Incidence of Chromophobe Adenoma after Chronic Diisopropylfluorophosphate Poisoning

It is widely assumed that changes in brain cholinesterase would have far reaching consequences on brain function. In practice, however, it has been found that, subject to the animal surviving the initial toxic consequences of the cholinesterase reduction, the ensuing effects are not seriously incapacitating.

Using an organophosphate—diisopropylfluorophosphate (DFP)—as the chief irreversible inhibitor of cholinesterases, we have maintained animals on 20–30 per cent of their normal concentration of brain acetylcholinesterase[1]. These animals have been used for behavioural testing and for concomitant biochemical study[2,3]. During the experiments, batches of normal and chronic DFP treated rats had to be kept for increasingly longer periods ranging from weeks to 2 yr.

Pituitary tumours were found in a batch of rats aged approximately 12–16 months, which had been on a chronic DFP regimen. Because pituitary tumours had never been observed before, closer attention was given to the incidence of this tumour and other tissue abnormalities in rats treated chronically with DFP.

Female Wistar rats were introduced to the laboratory in batches of twenty at 100 days of age. They were caged singly in an air-conditioned colony room at 70° Fahrenheit with a 12 h cycle of light and dark. A dose of 1 mg/kg body weight of DFP was administered at first, followed by booster doses of 0·5 mg/kg every 72 h. The DFP was made up in arachis oil and was injected intramuscularly (gastrocnemius).

Out of five batches consisting initially of one hundred rats, sixteen confirmed cases of pituitary abnormality were recorded, 12, 16 and 18 months after the onset of treatment. This figure does not include those animals which died from causes not established at the time, or which were destroyed because of disease.

The tumours ranged in size from a barely recognizable increase of tissue mass to one with a diameter of 8 mm and with a wet weight of 99·7 mg. The pituitaries were sectioned and stained with haematoxylin and eosin, PAS and Gomori's aldehyde-fuchsin stain. Hyperplasia of the chromophobes was the pathological picture in all but three cases; the remainder showed hypertrophy of the chromophobes. In one specimen cellular pleomorphism and the presence of mitotic figures suggested early malignancy.

Routine post-mortem examinations of all normal and DFP treated rats were conducted in the last 12 months. All pituitaries, whether normal or abnormal in appearance, were sent to the Institute of Medical and Veterinary Science, Adelaide, South Australia, for a pathologist's report. The post-mortem examinations suggested that some of the rats which had been chronically treated with DFP for long periods and which had been recorded in the past as having died from causes unknown may have died because of increased intracranial pressure and brain displacement caused by pituitary overgrowth. All pituitaries, irrespective of appearance, taken from rats which had been chronically treated with DFP for more than approximately 12 months contained hyperplastic chromophobes. The incidence of macroscopic abnormalities approached 100 per cent after chronic treatment with DFP for 21 to 23 months. There are now eleven animals in this latter category. The incidence of non-pituitary tumours was found to be compatible with published observations, indicating an age dependency in their natural occurrence[4,5].

For experimental purposes not directly associated with this communication, five groups of six female Wistar rats, aged 3 months, were treated with various regimens of DFP. For the first 12 weeks the animals were treated with the chronic regimen already stated. This was followed by a series of injections, spaced 72 h apart, containing higher concentrations of DFP. The five groups received their final injections in concentrations of either 0·5, 0·75, 1·0, 1·5 or 2·5 mg/kg body weight. The animals were decapitated and the pituitaries sent, identified only by number, for a pathologist's report. In the groups which received 0·5 and 0·75 mg/kg body weight of DFP there were no abnormalities. The group receiving 1·0 mg/kg contained two pituitaries with vacuolated cells; that receiving the 1·5 mg/kg had no marked abnormalities, and in the group receiving 2·5 mg/kg all six pituitaries had areas where chromophobes appeared more dense, suggesting hyperplasia.

The literature suggests that the incidence of spontaneous chromophobe adenoma in the rat is rare[4,5]. Those reported may have been related to the strain of rat used and to the fact that the animals were carrying experimentally induced tumours[6,7]. It is of interest to note in this context that in human carcinoma an associated increase in the size of chromophobe cells has been noted[8].

There has been one specific and detailed histological study of the anterior pituitary of old female rats which is pertinent to this report[9]. Wolfe *et al.* examined the pituitaries from twenty-six old breeding females, ranging in age from 543–848 days. Two anterior pituitaries were "enlarged, congested and soft". Both were thought to be adenomata of the pituitary. In fifteen rats, 58 per cent of the population, "small areas often consisting of only a few cells" were found which were histologically different from the rest. The difference, in most cases, consisted of "hypertrophied chromophobes". The authors suggest that in their strain of aged breeding females "there is a tendency for atypical areas of hypertrophied cells to appear". In contrast the pituitaries reported here usually showed chromophobe hyperplasia; there was a remarkably high incidence of this abnormality in the animals which received DFP as compared with only one tumour in a control group of equivalent age and sex.

The data obtained by treating some animals with a standard DFP regimen, and treating others with higher concentrations of DFP, suggest that chromophobe abnormalities may be induced in younger females by this treatment. It is possible that DFP has a specific oncogenic effect on the female rat pituitary, or perhaps uncovers or accelerates a change latent in that gland.

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Neurological Illness after Inoculation of Tissue from Tumour Bearing Animals

Certain transmissible infectious agents may remain in tissues for prolonged periods before leading to pathological processes and disease ("slow" infections)[1]. Riley

agent (lactate dehydrogenase elevating virus)[2] is associated with many experimental tumours of mice and is said to persist in the blood of an infected animal throughout life[3]. Because scrapie belongs to the group of "slow" infections and Pattison and Jones[4] have recently claimed that scrapie may sometimes result from inoculation of mouse tumour material into normal mice, we have tried to determine whether prolonged infection with Riley agent might initiate the scrapie process, especially as mouse scrapie has certain features resembling astroglial neoplasia[5]. Although Riley agent was not found to produce scrapie, we obtained some highly unexpected results.

Mice were infected with Riley agent by intraperitoneal injection of 0·2 ml. of a ten-fold dilution in saline (10^{-1}) of plasma from a mouse bearing Ehrlich ascites tumour which had been serially maintained by one of us (D. H. A.) in this unit. Three months later plasma was withdrawn from a mouse and 0·1 ml. (10^{-1}) inoculated intraperitoneally into seventeen Swiss mice aged 4–6 weeks. Fifteen of these developed a neurological illness after an interval of 6–11 months, four showing well marked paralysis of the hind limbs. There was marked astrogliosis in the grey matter of the spinal cord, particularly where it adjoined white matter, and scattered vacuolation of white fibre tracts. The brain, however, was normal. All sick animals had a considerably enlarged spleen infiltrated by myeloid leukaemic tissue, and blood films from two indicated leukaemia.

Similar results were obtained in two more experiments with plasma derived from other mice carrying Riley agent (passage animals of the original Ehrlich tumour)—neurological disease after 6–11 months accompanied by enlarged spleen and lymph nodes (leukaemia) and the disease process localized in the nervous system to the spinal cord, the brain appearing normal. On passage it produced disease with the same localization in the spinal cord, together with leukaemia, but the (histologically positive) spinal cord failed to do so (after 12 months).

In a fourth experiment, the brain of an Ehrlich ascites bearing mouse was injected (10^{-1}) intracerebrally into five mice. Within 5 months all developed a neurological illness with lesions restricted to the cord. Spleen and mesenteric lymph nodes were enlarged (leukaemia) in every case. The brain (histologically negative) of one of these mice on passage (by intracerebral injection) again produced lesions in the cord, but not in the brain, after 9 months. Spleen and mesenteric lymph nodes of these passage animals also showed leukaemic changes.

In these experiments a disease limited to the spinal cord emerged from the passage of serum or brain from Riley infected or Ehrlich bearing animals. It is interesting to note that Stansly *et al.*[6] reported the recovery of a mouse leukaemia virus from Ehrlich's ascites tumour cells and that it could be passaged with nearly 100 per cent success by cell free filtrates of infected splenic tissue. It had a latent period of 5·5–8 months. These results were so unexpected that we have brain and cord passaged another group of Ehrlich ascites tumour bearing mice (obtained from Dr F. J. C. Roe). The recipients have all remained well. Ehrlich tumour bearing mice from which cord disease emerged may have been contaminated by scrapie during passage, but this may not have taken place with the second set of Ehrlich bearing mice. Passaging was, however, done in a non-scrapie animal house and the possibility of contamination is remote. It is also possible that some hitherto unrecognized disease pathologically similar to scrapie has emerged, and might be a source of confusion. Some of the vacuolation in white fibre tracts is comparable with the much more severe lesions described by Pappenheimer[7] in a spontaneous demyelinating disease of adult rats. A condition with some resemblances to this has recently been found by one of us following neonatal thymectomy of mice (unpublished results of E. J. F.), and a full report will shortly be published.

The neurological disease observed presented several interesting features. First, transfer by means of plasma was consistently achieved and was always associated with the presence of leukaemia. Ehrlich tumour cells may be a particularly good medium for the growth of the agent concerned and we are currently testing this possibility. Virus may grow and multiply in leukaemic and neoplastic cells to which it has no aetiological relationship[8,9]. On the other hand, it may be that the adventitious presence of leukaemia facilitates the establishment of the neurological disease and it is known that brain may be a good source of leukaemia virus[10]. Pre or concomitant infection with murine leukaemia viruses has also been reported to result in increased oncogenicity of murine sarcoma virus (Moloney) perhaps by inducing depressed antibody response[11–15]. Chirigos *et al.*[11] also suggest that leukaemia virus may act as a "helper".

Second, localization of disease to the spinal cord after intracerebral inoculation of material under test is most unusual. Localization might result from a modification of the agent by leukaemic virus or be a result of peripheral nerve stimulation as leukaemic lesions develop[16].

In view of the well marked hind leg paralysis which occurred in a proportion of animals, it seems more likely that if the condition were due to scrapie contamination then the latter agent must have undergone some considerable modification. It must be emphasized, however, that failure to passage the positive cords after 12 months suggests (especially in view of the other unusual features of the condition) that some quite different disease is present with only some resemblances to scrapie.

In any case, this work suggests that the possible effect of mixed scrapie and other infection be further studied, and that the suitability of some neoplastic cells (such as Ehrlich tumour) as a growth medium for scrapie agent *in vitro* be explored. The finding reported by Pattison and Jones[4], that scrapie agent may be present in mouse tumours, also points to this possibility. Their observation that neurological illness may be produced by inoculation of tissue from tumour bearing mice emphasizes the complexity of the problem and the great caution with which interpretations should be offered. It also suggests that murine leukaemia (as indeed other tumours) might be a source of the linkage substance recently postulated as important for the development of scrapie[17].

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