AGRICULTURAL RESEARCH COUNCIL

REPORT OF THE ADVISORY COMMITTEE ON SCRAPIE

Office Note

The attached report (ARC 196/77) makes reference to a number of ARC papers which provide more detailed information from which the Committee was able to arrive at its conclusions and recommendations. Copies of these papers have been sent to those members with a particular interest in the subject and will be available to other members on request, either before or at the meeting.

ARC 196/77

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REPORT OF THE ADVISORY COMMITTEE ON SCRAPIE

CHAIRMAN: PROFESSOR PETER WILDY

Introduction

At its meeting on 12 October 1976, the Agricultural Research Council agreed to authorize the Secretary to the Council to reconstitute the expert committee responsible for advising him on the Agricultural Research Service programme on scrapie. The previous committee, the ARC Technical Committee on Scrapie Research, had last met in 1969 and had been formally wound up when the Joint Consultative Organisation was established.

Among the factors which influenced Council's decision were the recently-imposed ban by the United States Department of Agriculture on the use for human food not only of sheep affected with scrapie but also of related or exposed sheep or goats, the need to develop a test for the early diagnosis of scrapie in the clinically healthy animal, the increasing recognition of the usefulness of scrapie as a model for certain degenerative diseases of the central nervous system of obscure aetiology in both man and animals, and the complex and specialised nature of scrapie research with the probability of requests for substantial financial support.

The Committee was set up with the following terms of reference:

To advise the Secretary to the Council on:

(i) the current Agricultural Research Service programme on scrapie, including the progress being made towards its objectives;

(ii) the future programme;

(iii) the level of support; and

(iv) liaison with other research workers, particularly in the medical field.

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A The Present Position with respect to Scrapie

A1 The Problem

Scrapie is a natural disease of sheep and goats. It is a slow and inexorably progressive degenerative disorder of the nervous system and it is fatal. It is enzootic in the United Kingdom but not in all countries.

The field problem has been reviewed by a MAFF working group (ARC 35/77). It is difficult to assess the incidence in Britain for a variety of reasons but the disease causes serious financial loss; it is estimated that it cost Swaledale breeders alone £1.7 M during the five years 1971-1975. A further inestimable loss arises from the closure of certain export markets, in particular those of the United States, to British sheep.

It is clear that scrapie in sheep is important commercially and for that reason alone effective measures to control it should be devised as quickly as possible.

Recently the question has again been brought up as to whether scrapie is transmissible to man. This has followed reports that the disease has been transmitted to primates. One particularly lurid speculation (Gajdusek 1977) conjectures that the agents of scrapie, kuru, Creutzfeldt-Jakob disease and transmissible encephalopathy of mink are varieties of a single "virus". The U.S. Department of Agriculture concluded that it could "no longer justify or permit scrapie-blood line and scrapie-exposed sheep and goats to be processed for human or animal food at slaughter or rendering plants" (ARC 84/77). The problem is emphasised by the finding that some strains of scrapie produce lesions identical to the ones which characterise the human dementias.

Whether true or not, the hypothesis that these agents might be transmissible to man raises two considerations. First, the safety of laboratory personnel requires prompt attention. Second, action such as the "scorched meat" policy of USDA makes the solution of the scrapie problem urgent if the sheep industry is not to suffer grievously.

A2 The concept of Scrapie

Scrapie is associated with a transmissible filterable agent, of which there are several strains. Some of these can be transmitted to a variety of animals which develop characteristic disease after varying periods of incubation. At present this remains the only way of recognising and quantifying the agent. The slowness of the disease is thought to be the outcome of a rate-limiting step which is controll by the host. It is possible that the same restriction operates in those cell lines in which scrapie replicates in vitro.

The agent is highly resistant to a wide range of physicochemical treatments including irradiation. It appears to be associated with cell membranes. There is no evidence that infectivity is associated with a virus-like particle or antigen.

It evidently replicates in the brain and in some lymphoid tissue: but it is not known which cell types are involved. Certain features (

pathogenesis, e.g. effect of age of the host and effect of steroids are opposite to those of conventional virus infections. The histological pattern is one of degeneration with no inflammatory element. Different strains give characteristically different histopathological patterns.

This short statement represents the distillate from a tremendous amount of work which has included many erroneous conclusions.

A3 Special difficulties of Scrapie Research

Research on slow virus diseases calls for exceptional qualities in those undertaking it. Even now, when the duration of experiments has been substantially reduced for certain scrapie strains the prolonged nature of the experiments seriously modifies the strategy and tactics of research. Several considerations follow:

a) There is an adverse effect on critical work. Because of the obstacles to repeating experiments many times, work may be reported prematurely and it frequently is. The psychological consequence is that those working in the area may lay so much stress on the importance of particular findings that they are heralded as "the Great Breakthrough". They seldom are.

b) The frequency of performance of any particular test or investigation (e.g. nucleic acid hybridisation) tends to be low compared with that in e.g. virus research. Unless the investigator is aware of his unfamiliarity with the techniques involved he may well draw naive conclusions. He frequently does.

c) Because of the desire for more rapid progress, many workers choose to study only those agent/host/strain combinations that give accelerated responses in small animals. Such models may be so atypical as to provide misleading information.

Such considerations suggest first that those responsible for work with scrapie should be selected with as much care as are astronauts. They should be experienced, mature investigators and research students should only be brought in for projects where the quality of their work will not be compromised by the time-scale involved. Secondly it would seem reasonable that investigators be permitted to work also on cognate problems other than scrapie if they wish. In this way the difficulties listed above would be much reduced in importance. Finally, it would be particularly useful if there were periodic exchanges of research workers between scrapie and other laboratories. This might take the form for instance of a 1-3 month visit by a biochemist working with conventional viruses. Funds would have to be made available for such exchanges.

B The Current Agricultural Research Service Programme on Scrapie

B1 Scientific aspects

Recent work at Compton, ABRO and Moredun is summarised in the Papers (ARC 33/77; ARC 34/77; ARC 38/77; ARC 39/77). The major areas of scrapie research are being covered at these Institutes without duplication of effort. These are Epidemiology, Pathogenesis, Agent-cell interactions and investigation of the Nature of the agent.

Attempts to control scrapie by flock recording and culling are promising (cf MAFF working group report ARC 35/77). It must be noted that there is a need for a rapid diagnostic test to increase the effectiveness of the programme.

B2 Financial aspects

ARS expenditure on scrapie research for 1975/1976 was about £260,000 distributed among ABRO, Moredun and Compton. About £5,000 was provided in addition by the Multiple Sclerosis Society. The overall costs of scrapie research are small in relation to the present financial losses that the country suffers (cf A1). These losses may well increase considerably in the future.

There are 16 scientists working on scrapie in the United Kingdom. A rough breakdown shows that their effort is distributed thus: Epidemiology 2, Pathogenesis 6, Agent-cell interactions 3, Nature of agent 5, (ARC 133/77).

C Future Programme for Scrapie Research

C1 Objectives

a) The eradication of scrapie from our flocks remains the overall objective.

b) Now that there is a serious suggestion that man might be affected by scrapie there are putative public health considerations. Even if the hypothesis is ill-founded, there will be a public demand for action. It has therefore become important to determine the relationship between agents causing slow disease in animals and man even though the scrapie programme cannot be accelerated much because of financial restrictions.

c) There is an urgent need to develop a quick reliable test for scrapie infection in sheep.

d) The investigation of scrapie opens up new areas in fundamental biology.

C2 Projects

a) The natural disease

Field and laboratory studies of natural outbreaks (e.g. scrapie at Compton and at Edinburgh; Rida in Iceland) to investigate the persistence of infection in the environment and the natural routes of spread to sheep.

Identification of changes in the population of agent strains in the development of natural outbreaks; the importance of blocking phenomena between strains and the possibility of a carrier state.

Feasibility of using genetically selected rams to control the natural disease.

b) Biological nature of the transmissible agent(s)

Studies of the stability of different agents on passage to investigate whether they possess the classical biological properties of mutation and recombination.

Studies of the factors affecting titrations of infectivity in animal hosts to improve the efficiency of assays and aid the interpretation of data.

c) Physicochemical nature of the transmissible agent(s)

Physicochemical characterisation of different agent strains to develop ideas on the composition and structure of the agent.

Search for scrapie specific molecules which may form part of the infectious agent or are direct products of it.

d) Agent-cell interactions

Development of methods for reproducibly infecting cells with scrapie *in vitro*.

Search for changes in infected cells, e.g. cell surface antigen.

Biological investigation of multiplication of agent.

Investigation of biochemical events accompanying replication.

In vitro studies with a variety of cells to identify types which can inactivate scrapie or support multiplication of agent.

e) Pathogenesis of disease

Studies of the dynamics of agent multiplication in a variety of extraneural organs with particular reference to the nature of the "zero" phase when infectious agent appears to vanish; mode of entry of agent into the CNS; significance of variation of infectivity titres in different regions of brain to the development of disease and investigation of the causes of cell death in the CNS.

Studies of the mechanism of blocking of replication sites; determination of the normal function of the *sinc* gene which may code for replication sites.

Investigation of the species barrier to find ways of increasing the efficiency of agent isolation in laboratory animals for strain typing.

Identification of specific cell types within the lymphoreticula system which are relevant to the elimination or development of infection in the host.

Biochemical studies of the cellular events associated with infection with particular reference to early changes in the metabolism of polyadenylated RNA, protein factors that may control it, and the genesis of amyloid plaques.

f) Diagnostic test for scrapie to identify sub-clinically infected sheep

A suitable test would immensely improve the efficiency of the flock recording scheme which is the only practicable method of controlling the natural disease.

g) Relationship with human diseases

Comparative investigation of the agents of Creutzfeldt-Jakob disease and scrapie. This would involve histopathological comparisons and transmission experiments. Biochemical comparisons would be the eventual objective.

C3 Strategy, Tactics and Requirements of the Programme

With most infectious diseases it is an easy matter to plan a logical sequence of investigational steps. Because of the slowness of scrapie (A3) it is difficult to formulate such a plan and to assign priorities; and these are not implied in the following classification.

a) Projects for which the way is clear

Some of the projects listed under C2 depend on biological assays which are already available if time consuming. Examples are study of the Natural Disease (C2a); Biological characterisation of the agent (C2b) and some projects for the study of Pathogenesis (C2e). These projects should continue: the lack of adequate mouse facilities is a major obstacle to some of them.

b) Projects which depend on other developments

(i) The physicochemical characterisation of the transmissible agent (C2c) requires procedures for purification to be worked out. To do this it is important to check the validity of early findings by the use of more than one strain of agent. Later, it may be expedient to concentrate on one experimental model. Many different purification schemes should be tried. Some measure of specific infectivity (e.g. titre/protein concentration) should be developed to gauge purification at each step although the problems of the titration assay (C2b) should be borne in mind.

Since recent attempts at agent purification have only been partly successful it should be recognised that the scrapie agent may not exist as a discrete particle. Therefore, alternative approaches should be pursued, for example, the isolation of a putative scrapie nucleic acid. The recent discovery of a DNA which may be specific to scrapie infection could provide a vital lead in the study of the disease. If it is indeed the genome of the agent then the specific activity of the DNA should increase with purification. This DNA should in any case be analysed in depth to establish its specificity for scrapie.

Until substantial purification is achieved, any attempt at chemical characterisation of the infective unit runs the risk of focusing on the wrong type of molecules. Therefore speculative interim work should only be undertaken on the basis of a balanced assessment of the cost of investigating particular options in depth.

(ii) Several items listed in C2d are being studied using cells isolated from infected tissues or established scrapie-infected cell lines. Nevertheless, these studies would be greatly facilitated by the development of reproducible methods for infecting cells with scrapie in vitro. While attempts should aim at producing high infectivity/cell ratios, it may prove to be that there is fundamental host restriction of scrapie replication at the cellular level.

c) Development of a diagnostic test for scrapie (C2f)

This is an urgent requirement essential for the practical control of the disease. It is of course to be hoped that the improved understanding of scrapie at the fundamental level will beget logical tests. This is extremely unlikely in the near future and if a test evolves soon it will probably result from empiricism. So although three recent tests have failed it is important to follow any likely lead. When a finding becomes encouraging, it is vital that double-blind checks are carried out. These can be arranged between Institutes.

d) Comparisons of agents of scrapie and Creutzfeldt-Jakob disease (C2g)

This is dealt with in document ARC 132/77 which outlines the urgent reasons for carrying it out, indicates the immediate investigations needed and considers safety requirements. With respect to the last, Sir James Howie's consultative code of practice deems Creutzfeldt-Jakob agent a B1 pathogen. This may necessitate some expenditure on structural alterations to provide special containment facilities.

A further important consideration which is considered later (E) is the interaction needed with other workers in the medical field and the corresponding need for long-standing liaison between the MRC and ARC.

D Level of Support

The following recommendations were prepared with a vivid awareness of the current financial restrictions. The present scrapie research programme is well balanced with good liaison between Compton and Edinburgh. In general, the projects listed for the future are natural extensions of this work and the present level of support should therefore be maintained. However, we have concluded that the research effort in the area C2d (agent-cell interactions) is under-supported and is the most in need of additional staff.

The Creutzfeldt-Jakob disease proposal (ARC 132/77) will require new support. Initially this will be modest since it is possible that the special facilities needed for the transmission experiments with animals could be obtained from existing budgets. If the transmission experiments are successful increased funding would definitely be needed to meet safety requirements.

E Liaison with other Bodies

Events have forced upon us the urgent requirement for comparative studies between Creutzfeldt-Jakob disease and scrapie. There might be equal benefit if other comparisons were made; indeed there may be hitherto unrecognized diseases of man and other animals which might shed light on the scrapie problem.

Important links have been discovered between some models of mouse scrapie (those which develop cerebral amyloid plaques) and the human presenile and senile dementias and these studies should be encouraged.

It is therefore both scientifically and financially desirable that adequate liaison occurs between workers in these fields and between Research Council officers.

At the scientific level it is probably sufficient to stage a number of symposia on slow virus disease and to foster communications between institutions.

At the financial level it seems important to maintain a joint advisory committee where workers in the field and officers of the ARC and MRC regularly meet to discuss progress.

F Recommendations

1. It is important to maintain the considerable momentum of the present programme of scrapie research by continued financial support at not less than the present level. At the moment the main constraint on the progress of research is financial rather than a shortage of ideas.

2. An urgent requirement has emerged to investigate the relationship between Creutzfeldt-Jakob and scrapie agents. The provisional scheme is that the pilot work is a collaboration between Compton (animal work: R H Kimberlin) and Edinburgh (histological studies: H Fraser). This work should receive the additional funding necessary. It may be appropriate for MRC and ARC support and liaison between the councils is important.

3. An advisory group should be set up. It should consist of scientists from within the scrapie field and also from outside it, together with ARC and MRC officers. It might initially concern itself with scrapie research although the relationship between some models of mouse scrapie and the dementias suggests one obvious area of human disease for the joint committee to consider.

4. Studies of agent-cell interactions (section C2d) are under-supported both at Compton and at Edinburgh. As this is the part of the programme most likely to yield the information required to devise a diagnostic test for scrapie based, for example, on changes in infected cells, there should be two post-doctoral appointments to accelerate progress. One should be at Compton to devise methods for infecting cells in vitro with scrapie agent and the other at Edinburgh to provide expertise on cell separation techniques to identify cell types that can support agent replication in a variety of tissues.

5. Those directing scrapie research should ensure (a) mature and experienced staff are brought into scrapie research and (b) that staff working on scrapie should be permitted to undertake subsidiary projects in other related areas if this is appropriat to making the best use of good scientists.

6. Funds should be made available to bring scientists with particular expertise to the centres of scrapie research and to send scrapie workers to other laboratories.