15	9.22	0.76
18	8.0	9.45	9.75	9.75	9.75	9.9	9.43	0.72
22	8.85	9.3	9.45	9.6	10.0	9.45	9.44	0.38
25	7.4	8.25	8.6	9.15	9.75	8.0	8.52	0.84
29	7.4	8.8	9.45	8.4	9.0	8.3	8.55	0.72
32	7.4	9.0	9.75	9.45	10.3	8.85	9.12	0.99
36	7.8	8.1	9.75	8.7	9.7	8.25	8.71	0.82

TABLE 11 - Haemoglobin Values in gm./100 ml. of the Calves in Group 2

Day	Calf Number						Mean	S.D. ±
	58	48	61	46	50	59		
4	9.9	10.5	9.45	10.0	9.45	9.9	9.86	0.39
8	8.3	10.0	8.0	9.7	8.8	8.0	8.8	0.87
11	9.2	9.5	9.3	10.3	8.6	9.2	9.35	0.55
15	11.5	10.0	9.9	10.9	9.3	9.6	10.2	0.83
18	9.45	9.45	9.0	11.1	9.3	9.6	9.65	0.74
22	10.2	10.9	9.75	10.75	9.75	10.35	10.21	0.40
25	9.15	8.85	9.0	10.0	9.75	10.3	9.5	0.59
29	8.7	8.6	8.8	10.3	9.6	9.7	9.28	0.68
32	9.75	9.75	9.3	9.75	9.9	10.9	9.89	0.53
36	9.3	9.15	9.0	10.3	10.0	10.0	9.62	0.54

TABLE 12 - Haemoglobin Values in gm./100 ml. of the Calves in Group 3

Day	Calf Number						Mean	S.D. ±
	62	53	52	49	51	47		
4	10.2	9.3	9.75	10.5	10.75	9.45	9.99	0.58
8	8.6	8.3	8.8	8.8	9.5	8.8	8.8	0.39
11	9.2	9.4	9.7	10.4	9.2	8.3	9.36	0.69
15	10.0	9.0	9.9	10.35	10.35	9.9	9.21	0.28
18	9.75	10.0	9.75	11.2	9.75	9.15	9.93	0.68
22	11.2	10.2	9.9	11.2	10.65	10.2	10.55	0.55
25	10.0	9.9	9.0	11.35	9.3	9.15	9.78	0.87
29	10.0	9.45	8.8	10.3	9.7	9.7	9.65	0.51
32	10.3	10.0	9.45	9.9	10.0	9.9	9.92	0.27
36	9.7	9.15	9.0	9.9	9.7	9.45	9.48	0.35

TABLE 13 - The Number of Leukocytes per c.mm. of Blood of the Calves in Group 1

Day	Calf Number						Mean	S.D. ±
	45	54	55	56	57	60		
4	11,150	9,750	7,200	9,700	8,450	5,600	8,641	2,000
8	11,200	10,000	7,250	7,500	8,050	5,850	8,308	1,950
11	13,450	10,650	8,050	5,550	7,200	4,850	8,291	3,480
15	12,150	10,000	8,950	4,800	8,000	4,200	8,033	3,070
18	13,150	10,550	8,450	6,750	8,450	5,450	8,800	2,740
22	13,850	11,150	9,750	4,900	8,750	5,950	9,058	2,530
25	10,850	8,950	8,650	4,800	8,600	7,400	8,208	2,040
29	14,050	9,900	8,200	5,500	9,300	7,200	9,025	2,940
32	11,650	9,000	8,000	5,650	7,550	6,250	8,016	2,140
36	9,700	8,500	7,400	4,700	8,100	6,100	7,416	2,200

TABLE 14 - The Number of Leukocytes per c.mm. of Blood of the Calves in Group 2

Day	Calf Number						Mean	S.D. ±
	46	48	50	58	59	61		
4	10,000	6,400	11,850	11,700	4,850	5,900	8,450	3,410
8	10,950	7,800	10,000	10,500	4,500	5,900	8,275	2,540
11	9,700	7,950	10,950	11,400	5,300	6,600	8,650	2,440
15	8,400	7,500	10,550	12,000	6,450	6,000	8,483	2,360
18	7,600	6,800	9,400	11,050	6,950	7,100	8,150	1,710
22	7,600	6,950	9,100	9, 600	7,200	7,600	8,008	1,080
25	6,850	5,950	8,450	6,900	5,300	6,950	6,733	1,060
29	7,750	5,200	10,000	7,700	6,200	7,100	7,342	1,660
32	6,000	4,950	9,600	6,900	6,200	6,500	6,692	1,560
36	5,600	6,500	10,650	7,900	5,100	5,700	6,908	2,010

TABLE 15 - The Number of Leukocytes per c.mm. of Blood of the Calves in Group 3

Day	Calf Number						Mean	S.D. ±
	47	49	51	52	53	62		
4	8,850	6,200	7,900	8,250	7,600	6,700	7,583	935
8	6,000	6,300	7,950	8,200	7,000	6,300	6,958	825
11	6,850	7,700	8,000	7,600	7,900	7,000	7,508	475
15	6,200	7,900	8,300	7,500	8,000	7,350	7,542	740
18	5,850	8,200	8,850	7,050	6,900	6,600	7,242	1,090
22	5,700	5,450	7,050	5,500	6,750	7,000	6,242	760
25	6,550	5,800	5,800	4,800	5,250	6,000	5,700	600
29	4,700	5,700	6,950	6,050	7,050	6,150	6,100	860
32	5,800	6,100	5,800	5,700	6,150	5,300	5,808	300
36	5,350	5,200	7,300	5,700	6,150	8,000	6,283	1,120

TABLE 16 - Percentage of Eosinophils in Differential Cell Count of Calves in Group 1.

Day	Calf Number						Mean	S.D. ±
	55	54	56	57	45	60		
4	0.6	1.0	6.0	0	0	0.75	1.39	1.51
8	4.3	0.6	4.5	1.06	0.25	1.0	1.95	1.92
11	0	0	0	1.5	0	0	0.25	0.61
15	1.0	7.75	5.0	2.5	1.25	2.0	3.25	2.6
18	12.0	6.5	7.3	6.0	4.75		7.31	2.78
22	5.75	6.75	12.5	4.0	2.5	1.5	5.5	3.89
25	9.7	1.0	0.75	0.25	2.5	0.5	2.49	3.64
29	0.75	1.5	0.67	0.75	2.0	1.0	1.11	0.53
32	0.25	1.5	3.5		0.5	0	1.15	1.43
36	1.75	1.75	3.6	0	0.75	0	1.31	1.36

TABLE 17 - Percentage of Eosinophils in Differential Cell Count in Calves of Group 2

Day	Calf Number						Mean	S.D. ±
	58	48	61	46	50	59		
4	0	0.25	0	0.25	0.75	1.5	0.45	0.58
8	1.25	1.0	1.5	0.25	1.25	1.0	1.04	0.43
11	3.0	2.0	1.5	3.5	0	0	1.6	1.47
15		12.0		5.75	2.6	2.5	5.71	4.45
18	7.0	9.0	0.75	2.0		5.75	4.9	3.45
22	5.7	9.0	1.25	2.0	11.7	4.25	5.65	4.07
25		9.0	0.75	2.0	6.6	0.5	3.77	3.82
29	5.75	11.0	2.5	2.25			5.37	4.07
32	5.75	8.0	3.5	3.25	13.75	1.75	5.5	4.41
36	8.0	15.5	4.75	9.0	1.5	2.5	6.87	5.15

TABLE 18 - Percentage of Eosinophils in Differential Cell Count of Calves in Group 3

Day	Calf Number						Mean	S.D. ±
	62	53	52	49	51	47		
4	1.0	0	0	0	0.8	0	0.3	0.47
8	2.0	1.0	0.5	0	0	0	0.58	0.80
11	1.0	0	0	0.5	0	0	0.25	0.42
15	2.0	0.5	1.0	1.0	3.25	1.0	1.45	1.0
18	5.0	2.7	3.0	2.5	9.0	2.6	4.13	2.56
22	6.7	2.0	3.5	1.25	4.25	1.75	3.24	2.03
25	3.75	2.0	5.75	1.0	5.5	5.2	3.86	1.73
29	7.0	6.0	4.7	0.33		11.0	4.83	4.01
32		1.5	1.5	0	4.5	3.6	2.22	1.81
36	3.5	1.75	4.0	0.25	1.5	3.5	2.2	1.49

TABLE 19 - Number of lympho-reticular nodules visible on the pleural surface of the lungs of each calf, together with the 'lesion score'.

Calf group.	Calf number.	Sub-pleural nodules.	Lesion score.
1. Treated early with diethyl-carbamazine.	45	60	0
	54	65	0
	55	40	0
	56	25	1
	57	20	0
	60	45	0
2. Treated later with diethyl-carbamazine.	46	1	5
	48	5	10
	50	0	8
	58	0	8
	59	0	4
	61	0	6
3. Untreated controls.	47	5	5
	49	1	10
	51	0	6
	52	0	3
	53	0	6
	62	0	9