Spread of Scrapie from Inoculated to Uninoculated Mice. (30458)

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Experience with scrapie in our laboratory (1) and in other laboratories in this country (2) and in Great Britain(3) indicates that inoculated and control mice can be housed in the same room for prolonged periods without transfer of the infection from mouse to mouse. There are, however, 2 recorded occurrences of spread of scrapie from experimentally infected mice to uninoculated control mice. Pattison(4) reported occurrence of scrapie in 15 of 49 mice held in direct physical contact for 8 to 14 months with successive batches of scrapie inoculated mice; Dickinson, Mackay and Zlotnik(5) reported the appearance of scrapie in 2 of an unprescribed number, but less than 24, uninoculated mice housed in the same cages for 11 and 14 months with a series of other mice with experimentally induced scrapie. In Pattison's work, fighting occurred between inoculated and uninoculated animals: because of this Pattison suggested that scrapie might have spread to uninoculated mice by ingestion of tissue fragments containing the scrapie agent. In Dickinson's study it was reported that one of the pair of scrapie affected control mice was bitten 9 months before onset of its scrapie illness by an experimentally infected cagemate, but for the other mouse there was no evidence for either fighting or for cannibalism.

In this note are reported our observations over a 3½-year period which suggest that scrapie, after its experimental introduction into mice, might become a contagious infection of low order for this animal.

Materials and methods. Mice employed were of 5 breeds: Swiss GP, Swiss NIH, CFW, CDF_1, and C_{57} black, all obtained from the Animal Production Unit, National Institutes of Health. The breeding history of each strain is known. The occurrence of scrapie-like illness and scrapie-like lesions in uninoculated mice at the National Institutes of Health has not been recorded. For our purposes here, the 5 breeds are not differentiated.

The mice were housed in plastic cages of the shoebox type fitted with perforated metal lids. Seventy cages, each holding 5 mice, were kept on a single rack. Mice inoculated with scrapie materials and uninoculated control mice were housed in separate cages on the same rack. An effort was made to keep control mice on the upper shelves and inoculated mice on the lower ones. Bedding in the cages was changed once and sometimes twice a week. About once a month the plastic cages were washed in water at 180°C. No effort was made to return the mice to the same cage from which they were removed. Mice were handled with forceps which were periodically disinfected by dipping in saponified cresylic acid.

The scrapie material employed was from 3 sources: brain from an experimentally infected goat obtained from Dr. Pattison, Agriculture Experiment Station, Compton, Berkshire, and 2 brains from naturally infected sheep obtained from workers in the U. S. Department of Agriculture. Inoculation procedures were carried out as previously described(1).

Diagnosis of scrapie in all affected mice was made on the basis of clinical observation. In addition, microscopic examination was made of the brain and cord of a small number of scrapie affected mice.

Results. Brain material from the experimentally infected scrapie goat was inoculated into 200 mice in August, 1961(1). One hundred uninoculated mice were maintained as shelf controls. The infectious material used represented the ninth passage of the scrapie agent in goat brain. It induced in the inoculated mice a central nervous system disorder associated with clinical scrapie signs which have already been described by Chandler(3) and by us(1). The incubation period ranged from 7 to 13 months and was dose depen-

dent. By October 1962, or 14 months after the beginning of the experiment, 139 mice had died with scrapie signs, 28 had died of nonspecific causes and 33 had been killed for harvest of tissue and serum.

In October, 1962, brain material from a naturally infected sheep was inoculated into 100 mice. Inoculation of a 10% saline suspension of this material was followed by development of scrapie illness in the test mice with incubation periods ranging from 9 to 13 months. By the end of the 15th month in this group of 100 mice, 74 had died with scrapie signs, 19 had died from nonspecific causes and 7 had been killed for harvest of tissue and serum. Passage of brain material from one of the affected mice in January, 1964, to 100 other mice was followed at approximately 5 months by development of scrapie illness in 89 of the passage mice. The remaining 11 mice were killed for tissue or serum harvest or died of nonspecific causes.

Finally, in November, 1962 brain material from another naturally infected sheep was inoculated into 150 mice. At this time 100 uninoculated shelf controls were established. Inoculation of the material was followed in the test mice at approximately one year by development of scrapie illness. Seventy of the 150 mice died with scrapie signs, 20 died of nonspecific causes and the remainder are still alive or have been sacrificed for study purposes.

In uninoculated mice scrapie illness was first observed in animals set up to control the goat brain study. In February and March, 1963, or 18 and 19 months after scrapie was introduced into our laboratory and approximately 6 months after the last scrapie death had been recorded for the mice inoculated with the infected goat brain, 3 control mice exhibited scrapie illness. Brains of 2 of the 3 control mice were examined microscopically and the lesions were consistent with those found in brains of mice inoculated with the goat brain material. Because of unrelated circumstances, it was not possible to hold these control mice longer and they were sacrificed in April, 1963. However, mice inoculated with sheep brain material together with control mice were kept under prolonged observation. Of the 100 mice set up in November, 1962, to control the sheep brain study 4 have developed scrapie illness in the 2 years they have been held for observation. Clinical evidence of scrapie occurred in these mice 16 to 22 months after initial exposure. Brain and cord from one of the mice in this group have been examined microscopically and the lesions are consistent with those of scrapie. Uninoculated mice used to control the October, 1962, inoculation when discarded in December, 1963, were not showing signs of scrapie.

Discussion. It seems highly probable that scrapie after experimental introduction into mice housed under conditions of our work might become a contagious agent for this animal. If this is the case, then, precise experimentation with this agent cannot be done in the absence of strict isolation facilities. We now have studies underway to determine whether or not the precautions taken by Rowe and his coworkers(6) and by us to prevent spread of viral hepatitis in mice are applicable in prevention of spread of scrapie. This involves use of Trexler isolators and jars with fiberglass filters in the lids(6). It seems likely that virus might have been carried from cage to cage by forceps, scattered bedding, or 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. Thus, airborne infection, while not ruled out, would appear to be unlikely in view of the small number of control mice who have acquired scrapie. It is established that scrapie is readily transmissible to sheep, goats and mice by feeding(4,7).

Pattison(4), in interpretation of his findings in mice, emphasized the necessity for caution in extrapolating experimental data obtained with one species in terms of another. He has presented convincing evidence that under the condition of his experiments, scrapie did not spread after prolonged and intimate contact from sheep to sheep or from sheep to goats. Our prime concern, of course, is the behavior of man following prolonged exposure in the laboratory to the scrapie

agent. There is no information as to whether or not humans will react to contact with scrapie agent as do sheep or goats or in a manner similar to the behavior of mice, or in a manner like none of these hosts. We have inoculated 4 Macaca (rhesus) monkeys both intracutaneously and intravenously with the mouse scrapie agent (10% brain in buffered saline) and to date, after 14 months of observation, no signs of disease have appeared in the monkeys. It seems appropriate to emphasize that precautions employed to prevent spread of conventional viral agents to man and mice might not be sufficient in work with scrapie. In this connection the still unverified observations of Palsson, Pattison and Field(8) of the emergence of a scrapie-like condition in sheep inoculated with brain material from an acute case of multiple sclerosis demands attention. Either there is more spontaneous scrapie in "control" sheep than the investigators suspect, or the multiple sclerosis brain material has provoked or activated a scrapie-like process.

The total experience with scrapie emphasizes the urgent need to develop a specific serologic test for scrapie. Such a test will not only find wide use in veterinary medicine but might also be of help in determining whether or not scrapie is of potential or real danger to man.

Summary. Scrapie occured in 7 of 200 uninoculated control mice housed in the same room with, but caged separately from, successive groups of scrapie inoculated mice. Clinical evidence of the disease appeared in controls as early as 16 months and as late as 22 months after initial exposure to scrapie inoculated mice.

1. Morris, J. A., Gajdusek, D. C., Nature, 1963, v197, 1084.

2. Eklund, C. M., Hadlow, W. J., Kennedy, R. C., Proc. Soc. Exp. Biol. and Med., 1963, v112, 974.

3. Chandler, R. L., Lancet, 1961, v1, 1378.

4. Pattison, I. H., Vet. Rec., 1964, v76, 333.

5. Dickinson, A. G., Mackay, J. M. K., Zlotnik, I., in A Symposium on Scrapie, Publication of U.S.D.A. and Nat. Acad. of Sci., Nat. Res. Council, 1965, Washington, D. C.

6. Rowe, W. P., Hartley, J. W., Capps, W. I., Proc. Soc. Exp. Biol. and Med., 1963, v112, 161.

7. Pattison, I. H., Millson, G. C., J. Comp. Path. and Therap., 1961, v71, 171.

8. Palsson, P. A., Pattison, I. H., Field, E. J., in: Slow, Latent and Temperate Virus Infections, Gajdusek, D. C., Gibbs, C. J., Jr., Eds., Nat. Inst. of Neurol. Dis., and Blindness, N.I.H., in press.

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Magnesium and Potassium Metabolism in Patients with Idiopathic Cardiomyopathy and Chronic Congestive Heart Failure.* (30459)

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Magnesium and potassium are among the most abundant cations in the human body. Both are predominantly intracellular and both have many pathophysiologic and pharmacologic similarities. It has been shown for human skeletal muscle that primary excess or deficiency of potassium is usually accompanied by concordant changes in magnesium content(1). Primary magnesium deficit has been reported to induce an increase in excretion of potassium(2). In acute renal diseases both serum magnesium and potassium are increased(3-5). In diabetic acidosis and also during recovery, magnesium follows a metabolic pattern similar in many ways to that of potassium(3,6,7). Both magnesium and potassium ions depress the cardiac ac-

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