The effect of *P. falciparum* infection is more pronounced in the present series than in previous groups collected in Southern Nigeria.

The series showed an unusually high proportion of mothers with both blood and placental infections detectable at the same time.

It is postulated from the last two observations that the mothers in the present series were not succeeding in mastering their parasitaemia, and this is thought to strengthen earlier impressions that malarial immunity is not firmly established in the population of Northern Nigeria.

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ISOLATION OF E.C.H.O. VIRUS TYPE 9 DURING AN OUTBREAK OF MENINGO-ENCEPHALITIS

BY

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During July, 1956, an epidemic of lymphocytic meningo-encephalitis with marked myalgia occurred in Newton-le-Willows, Lancashire (Lyle, 1956). At the same time type I poliomyelitis was occurring in Manchester and Coxsackie B meningitis in Stockport. Neither the epidemiology nor the symptomatology of the Newton-le-Willows outbreak suggested non-paralytic poliomyelitis or the clinically indistinguishable Coxsackie meningitis, but attempts were made in tissue cultures to isolate an agent from the faeces of seven patients.

Virus Studies

Faecal suspensions were prepared in saline and purified by high-speed centrifugation to remove contaminating bacteria. The resulting supernates were inoculated into stationary monolayer tube cultures of monkey-kidney cells prepared by a modification of Youngner's (1954) method.

Two specimens were tested shortly after receipt and both were found to be highly toxic to the culture cells, non-specific degeneration occurring within 24 hours of inoculation.

The residue of these, together with five other prepared specimens, were stored in a low-temperature refrigerator (-25°C .), as prolonged storage has been found to diminish the toxicity of faecal extracts.

At the end of four months all the specimens were tested in monkey-kidney cultures as before, and also in stationary HeLa-cell cultures. Negative results were obtained in both types of culture with specimens from two adults and one child taken on the third, seventh, and sixteenth days of illness respectively. Of the other four specimens from children, two showed slight degenerative change in kidney cells with no effect in the HeLa strain, while the other two showed no change in either type of culture. In all four cases subinoculation of culture fluid was made to fresh kidney- and

HeLa-cell cultures. It was not until the fourth blind passage that definite cytopathogenic degeneration was noted in HeLa cells, although it was evident in all the monkey-kidney cultures at the second passage. A further series of blind passages in tissue culture was made with the three negative specimens previously referred to, without results at the fifth passage, when the material was abandoned. Lesions were not produced in 1-day-old mice either by original faecal suspensions or by tissue-culture fluids from cultures showing cytopathogenic effect inoculated by the intracerebral or subcutaneous routes.

Neutralization tests on tissue-culture fluids were carried out with antisera against poliovirus types 1, 2, and 3, and Coxsackie virus types B1-B5, with negative results. Tests with E.C.H.O. virus type 9 antiserum gave positive results identifying the agent.

Significant rises in neutralizing antibody in E.C.H.O. 9 virus were demonstrated by Dr. D. A. J. Tyrrell, of the Virus Research Laboratory, Sheffield, and by Dr. A. D. Macrae, of the Virus Reference Laboratory, Colindale, in 8 out of 10 cases from which paired sera were available, including one with negative faeces, and three of the four cases from which isolations were achieved. The fourth case had a high neutralizing antibody titre in the first serum specimen, and this was unchanged 15 days later.

Clinical Notes.—The ages of the children from whom isolations of virus were made were 5, 6, 7, and 12 years. All had fever, headache, and enlargement of posterior cervical lymph nodes. In three of the cases axillary glands were also involved, and two of these had inguinal gland enlargement as well. Central nervous symptoms were not prominent, but one child had a positive Kernig sign with slight neck rigidity, one had a transient loss of knee-jerks, and a third had some neck stiffness.

Discussion

The association of E.C.H.O. virus type 9 with cases of aseptic meningitis has been reported from West Virginia (Committee on E.C.H.O. viruses, 1955), and Belgium (Nihoul *et al.*, 1957). In the United Kingdom, Tyrrell and Snell (1956) and Tyrrell (1957, personal communication) recovered this agent from cases similar to Lyle's occurring in the Sheffield area in September, 1956, and a closely related agent with the additional property of pathogenicity for suckling mice has been isolated during outbreaks in East Suffolk (Boissard *et al.*, 1957), Leicester (Rotem, 1957), and Sweden (Wesslén *et al.*, 1958).

The initial negative findings in HeLa-cell cultures suggest that these are less sensitive than monkey-kidney cells for the primary isolation of this virus, and this is confirmed by the findings of Jamieson *et al.* (1958), who isolated five strains in monkey-kidney cells, but could not detect any virus using HeLa-cell cultures.

Although repeated passage in HeLa cells may lead to adaptation to this cell strain (Archetti *et al.*, 1957), this is not normally a practicable diagnostic procedure.

Summary

An agent serologically identical with E.C.H.O. virus type 9 has been isolated from four cases of lymphocytic meningo-encephalitis with myalgia and a rash occurring in an epidemic outbreak.

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