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Discussion

Chairman: DR F. O. MACCALLUM

DR A. H. TOMLINSON: As Dr MacCallum is in the Chair, I would like to present briefly his work on antibody in the cerebrospinal fluid.

The slide shows antibody titres for three patients. For the first patient complement-fixation titres to herpes virus were 1 : 64 in the serum and 1 : 32 in the CSF, giving a ratio of serum to CSF equal to 2 : 1, whereas the ratio of titres of antibody to poliovirus in the same serum and CSF was 64 : 0. For the second patient the herpes titres were: serum 1 : 512 and CSF 1 : 256, giving a ratio of 2 : 1, and the ratio of poliovirus titres was 64 : 0. Antibody in the CSF could be due to 'leakage' from the blood stream or to local production, but the difference in ratios for poliovirus and herpes virus suggests production of antibody to the latter within the central nervous system.

In summary, Dr MacCallum's observations on antibody in the CSF showed a rise of titre in seven patients; a fall of titre over a period of time in ten patients; a single sample with antibody from one patient, and a single sample with no antibody from another patient. This last sample was taken only 7 days after onset.

It appears, therefore, that the antibody titre in the CSF determined at several points in time and compared with the serum titres, is a useful diagnostic aid.

PROFESSOR A. W. DOWNIE: I think that it has been suggested by one of the other speakers, and possibly everyone will agree, that most cases of herpes encephalitis are primary infections. That is to say, encephalitis does not often complicate recurrent herpes. That being so, the virus presumably reaches the brain from somewhere, possibly from the oropharyngeal region. What I would like to ask of those who have obviously seen many cases is, have there been any observable primary herpetic lesions in the mouth, lips, cheeks, tongue or anywhere else?

CHAIRMAN: Would anybody like to answer for their cases?

DR L. A. LIVERSEDGE: In our first case, Dr Longson did recover *Herpesvirus* from the oropharynx.

CHAIRMAN: But there were no visible lesions?

DR L. A. LIVERSEDGE: Not that we observed, but it may well have been there of course, but it had gone by the time the patient was admitted to hospital.

CHAIRMAN; What about yours, Dr Rappel?

DR M. RAPPEL; I have been interested in this question and I have searched the literature. I have been through about 300 cases and I think that in only ten of these, certainly not more, have recurrent herpetic lesions of the skin been described.

PROFESSOR A. W. DOWNIE: Yes. It may be of course that if the disease is of insidious onset and the virus travels by way of the nerves, as many people believe, the oral lesions may well have disappeared by the time the patient is hit with the encephalitis.

CHAIRMAN: I think that Dr Longson has been doing some work recently which is relevant to this.

DR M. LONGSON: I would like to challenge the suggestion that herpes encephalitis is a primary infection. I have to admit that I have very little evidence for this statement other than a personal conviction, influenced, I believe, by a very careful search through the literature. I have become convinced that, although the disease can occur as a primary infection, it rarely does so. I submit that herpes encephalitis should be considered, at least in the adult, as a manifestation of recurrent herpes, and as the human counterpart of the interesting rabbit experiments with which I am sure everybody is well acquainted. You will recall that rabbits were inoculated on the eye with *Herpesvirus*. Some animals died, but others recovered and became immune. The surviving rabbits could be sacrificed any time during the following 2 years and no virus would be recoverable from any part of their anatomy, including the brain. If the same rabbits were allowed to survive for up to 2 years and given an anaphylactic shock, or injected with adrenalin, many succumbed to a fulminating herpes encephalitis with virus present in cerebral tissue. I admit that the animal model may not be applicable to man, but I think it is nevertheless a very revealing one. After all, since the work of Dr Hope-Simpson, we all accept shingles to be a reactivation, in nerve tissue, of a *Herpesvirus varicellae* infection acquired much earlier in life; so why should not herpes encephalitis be a manifestation of a similar phenomenon?

The second question I would like to raise concerns the significance one can attach to the presence of herpes simplex virus in the pharynx or in the saliva. We know that very many people, whether they suffer from recurrent lesions or not, can excrete virus from the oropharynx; there have been some quite interesting studies in the United States on this phenomenon. In prisoners, it has been shown that many shed virus in the saliva at odd times in their lives, either in periods of stress, menstruation, or otherwise. On the other hand, we have isolated the virus from the saliva of patients during herpes encephalitis, but I therefore doubt whether these isolations were of any real significance. It occurred to us that a more rewarding approach to the problem might be to use the technique pioneered by Dr Gardner in Newcastle-upon-Tyne, which consists in the aspiration of mucus from as far down the nasopharynx as possible. In the case of herpes encephalitis described earlier this morning by Dr Liversedge, and in a number of later cases, we have been successful in recovering virus from the posterior nasopharynx; this virus we have detected both by immunofluorescence and by isolation techniques. Whether the presence of *Herpesvirus* in the nasopharynx is of any more significance than its

presence in saliva, remains to be established by suitable searches in control cases. Dr Tomlinson in Oxford, and I, have this currently in hand.

PROFESSOR A. W. DOWNIE: Didn't one of the speakers talk about a fourfold increase in the amount of serum antibodies? This is not the sort of thing likely to occur in recurrent herpes. I have always found that with recurrences, the antibody titre does not vary very much—it stays high all the time. On the other hand, in a primary infection, the antibody starts from nothing and rises about 20 times. Now, if you are getting cases of herpes encephalitis showing fourfold and higher increases in antibody, this doesn't sound to me to suggest that herpes encephalitis is occurring in patients with recurrent herpes.

DR M. LONGSON: I think that it must be remembered that the work showing that antibody recall is not a feature during recurrences of herpes simplex, concerns patients with superficial skin lesions. I wonder whether, in the face of a fulminating infection of the brain, when a large antigenic mass is being produced in a deep seated organ, antibody recall could not be expected?

PROFESSOR A. W. DOWNIE: Well, I don't know. I am thinking of Buddingh's work on antibodies and of a number of cases we have studied over many years.

CHAIRMAN: I think, Professor Downie, that we have to think again. The views you are expressing are the traditional ones which we have all accepted. It was not until the report of three cases from San Francisco in 1965, in which there was a clear history of cold-sore in the patient previous to the onset of CNS disease, and who then showed greater than fourfold rise in the serum antibodies at the time of the encephalitis, that we began to think of this problem again. I now accept that the possibility is there, such as Dr Longson has mentioned. One can accept the possibility that the virus has been lying dormant for a long period of time. It may well be possible that the virus is present in the nervous system where antibodies cannot get at it and where it can multiply. In such an instance, one may get sufficient virus produced for an antigenic stimulus to produce a rise in titre. I would agree with you, however, that we have never in all the years that we have been doing the tests, demonstrated a rise in antibody titre in the recurrent herpetics. I have done these tests hundreds of times. Nevertheless, I do believe that the other mechanism is a real possibility.

PROFESSOR A. W. DOWNIE: There have now been many reported cases of herpes encephalitis. Has nobody been able to find out whether this disease is occurring in chronic herpetics or not?

DR J. DOUGLAS MILLER: One out of our series of twenty-two Glasgow cases.

TWO UNIDENTIFIED SPEAKERS: (One out of sixteen—One out of forty).

DR G. D. W. MCKENDRICK: Well, I don't want to disagree with the Chairman's pertinent suggestion that the virus may remain latent in the brain for years; but, I would like to ask what evidence there is about the recovery of *Herpesvirus* from healthy brains. What evidence, in fact, is there that recovery of virus from the brain is associated with disease?

DR T. FLEWETT: We have in the past at various times attempted to culture virus, I suppose, from very many brains (I could not possibly tell you exactly how many without going through the records, but during poliomyelitis epidemics, people quite frequently died and we got an awful lot of brains). I cannot ever, ever remember isolating *Herpesvirus* from a patient who had not got herpes encephalitis.

DR A. H. TOMLINSON: We have cultured tissue from quite a number of brain biopsies but the seventeen specimens which yielded herpes simplex virus were all from cases of encephalitis.

CHAIRMAN: I think it is relevant, Dr McKendrick, and I am sure that the Glasgow group will have even larger figures, that there are about 250 patients a year who have brain surgery in Oxford. In the last 10 years, therefore, this means that 2500 people have had damage done to their brain and have had biopsies. There has been no evidence of encephalitis arising from this, nor have we made any virus isolations. I wasn't suggesting necessarily that the virus was latent and persistent in the brain, but rather that it may be in nuclei, nerve endings, ganglia or something like that.

DR G. D. W. MCKENDRICK: It wasn't a very serious suggestion! I would like to make one other point, however. I think it has become clear that herpes encephalitis is a rare disease. Of the last 159 patients with encephalitis which I have handled, I have only had three patients of proved herpetic origin and we have looked fairly hard. Admittedly, something between seventy and eighty patients were of unknown aetiology, but only two of these died, so, if this herpes encephalitis is really the sort of disease described this morning, we have been very lucky in London.

CHAIRMAN: I think this is very important. Dr McKendrick wrote a letter to the journal some time ago, shortly after Dr Miller and Dr Ross had reported their cases. As most of you know, Dr McKendrick is at one of the largest hospitals in London as a Consultant in Infectious Diseases. Many of us who are concentrating on CNS diseases see these cases, and it may be that people get the wrong impression about the frequency of the condition, but I am sure that Dr McKendrick will agree that this does not detract from the importance of the individual case, which is what we are particularly concerned with this afternoon.

DR T. FLEWETT: May I raise a point which has not yet been mentioned? That is, the rather peculiar periodicity in the diagnosis of these patients. For example, in Birmingham, the disease was excessively rare and then, all of a sudden, it became quite common and we saw several cases in 1966 and into 1967. Last year, however, it seems to have completely disappeared. Has this been other people's experience, and what can be the explanation?

DR M. RAPPEL: We have also noticed this periodicity; however, it must be stressed that the patients in each 'cluster' came from places geographically far apart. This phenomenon has also been observed in other neurological centres in Belgium. Only a detailed analysis will answer the question whether this pseudo-periodicity is genuine or purely an artefact.

DR L. A. LIVERSEDGE: What is so fascinating to those of us with clinical interest is the extraordinary way in which you encounter localization of dysfunction. This can't be entirely due to intracellular attack. Let us

assume that the concept of invasion of the olfactory pathway, then spread to the rest of the brain, is correct; it is then possible that the so-called neurosurgical type of case is due, not to the primary viral invasion of the nerve cells, but is the result of a secondary interaction in the white matter between viral particles being released from infected brain cells and the antibody? This process may then go on to the necrosis and the long-term disability which we have seen. In other words, what I am implying is, if only we could attack whilst the virus is still in its intracellular phase and before it is released, we would do very well. In such circumstances, the therapeutic approach would be much more medical than surgical. In some cases, you may need decompression, in others, this need not come into it.

PROFESSOR T. C. HALL: I was interested in asking Professor Adams two questions. One concerned spotty distribution of the lesion and what the implications of this might be, both diagnostically and cytogenetically. The distribution was so spotty that one wondered when one one saw the little red shapes in particles if this was an end-arterial manifestation of a haematogenous spread. If so, you would not necessarily have to look diagnostically at the lower pharynx. Contrariwise, there may be something neuropathological about it that would indicate that these particular loci within the brain are related to specific foci for perineural transmission from sites that would not be known to me, for example, the ear, or temporal lobes. The other question deals with the problem of early diagnosis. No-one wishes to rush unthinkingly into a brain biopsy and one should reflect on whether in the sequence of inflammation and necrosis, the reactive glial cells have to go to the site because of the existence of some material which is ectopic and therefore is communicating its presence to these cells. It would be interesting to know whether the nature of such material is strongly related to this process. The material might be normal brain substance or some specific viral product which one could look for in cerebrospinal fluid. For example, the substance might be a particular brain cerebroside, which would be diagnostically helpful if, by chemical means, it were found free in the CSF.

PROFESSOR J. HUME ADAMS: The question of virus distribution is difficult. I look particularly for lesions in the olfactory tracts and trigeminal ganglia and trigeminal nerve, but the results of this have not been particularly helpful. Could I ask just one point? Dr Flewett, you said that you isolated virus from the right olfactory tract. Did you by any chance recover virus from many different sites of the brain? Did you, for example, sample eight, ten, twelve, or fourteen parts of that brain? What is the significance of the isolation from the right olfactory tract?

DR T. FLEWETT: I don't know the answer to that—I didn't do the autopsy; this was done by the late Dr Woolf. On this particular occasion, he sent us bits of brain from different parts including the temporal lobe and the necrotic olfactory lobe, together with some olfactory mucosa, but this was an isolated observation and it might be worth looking at the distribution of virus in further cases.

CHAIRMAN: In Oxford, we have only had one case where the virus has been found in the olfactory tract. Dr Tomlinson happens to have some slides in his pocket which will go part of the way to answer your questions.

DR A. H. TOMLINSON: These slides show published (see refs.) tables of the distribution of virus in the post-mortem brain of two cases. Patient G.L. died after 5 days of idoxuridine therapy; thirteen sites in the brain were sampled and the virus was found by culture and/or immunofluorescence in ten samples. Both hemispheres were infected, the left more heavily, and virus was present in both olfactory tracts. Immunofluorescence showed that virus had grown in the cells of the left olfactory tract. Patient W.B. died after 4 days of idoxuridine therapy and virus was present in nine of the thirteen sites sampled; both hemispheres were infected, the right more heavily than the left. The olfactory tract was not examined. Virus was not recovered from cerebellum or medulla in either case. This further slide (Plate 1) shows the immunofluorescent appearances of a typical area of biopsy from one of these patients.

In the hope of getting information on the route of infection, it is important to examine, by culture and immunofluorescence, the olfactory tracts and trigeminal nerves of as many cases as possible.

PROFESSOR J. HUME ADAMS: The distribution of virus in the brain in acute necrotizing encephalitis has always intrigued me, and one wonders if the asymmetry of the pathology is related in any way to the distribution of virus. If it could be established, however, that virus is always distributed widely throughout the brain, it might be in order to take a frontal lobe biopsy rather than tissue from the temporal lobe for the virologist. This would probably be less traumatic for the patient, as the biopsy would not then have to be taken from a soft and haemorrhagic part of the brain. In one of our series of cases, virus was isolated from a histologically normal piece of frontal cortex.

DR I. M. S. WILKINSON: I was going to make exactly the same point; perhaps we could do it by frontal biopsy.

CHAIRMAN: Could I ask Dr Rappel a question which is relevant to the query raised by Dr Hall? This concerns the use that could be made of the cerebrospinal fluid. I believe that you, in Brussels, and some of your colleagues, have been looking at some specific globulin. Have you found some unusual globulin in the CSF?

DR M. RAPPEL: Yes, we are looking at the chemistry of the fluid to find out what kind of globulins there are in the CSF. We have found a large quantity of alpha-globulin and of gamma-globulin which have unusual fractionation pattern. The same thing has happened in the sera. The picture we have seen is similar to the picture which you see in sub-acute sclerosing panencephalitis, but it is not identical, just very similar. It appears so obvious that it might be another sign useful in the diagnosis of the disease, but it appears to be more like a hyper-immune disease which we are looking for (Rappel *et al.*, 1971).

DR L. A. LIVERSEDGE: Could I ask Dr Rappel two points there? Was the total CSF protein raised?

DR M. RAPPEL: The total CSF protein was in some cases normal, but the gamma-globulin fraction was increased.

DR L. A. LIVERSEDGE: How late in the disorder were you getting these changes?

DR M. RAPPEL: Well, it varies. Sometimes it was early; when I say early, I mean the first week of the disease.

DR L. A. LIVERSEDGE: I think it is important to know whether there is time for this to be a by-product.

DR M. RAPPEL: Yes, I agree. Sometimes, after 6 months, there was still some abnormality in the CSF, but it slowly disappeared after a while.

References

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