depression of heat production. The drop in body temperature of the restrained rat, in fact, appears to be the cause of the drop in oxygen consumption (metabolism), for if the reverse were true the I^{131} uptake (thyroid metabolism) should have been higher in the nonrestrained than in the restrained animals.

Summary. Thyroid I^{131} uptake by restrained rats was higher at one hour, and lower at 4 hours, than that of nonrestrained control animals. The lower the body temperature of the restrained animals at 4 hours, the lower the I^{131} uptake by the thyroid. Restrained hypothermic rats had a significantly greater thyroid uptake of I^{131} than equally hypothermic nonrestrained animals. It is concluded that hypothermia of restrained animals can not be ascribed to depressed basal metabolism.

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Association of Mouse Pathogenic Strain of Echo Virus Type 9 with Aseptic Meningitis.* (23043)

DONALD M. MCLEAN† AND JOSEPH L. MELNICK

Department of Pathology, University of Cambridge, England, and Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

Echo viruses are a new group of agents which have been isolated from stools of aseptic meningitis cases and of normal children(1). Although one of the 14 antigenic types, echo-6, has repeatedly caused aseptic meningitis(2-5), the etiological relationship of most types to human disease remains obscure. The present paper offers evidence that echo-9 virus has caused one epidemic of typical aseptic meningitis near Cambridge, England, during 1955, and two epidemics of this syndrome with milder symptoms in other nearby areas during 1956.

Methods. Virus isolations and antibody studies were performed in cultures of human amnion cells(6) which had been grown for the first 3 days in medium containing 20% human serum and 0.5% lactalbumin hydrolysate in Gey's balanced salt solution followed by 3 days in medium containing 10% inactivated rabbit serum and 0.5% lactalbumin hydrolysate in balanced salt solution. Immediately prior to inoculation, the cell sheets were washed twice in salt solution and a maintenance medium, containing 5% rabbit serum and 0.25% lactalbumin hydrolysate in salt solution, was added. Uninoculated cultures remained intact in maintenance medium for 10 days. Monkey kidney (MK) cell cultures were also used. Those in Cambridge were kindly provided by Dr. J. O'H. Tobin, while those used in New Haven were prepared by methods described elsewhere(7). Virus isolations were attempted from feces obtained from patients between 2 and 7 days after onset of illness. In the third outbreak, virus isolation was attempted from both throat washings and rectal swabs. Samples of serum were collected from each patient between 2 and 7 days and again between 12 and 21 days after onset of illness. Neutralization tests on paired sera were performed on serial

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† Harrison Watson Student, Clare College, Cambridge, England; present address, Commonwealth Serum Laboratories, Melbourne, Australia.

TABLE I. Clinical Features of Eleven Cases of Aseptic Meningitis in 1955.

Clinical features	No. of cases
Symptoms and signs	
Fever	11
Stiff neck	10
Headache	10
Vomiting	8
Backache	8
Sore throat	3
Limb pains	2
Photophobia	2
Paralysis	0
CSF findings (2 to 10 days after onset)	
<10 cells	1*
10- 24 "	2
25- 99 "	2
100-300 "	2
>300 "	4
> 10 "	10

* This patient had no cells in the CSF 2 days after onset, but on seventh day the CSF contained 282 cells/mm³.

fivefold dilutions of sera using 300 to 1000 TCD_{50} of virus in amnion cultures and 320 TCD_{50} in MK cultures. Virus typing was carried out in MK cultures using antisera against each of the 14 echo types, against the cytopathogenic Coxsackie types, and against the 3 polioviruses.

Results. Bourn outbreak. Between Oct. 17 and Nov. 11, 1955, 8 cases of typical aseptic meningitis occurred amongst members of 4 families who lived in an emergency housing area at Bourn, Cambridgeshire. Three additional cases occurred in people living in Cambridge who were in close association with the first 2 Bourn cases. The incubation period was between 8 and 11 days. The clinical features are listed in Table I. All at some stage had increased lymphocytes in the cerebrospinal fluid (CSF), ranging between 10 and 1180/mm³, and 8 cases also had between 6 and 48 granulocytes/mm³. In the case of L.R., the CSF contained no cells on second day of illness but on the seventh day it contained 261 lymphocytes and 21 granulocytes/mm³.

Virus was isolated in amnion cultures from feces obtained from two patients, L.M. and L.R., on second and third days of illness, respectively. Virus was also isolated from L.R.'s feces by inoculation of MK cultures. At the Virus Reference Laboratory, Colindale, virus was isolated from the feces of 2 more patients, E.K. and C.W. (G.P.B. Boissard, personal communication). These 4 virus strains could not be distinguished from each other on the basis of neutralization tests using 1000 TCD_{50} of virus against the acute and convalescent sera from patient L.R. (Table II).

Virus from patient L.R. (Bourn virus) in its first passage in amnion culture had a titre of $10^{7.0}$ TCD_{50}/ml when titrated in cultures of human amnion. This material was used in most neutralization tests. After 6 passages in MK cultures, the titre increased to $10^{8.5}$/ml. Bourn virus was not neutralized by antisera against the Coxsackie viruses or polioviruses. In repeat tests it was neutralized by antisera against echo-9 virus but not by antisera against any of the other 13 types of echo virus (see Table III). Both Bourn and Quigley strains produced irregular-shaped plaques with diffuse boundaries on MK cells under agar, typical of group A echo viruses (8). Paired sera of 2 patients were also titrated in MK cultures against the Quigley strain of echo-9 virus. The results are comparable, as shown for patient L.M. in Table III, with those obtained when the same sera were tested against the Bourn strain.

TABLE II. Serological Relationships of Viruses Isolated from Cases of Aseptic Meningitis. (Each virus was tested against sera of the patient yielding the virus and also against the paired sera of patient L.R.)

Date of epidemic	Patient	Days after onset	Serum titres against 1000 TCD_{50} of virus isolated from each patient: L.R.'s sera	Homologous sera
Oct. 1955	L.R.	3	0*	0
			50†	50
"	L.M.	2	0	0
			>100	>100
"	E.K.	6	0	50
			250	>250
"	C.W.	7	0	50
			>50	50

This legend pertains for Tables III through VI, also.

* Titre of acute serum. 0 indicates < 10.

† Titre of convalescent serum.

TABLE III. Cross Reactions between Echo-9 Sera and the Bourn Virus.

Virus	Serum titres (50% endpoint against 100 TCD_{50} of virus) Echo-9, Hill strain*	Echo-9, Quigley strain*	Bourn patient L.M. Acute	Conval.	Other†
Hill‡	15,625	1,500			<10
Quigley§	15,625	2,500	0	250	<10
Bourn	5,380	220	0	>100	<10

* The echo-9 typing sera were prepared by immunization of monkeys, the Hill serum by Wenner and the Quigley serum in New Haven.

† Includes echo viruses 1-8, 10-14, Coxsackie A9, B1-5, polio 1-3.

‡ Hill strain, the echo-9 prototype, was isolated from a normal child in Ohio(10).

§ Quigley strain was isolated from a patient with aseptic meningitis, in W. Va.

Acute and convalescent sera were collected from each patient. Sera were also collected from 14 family contacts 23 days after the epidemic began, and 9 of these were bled again on the 43rd day. Neutralization tests which were performed against 1000 TCD_{50} of Bourn virus showed that 5 cases and 2 contacts had no antibody in the first serum but acquired antibody by the time the second serum was taken. Two cases with a low antibody titre in the first serum showed fivefold or greater rise in antibody titre in the second serum, and 4 cases and 5 contacts had a high antibody titre in the first serum with no increase in the second serum (Table IV). In addition, 3 contacts had antibody in single specimens of serum taken 3 weeks after the epidemic began, one contact had a low antibody titre in both serum samples, one contact had no antibody in either early or late serum, and 2 contacts had no antibody in single serum samples. Thus, of a total population of 23 at risk, 17 subjects were infected with echo-9 virus and 11 of these became ill with aseptic meningitis.

Huntingdon outbreak. During late July and early Aug. 1956, many servicemen and their families who lived on Royal Air Force Station near Huntingdon suffered from severe headache and fever and in some cases this was accompanied by a morbilliform rash. No patient had neck stiffness, and lumbar puncture was not performed. In 3 families who were

TABLE IV. Neutralization Tests on Sera Taken during the Bourn Epidemic, October, 1955.

Name	Virus isolation	Days after onset	Antibody titre against echo-9 virus
	Cases		
M.B.	n.t.	7	>250
		20	"
R.C.	0	4	50
		16	>250
M.M.	0	5	"
		20	"
I.M.	0	3	50
		16	250
C.W.	+*	6	"
		18	"
E.K.	+*	5	0
		17	50
G.R.	0	5	0
		18	250
L.R.	+	3	0
		16	50
L.M.	+	2	0
		13	250
H.D.M.	0	3	0
		13	>250
R.K.	0	5	"
		14	"
	Contacts		
B.M.		23†	0
		43	>50
H.Z.B.		24	0
		49	0
Ro.C.		23	>100
		43	"
E.A.C.		23	10
		43	"
P.L.		23	>100
		43	"
D.L.		23	"
		43	"
E.R.R.		23	"
		43	"
J.R.		23	"
		43	"
P.R.		23	0
		49	>50
B.R.		21	0
A.R.		21	10
A.C.		27	>50
L.C.M.		23	0
B.B.		24	50

* Virus isolated by Dr. G. P. B. Boissard.

† Echo-9 virus isolated from a rectal swab taken on this date.

n.t. = not tested.

TABLE V. Neutralization Tests on Sera Taken during the Huntingdon Epidemic.

Virus strain	Sera of patients: J.C.	M.R.	J.E.R.	D.R.	L.M. (Bourn)
Patient J.C.	10	20	0	n.t.	10
	>50	>250	>250	n.t.	>250
Patient M.R.	n.t.	0	0	0	0
	>250	>250	>250	>250	>250
Bourn strain (Echo-9)	n.t.	0	0	n.t.	0
	>250	>250	50	n.t.	>250

studied closely, stool specimens and paired sera were obtained from 2 patients, stool and convalescent serum was obtained from one patient, stool only was obtained from another patient, and from two further patients paired sera were obtained. The families lived some distance apart from each other. Echo-9 virus was isolated from 3 stools. Rising titres of neutralizing antibody to 2 current strains of virus and the Bourn strain of echo-9 virus were detected in all 4 serum pairs and a high titre was detected in the single convalescent serum. The results are shown in Table V.

Cambridge outbreak. During late Sept., 1956, a schoolboy, D.W., developed severe headache and fever but there was no neck stiffness and no rash. During the next 3 days, 3 of his studymates complained of the same symptoms. All had played a trumpet belonging to D.W. on the day prior to onset of D.W.'s illness, which was the date on which they resumed school after the summer vacation. Throat washings, rectal swabs, and sera were collected from each patient 3 days after D.W. became ill; further serum samples were obtained 16 days later. Echo-9 virus was isolated from throat washings obtained from J.A.C. A rising titre of neutralizing antibody against 300 TCD_{50} of this strain of virus was detected in paired sera from all 4 cases (Table VI).

Mouse pathogenicity. Newborn mice were inoculated with the 3 strains of echo-9 virus, Hill, Quigley, and Bourn, using undiluted tissue culture fluids containing 10^7, $10^{6.5}$, and $10^{8.5}$ TCD_{50} of virus, respectively. From 51 to 90 mice were inoculated with each strain, some injected intracerebrally and some subcutaneously. None of the mice inoculated with the Hill or Quigley strain succumbed to the virus, in confirmation of the earlier findings with these strains. However, the mice inoculated with the Bourn strain developed paralytic illness, with all 19 animals injected subcutaneously succumbing on the third and fourth days and 27 of 32 mice injected intracerebrally coming down between the third and sixth days. The virus was carried for 3 passages in mice. Eviscerated torsos of paralyzed mice yielded titers of more than $10^{8.5}$ and of $10^{7.2}$ per gram, respectively, in MK cultures and in newborn mice. Echo-9 (Hill) antiserum neutralized, both in TC and in mouse neutralization tests, large doses of Bourn virus harvested from infected mouse torsos; thus, a serum dilution of 125 neutralized 10^6 TCD_{50} of the second passage mouse virus in MK cultures, and over 10^4 paralytogenic doses of the same virus seed in the mouse neutralization test(9). Sections of paralyzed mice manifested myositis indistinguishable from that produced by group A Coxsackie viruses. No lesions were seen in the brain or fat pads.

TABLE VI. Neutralization Tests on Sera Taken during the Cambridge Epidemic.

Virus strain	Sera of patients: J.A.C.	C.W.C.	J.H.R.	D.A.W.
Patient J.A.C.	0	0	0	10
	250	>50	>50	50

Discussion. Echo-9 strains have been recovered from healthy children(1,10) and also from sporadic cases of aseptic meningitis(1).‡ However, the present study is the first association of this virus with a sharp epidemic of frank aseptic meningitis and subsequently with two outbreaks characterized by milder symptoms. Since echo-9 virus was the only virus isolated from the patients studied, it seems reasonable to infer that this virus caused each outbreak. Rising titres of neutralizing antibody to current strains of echo-9 in paired sera from patients further strengthen this view. From these results it would appear that echo-9 virus, as well as echo-6(2-5), must be added to the list of agents which may cause the aseptic meningitis syndrome. Sup-

‡ Unpublished results.

port of this view has recently come from several sources. A virus isolated in MK cells by Dr. P. DeSomer in Louvain from the spinal fluid of an aseptic meningitis patient, and sent to New Haven for typing, turned out to be echo-9 virus. DeSomer§ isolated the same virus from the spinal fluids of 7 cases of aseptic meningitis and from 26 fecal specimens. Serological studies showed eight-fold or greater antibody increases during convalescence. Tyrrell and Snell(11) in England have recently described an epidemic disease characterized by rash and aseptic meningitis, some cases of which were similar to the disease observed in babies in East London in 1954 by Crawford, *et al.*(12). Echo viruses were isolated in tissue culture by both groups, but only strains isolated in 1955 and 1956 were found to produce myositis and paralysis in infant mice. Similar viruses, isolated from cerebrospinal fluids, throat washings, and feces, have been recovered during the summer and fall of 1956 from other outbreaks in different parts of England(13). We have learned through personal communications from these investigators that the viruses isolated in their studies have now been typed and have turned out to be strains of echo-9 virus. It is noteworthy that several virus strains, recovered in tissue culture by Meyer§ from a large outbreak of aseptic meningitis which occurred in Germany during the fall of 1956, were also typed as echo-9 virus.

The observation that certain strains of echo-9 virus produce myositis and paralysis in infant mice raises the question of whether the echo-9 strains really belong with the echo or with the Coxsackie viruses. In addition to the mouse pathogenicity of certain echo-9 strains, the Quigley strain of this virus, isolated from an aseptic meningitis patient in West Virginia in 1954, has produced mild lesions in the spinal cord of monkeys inoculated directly into the lumbar area.‡ The lesions, including cellular infiltration and neuronophagia, were limited to the lumbar area of the spinal cord, but were present outside the area of traumatic injury produced by the injection. Benyesh recovered virus from the spinal cords of a number of monkeys for at least a week after inoculation.

Summary. From an outbreak of 11 cases of aseptic meningitis near Cambridge, England, during the autumn of 1955, echo-9 virus was isolated from 4 patients. In addition, 7 cases and two family contacts showed rising titres of neutralizing antibody to this virus. From 2 subsequent outbreaks of febrile headache in this region during the summer and autumn of 1956, echo-9 virus was isolated from 6 patients and rising titres of neutralizing antibody were detected in sera from 8 patients. These data, plus the recent echo-9 isolations from spinal fluids as well as from the alimentary tract of aseptic meningitis cases in other outbreaks, strongly suggest that echo-9 virus has caused severe and mild cases of this syndrome. English strains isolated in 1955 and 1956 were found to produce myositis and paralysis in infant mice indistinguishable from that produced by group A Coxsackie viruses.

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