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REFERENCES

- Bouvrain, Y., Slama, R., and Temkine, J. (1967). *Arch. Mal. Cœur*, **60**, 753.
Cowan, J. (1939). *Brit. Heart J.*, **1**, 3.
Froment, R., de Gevigney, D., Perrin, A., and Normand, J. (1959). *Arch. Mal. Cœur*, **52**, 481.
Fowler, P. B. S. (1962). *Brit. med. J.*, **2**, 1638.
Hewlett, A. W. (1907). *J. Amer. med. Ass.*, **48**, 47.
Lev, M. (1964). *Amer. J. Med.*, **37**, 742.
Merklen, P. (1908). *Leçons sur les Troubles Fonctionnels du Cœur*. Paris.
Rushmer, R. F. (1961). *Cardiovascular Dynamics*, 2nd ed. Philadelphia and London.
Stokes, K. H. (1909). *Heart*, **1**, 297.
Smith, K. S., and Zoob, M. (1961). *Brit. Heart J.*, **23**, 458.
Silverman, L. F., Mankin, H. T., and McGoon, D. C. (1968). *J. thorac. cardiovasc. Surg.*, **55**, 264.

Preliminary Communications

Maintenance of Encephalitogenic Viruses by Non-neuronal Cerebral Cells

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Summary: Mouse and human brain glial elements have been successfully tissue-cultured and inoculated with two highly encephalitogenic viruses without apparent cytopathogenic effect but with long-term continued production of virus.

INTRODUCTION

The role of viruses in the pathogenesis of disease of the central nervous system (C.N.S.) is under intensive study at the moment. The ways in which viruses may affect the C.N.S. have been discussed by Webb and Smith (1966). The following experiments are of great interest, as they show how non-neuronal cells of whole mouse-brain tissue can support for a long time, without apparent damage, the multiplication of two arboviruses, Kyasanur Forest disease (K.F.D.) and Langat virus. These are both of group B tick-borne complex and are highly encephalitogenic to mice and sometimes human beings (Webb and Lakshmana Rao, 1961; Webb *et al.*, 1966).

MATERIALS AND METHODS

Trypsin dispersed whole brain-cell suspensions were prepared from Swiss A₂G white mice 3-6 days old. These cell suspensions were used at concentrations of 4×10^5 /ml. in Eagle's nutrient medium adjusted at pH 7.2. Each tube had a 1-ml. inoculum and was incubated at 37° C. Langat virus was inoculated either at the time of seeding or after eight days of growth. K.F.D. virus was inoculated after eight days of growth.

Langat virus T.P.21 strain was used in its seventh mouse-brain passage diluted in sterile saline containing 0.75% bovine albumin phosphate and buffered at pH 7. Kyasanur Forest disease W3771 CDIV strain was used in its seventh mouse-brain passage and diluted in the same way. Both viruses were received from Dr. C. E. Gordon Smith, Microbiological Research Establishment, Porton. Each virus was used at a dose of about 50,000 intracerebral mouse LD₅₀.

Tubes of the viruses in Eagle's medium were also kept to see how long the virus remained active at the incubation temperature of 37° C. Control tubes of mouse brain tissue culture were also kept in each experiment. All tubes of mouse brain tissue culture with and without virus were examined regularly, the

medium being changed as necessary. When the medium needed renewing both test and control tubes were changed at the same time. At the time of changing the fluid suitable specimens were kept from each group of tubes and inoculated intracerebrally, 0.03 ml. into 3-4-week-old Swiss A₂G mice. Parallel specimens were stored at -60° C. for further titration if necessary. If all the mice became typically paralysed around the sixth to seventh days from this neat fluid inoculation, further specimens were unfrozen and titrated in tenfold dilutions to estimate the total amount of virus present. Brains from the inoculated mice were kept so that the virus could be identified as that originally inoculated into the mouse brain tissue culture.

RESULTS

Mouse brain tissue culture cells have been kept growing successfully for 160 days on their own without subculture and with both viruses inoculated into them after eight days of growth. Langat virus has been released from these cells for 58 days. On the 44th post-inoculation day fluids from four out of six tubes had virus present. Three of these titred 3.0, 3.4, and 2.3 logs of virus respectively. On the 58th day fluids from three tubes inoculated individually produced 2.4, 2.5, and 1.5 logs of virus respectively. On the 65th day fluids from three tubes were negative for virus, and to date no further virus has been isolated. K.F.D. virus was liberated from the cells up to the 51st day, when a pool of three fluids gave 2.0 logs of virus. On the 37th day a similar pool gave 2.3 logs. No virus was isolated from day 58 onwards. When Langat virus was inoculated at the time of seeding the results were very similar. Virus was still being liberated up to the 66th day, when four out of six fluids were positive, one titring 2.4 logs. Three tubes were negative on day 73, but one out of three fluids was positive for virus on day 80. Fluids from the tubes continue to be inoculated in case more virus is picked up at a later date. The other interesting feature has been that the cells in the infected tubes have appeared to multiply more rapidly than the controls. The viruses in the Eagle's medium survived for only 48 hours when used at the highest possible dose. Three points of interest arise from these findings:

(1) Neuronal damage is not a marked feature of encephalitis in immunologically mature mice inoculated with Langat virus intraperitoneally. It is much more pronounced the younger the mice and if the virus is given intracerebrally, when the death rate is 100% even after a very small dose. With large intraperitoneal doses of Langat virus in 6-8 week-old mice a small percentage have a clinical C.N.S. disturbance, but 100% show histological and virological evidence of encephalitis, the emphasis being on an inflammatory reaction (Webb *et al.*,

1968a). It seems that in the brain substance of these animals there are cells which can support virus multiplication for a long time without sustaining lethal damage. It may be the virus antigen from these cells reacting with antibody which produces the allergic type of inflammatory reaction, as suggested by Webb and Smith (1966) and Webb *et al.* (1968a, 1968b). Coombs (1968) refers particularly to the possibility of allergic reactions being involved in microbial pathogenesis.

(2) Price (1966) and C. R. Anderson and M. K. Goverdhan (personal communication, 1968) have shown that K.F.D. virus can survive 246 days and 937 days respectively in the brains of mice. We have recovered 3.5 logs of Langat virus from the brain of a mouse clinically well 36 days after infection. More and more viruses are being found to be latent in the C.N.S. (Rogers *et al.*, 1967), and perhaps one of the mechanisms involved in their persistence is continual and non-destructive multiplication in the non-neuronal elements (probably glia) of cerebral tissue.

(3) If our findings of an increased rate of multiplication can be conclusively confirmed, this would correlate well with the work of Zlotnik (1968). He has shown that the peripheral inoculation of several arboviruses causes, as their first cerebral histological abnormality, hypertrophy and proliferation of the astrocytes. Repeated inoculation of the same virus caused a dense astrocytosis in those animals which survive. One of the features of chronic C.N.S. disease thought to result from virus infection is hypertrophy and proliferation of the glial elements of the brain. Webb (1967) and many others have suggested that demyelination seen in these chronic neurological diseases is secondary to an abnormality of the glial cells. Both astrocytes and oligodendrocytes have been shown to be able to produce myelin (Wendell-Smith *et al.*, 1966; Peters, 1964). If there is a situation of increased multiplication of glial cells their increased metabolic requirements may not permit them to con-

tinue to support the myelin they have produced. These findings suggest that this hypothesis should be further examined.

Using exactly the same techniques, we have now grown cultures of foetal human brain and infected it with both viruses. On the 18th day after infection more than 5 logs of both viruses were being produced without obvious cytopathological effect. Soon we hope to report further on the effects of these and other viruses on both animal and human cerebral tissue cultures.

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REFERENCES

- Coombs, R. R. A. (1968). *Brit. med. J.*, **1**, 597.
 Peters, A. (1964). *J. Anat. (Lond.)*, **98**, 125.
 Price, W. H. (1966). *Virology*, **29**, 679.
 Rogers, Nancy, G., Basnight, M., Gibbs, C. J., jun., and Gajdusek, D. C. (1967). *Nature (Lond.)*, **216**, 446.
 Webb, H. E. (1967). *Proc. roy. Soc. Med.*, **60**, 698.
 Webb, H. E., and Lakshmana Rao, R. (1961). *Trans. roy. Soc. trop. Med. Hyg.*, **55**, 284.
 Webb, H. E., and Smith, C. E. G. (1966). *Brit. med. J.*, **2**, 1179.
 Webb, H. E., Wetherley-Mein, G., Smith, C. E. G., and McMahon, D. (1966). *Brit. med. J.*, **1**, 258.
 Webb, H. E., Wight, D. G. D., Platt, G. S., and Smith, C. E. G. (1968a). *J. Hyg. (Camb.)*, **66**, 343.
 Webb, H. E., Wight, D. G. D., Wiernik, G., Platt, G. S., and Smith, C. E. G. (1968b). *J. Hyg. (Camb.)*, **66**, 355.
 Wendell-Smith, C. P., Blunt, M. J., and Baldwin, F. (1966). *J. comp. Neurol.*, **127**, 219.
 Zlotnik, I. (1968). *Brit. J. exp. Path.* In press.

Medical Memoranda

Guillain-Barré Syndrome Associated with Typhoid Fever

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A syndrome characterized by the acute or subacute onset of distal paraesthesias, muscle pain and weakness, with a tendency for the symptoms and signs to spread proximally, and with increased spinal fluid protein and a normal cell count (albuminocytologic dissociation) is usually referred to as the Guillain-Barré syndrome or Landry-Guillain-Barré syndrome (Macfarland and Heller, 1966). A febrile respiratory or gastrointestinal illness often precedes or accompanies the symptoms of polyneuritis, and therefore the syndrome is also called acute "infectious" polyneuritis or infectious neuronitis (Von Hagen and Baker, 1953). Often no infectious agent can be isolated, but various viral agents have been recovered from the stools, nasopharynx, and even the cerebrospinal fluid. Hitherto no case of this syndrome has been reported to have occurred during the course of a salmonella infection. Below, we record a case of proved symptomatic typhoid fever which showed the features of the Guillain-Barré syndrome.

CASE REPORT

A 27-year-old farmer was admitted to hospital with a four-day history of fever and a two-day history of numbness and weakness

of the extremities. Before the onset of this illness he had been quite well. There was no history of a cough, of any urinary symptoms, or of contact with any insecticides. Examination showed him to be an ill-looking young man, flushed with fever, and presenting abnormal physical signs confined to the nervous system. These consisted of weakness of all four limbs, more marked peripherally, with areflexia and cutaneous hypaesthesia of glove-and-stocking distribution. The cranial nerves were normal and there was no bladder or bowel dysfunction.

Investigations on admission showed haemoglobin 14.5 g./100 ml.; white cell and differential counts normal; E.S.R. 75 mm.; blood for malaria parasites negative; urine normal. The cerebrospinal fluid contained 6 lymphocytes/cu. mm., proteins 200 mg./100 ml., and sugar 73 mg./100 ml. A diagnosis of Guillain-Barré syndrome was made, and prednisone and tetracycline were given.

The muscle weakness began to spread proximally, and by the second hospital day the patient was in respiratory embarrassment and had to be transferred to an "iron lung." By the fifth day the respiratory muscles, though weak, were able to maintain his ventilation unaided, and he asked to be removed from the iron lung; accordingly he was taken out of it. Thereafter his progress from the nervous system point of view was slow and steady. However, he still appeared ill and had persistent fever. There were no clinical or radiological signs of pulmonary infection, nor was there any suggestion of a urinary infection. A salmonella agglutination test at this stage gave the following results: H, 1:250; O, 1:250; Vi and parathyroid A, negative. This test was repeated on the sixteenth day with identical results. Prednisone and tetracycline were discontinued from the tenth day. On the twentieth day he was still febrile and toxic-looking, so blood was taken for culture.