PROGRESS IN MULTIPLE SCLEROSIS

RESEARCH AND TREATMENT

Edited by

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Proceedings of an International Symposium
12 to 13 May 1970, Jerusalem, Israel

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and Hebrew University–Hadassah Medical School,
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PREFACE

Multiple sclerosis is one of the most common of the so-called "primary" diseases of the nervous system. It constitutes one of the major causes of morbidity and invalidism among young adults in developed countries. It affects its victims at the very outset of their productive life in society and their families. It is a constant, perhaps even growing, source of personal tragedy as well as a constant and growing burden on community health and welfare resources. As such, it ranks as one of the most serious problems in medicine. The cause of the disease remains an enigma, although a century has passed since it was first recognized as a clinical entity.

In the last few years we have witnessed a dramatic increase in research activity in various fields related to multiple sclerosis. The interest and concern of the lay public have also greatly increased, as manifested by the growing number of multiple sclerosis societies in Europe and America. Recently, these societies organized in the International Federation of Multiple Sclerosis Societies, which set as one of its goals the coordination of international effort in research, treatment and rehabilitation of multiple sclerosis. Our group, the Multiple Sclerosis Research Committee, is also a manifestation of international cooperation in multiple sclerosis research.

One of the advantages of our group is the fact that it includes workers in many fields, such as pathology, neurochemistry, virology, immunology, epidemiology and—of course—clinical neurology. The conferences of this group, as well as the constant exchange of information among its members, form a meeting ground for workers in basic and clinical research. Publication of such symposia should spread the results of modern research among workers all over the world. I would like to mention just a few of the multiple sclerosis symposia which have been published recently: the 1964 meeting in Minneapolis, the 1967 Locarno conference, the 1968 Vienna symposium, and the 1969 meeting in Göttingen which will appear shortly.

In a recent article (*Medical News-Tribune* **2** (1): 11, 1970), Sir Francis Walshe asked whether neurology had kept pace with other fields of

medicine. He continued by saying: "A very wide range of therapeutic impotence is still a characteristic of neurological medicine. The 'dead weight' of chronic, disabling and incurable diseases that still confront us, still very imperfectly understood and wholly refractory to any mode of therapy, forces us to ask ourselves whether our research into the aetiology and pathogenesis of these maladies has been as wisely directed and forward-looking as it might have been." These words are certainly not true as far as multiple sclerosis is concerned. The advances in this area in recent years have been really impressive, and it would seem that the amount of effort invested in multiple sclerosis research equals the great challenge. Although we are still far from a full understanding of the pathogenesis of the disease and from an etiologic treatment, we have a consensus that environmental factors play a role in the etiology. I hope that the present conference will demonstrate our progress in both the basic and clinical research into multiple sclerosis.

The Jerusalem Symposium on Progress in Research and Treatment of Multiple Sclerosis, 12 to 13 May 1970, was organized by the Multiple Sclerosis Research Committee, World Federation of Neurology, and was sponsored by the Israel Academy of Sciences and Humanities. I would also like to thank the other institutions that helped us arrange the conference: the Van Leer Institute, the Hadassah University Hospital and the contributing pharmaceutical companies.

The help of the Editor-in-Chief, Dr. Moshe Prywes, the Manager, Mrs. Shulamith Toledano and the editorial staff of the *Israel Journal of Medical Sciences* in the publication of this symposium is gratefully acknowledged.

Jerusalem, May 1972 URI LEIBOWITZ

THE PROBLEM OF CONTAMINATION AND LATENT INFECTION IN THE STUDY OF "SLOW VIRUS" DISEASE OF THE NERVOUS SYSTEM

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INTRODUCTION

Since 1954, when the late Dr. Bjorn Sigurdsson (1) laid down the criteria of a "slow" infection, a great deal of work has been carried out on scrapie, widely recognized as a paradigm of such infections. Scrapie is a naturally occurring disease of sheep, known for more than 200 years in Europe (2) and deriving its name (surprisingly enough only as late as the middle of the 19th century) from the characteristic way in which affected animals rub themselves against fences, telegraph poles, etc. until bare patches appear over the rump or flanks. In 1961 Chandler (3) and in 1963 Chandler and Fisher (4) made a considerable advance in scrapie research when they succeeded in transferring the disease to mice and rats respectively, and so brought its study within the purview of several laboratories not equipped for work with sheep or goats. The use of small rodents has also made bio-assay—still the only means of titering scrapie agent—a practical proposition. Small rodents have also been used in attempts to passage some obscure human neurological diseases [e.g. multiple sclerosis (MS), kuru].

Until 1965 it was generally held that scrapie contamination from cage to cage did not occur within an animal house. However, in that year Morris et al. (5) reported the appearance of scrapie in seven of 200 normal animals which had been living in the same room as scrapie animals up to the age of 18 to 20 months. In diagnosing the disease they relied largely on clinical appearance—which can be deceptive in the old animal—and in some cases on a limited histological examination. More important, no passages were attempted. Passage (with reduction of incubation time to a standard four to six months) is really the only way in which the diagnosis can be

established with certainty in a really old mouse. Even the study of well-stained Cajal astrocyte preparations may not be enough to make a definite diagnosis. The observations of Morris et al. thus require independent and properly controlled confirmation. Since attempts to passage human disease into mice have been reported (6,7) the question of contamination by such a resistant agent as scrapie is of the greatest importance. Experiments are presented which are designed to test the risk of contamination.

MATERIAL AND METHODS

A mouse-adapted strain of scrapie agent (initially kindly given to us by Dr. R. L. Chandler) has long been used in this unit and has been employed throughout the present work. Scrapie mice were inoculated intracerebrally, but a few by the i.p. route. All were housed in a room set aside for scrapie animals.

Experiment 1. Normal mice (from the nonscrapie animal house in the unit) were transferred, immediately after weaning, to cages in the scrapie room for periods of more than two years. No special precautions were taken and they were treated in respect of cleaning, feeding, etc. exactly as other mice in the room.

Experiment 2. Normal mice were fostered to mothers recently injected with scrapie and allowed to suckle for the normal period. After weaning at three weeks they were kept in the scrapie animal room for their lifetimes.

Experiment 3. Male and female mice were injected intracerebrally with scrapie-infected mouse brain (0.05 ml of a 10^{-1} suspension, cleared by spinning at $1{,}500 \times g$ for 10 min) and allowed to breed. Two and occasionally three litters were sometimes obtained. In some experiments one or other parent was normal. Litters were allowed to grow up in the scrapie animal house.

Experiment 4. Sawdust from cages of scrapie-infected mice contaminated with fecal pellets, urine, etc. was transferred twice weekly to cages of normal weanling animals for periods in excess of two years. This experiment was designed to simulate the most gross contamination conditions. The scrapie mouse sawdust was collected from one month after inoculation and the experiment continued until the animals were severely affected by the disease.

Experiment 5. Six white female mice which had been infected either intracerebrally or i.p. one month earlier with scrapie agent were put into large cages with 24 female CBA mice. The color difference obviated all

confusion between exposed and infected animals. Care was taken not to leave the white scrapie mice to die in the cage, lest cannibalism, which is known to result in infection (8) occur. When the white mice showed advanced disease they were taken out and replaced by recently inoculated animals.

Experiment 6. The brain was removed from a mouse with advanced scrapie and stored in formalin in a universal bottle, approximately half full. After three weeks the contents were poured out and without rinsing (let alone autoclaving) the bottle was used to store a fresh normal brain in new formalin. In other words, the accident of using a contaminated bottle was simulated. After a further three weeks the normal brain was removed and a 10^{-1} suspension prepared. This was inoculated intracerebrally into mice to assay the presence of scrapie agent by the contamination procedure. The experiment was performed twice.

Experiment 7. An MSE macerator which had been used for homogenizing a scrapie brain was inadequately autoclaved. The normal procedure is to autoclave all instruments immediately after use (20 lb/inch^2 for 30 min), then to scrub them clean, and finally to reautoclave as before. Instead of undergoing this routine, the macerator was autoclaved only once at 20 lb/inch^2 for 30 min and then used to make up a normal mouse brain (10^{-1}) suspension which was then assayed for scrapie agent by intracerebral inoculation into mice.

Experiment 8. A 10^{-1} saline suspension of scrapie mouse brain was autoclaved for 30 min at 0.5 atm pressure and then titered by intracerebral inoculation in mice. Similar experiments were set up after autoclaving at 1.2 and 2.0 atm.

RESULTS

At no time did any normal mouse develop scrapie through being kept in the scrapie animal house or even through close mixing with scrapie animals. Progeny of scrapie animals likewise remained free from the disease for the 22 months over which the longest-lived animal survived, with one exception—an animal that was bled from the tail for lactate dehydrogenase estimation some weeks after birth.

Animals which received soiled litter from scrapie cages took on a prematurely aged appearance—ruffled hair, slightly hunched stance, slow movements—and were rather easily pushed over, so that after about a year they looked almost twice that age and on superficial examination their symptoms might have been mistaken for those of scrapie. Full histological

examination was carried out on all animals in these experiments (and on any which died unexpectedly and were not decomposed) and showed that no signs of scrapie had developed in any animal.

The contaminated bottle experiment (exp. 6) was negative, as was the imperfectly prepared macerator experiment (exp. 7).

In experiment 8, all six animals injected intracerebrally with 0.1 ml of the 10^{-1} scrapie brain preparation, which had been autoclaved at 0.5 atm for 30 min, developed the disease with an incubation period of eight to 12 months. At 10^{-2}, five of six went down within 13 months. At 10^{-3}, five of six developed scrapie within 14 months; at 10^{-4}, one of six after 13 months; and at 10^{-5} and 10^{-6} no animal became ill. After autoclaving at 1.2 atm 10^{-1} produced disease in one of four animals after 11 months in the first experiment, and in one of four mice after 13 months in a second experiment. Higher dilutions produced no disease. No scrapie developed after a single 30-min autoclave at 2.0 atm.

A second autoclave of the material which had already been autoclaved once at 1.2 atm for 30 min was carried out and the preparation now produced disease in four of five mice at 13, 14 ,15 and 18 months after intracerebral inoculation. All positive cases were confirmed by further passages with a five-month incubation period.

It is thus clear that infective scrapie material (capable of producing six out of six cases at 10^{-6}) is by no means completely destroyed by twice autoclaving at 1.2 atm for 30 min each time.

DISCUSSION

The specter of contamination is an abiding one with all who attempt transmission experiments. Ideally such attempts should be made in an institute not working with scrapie (6). However, the present work goes some way towards assessing experimentally the real hazards involved as opposed to opinions expressed. The suggestion (5) "that virus might have been carried from cage to cage by forceps, scattered bedding, unwashed hands, mixed water bottles or cages, or even insufficiently sterilized cages in the long period of over one year of daily handling and feeding of the animals" seems unlikely. The biggest hazard resides in the possibility that a) cage labels might be interchanged; b) an occasional escaped animal, despite the most vigorous animal house discipline, might be returned to the wrong cage; or c) the material used for inoculation may have been wrongly labeled. Contamination by sawdust, droppings, scrapers, etc. is further rendered