($P<0.01$) on the second, 95% ($P<0.001$) on the third and 120% ($P<0.001$) on the seventh day following Zn^{65} injection. Although not shown in the figure, essentially the same type of increased Zn^{65} retention was noted when the cadmium was administered on the same day as the Zn^{65}. When cadmium was administered 2 days following the Zn^{65}, increased Zn^{65} retention in liver and whole body became evident within 24 hours after the cadmium injection. In all of the cadmium-injected mice, there was also increased Zn^{65} retention in kidney and pancreas as noted in studies on the rat.

Discussion. Cotzias, Borg and Selleck(7) have recently reported that cadmium is capable of displacing zinc in certain tissues *in vivo*. In rabbits previously loaded with Zn^{65}, they noted 5 hours following cadmium a slight displacement of Zn^{65} from the small bowel wall and a more marked loss of radioactivity from the liver. They stated their work did not assess the amount of zinc replaced or the functional consequences of this replacement. The studies reported here have indeed confirmed that an initial displacement of Zn^{65} by cadmium does take place in the liver; but, subsequently, there is a marked pile-up of the radioisotope in liver as well as in kidney and pancreas. The results further demonstrated that cadmium caused an actual block in fecal excretion of Zn^{65} and an increased whole body retention of the radioisotope which could, in large part, be accounted for by the increased Zn^{65} levels in liver, kidney and pancreas. These results indicate, then, that one of the functional consequences of the initial displacement of Zn^{65} by cadmium previously reported(7) may be an interference with the normal excretory pathway for zinc.

Summary. The results of this study indicate that cadmium interferes with fecal excretion of Zn^{65}, resulting in an increased retention of radioactive zinc in liver, kidney and pancreas.

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Measles Antibodies in Multiple Sclerosis.* (27855)

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Serologic studies offer a new approach to the problem of pathogenesis of multiple sclerosis (MS) as a possible sequela of measles or measles encephalitis (ME). Although the exact etiology of measles encephalitis is not clear, the pathologic process is characterized by diffuse disseminated demyelinating pathology(1). A demyelinating process is considered characteristic of multiple sclerosis (2-4). In this study measles antibody titers were determined in sera of over 100 patients with MS. Spinal fluid titers were similarly determined in 35 MS patients. The main purpose of this preliminary report is to stimulate further investigation in this important and unsolved problem in medicine.

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TABLE I. Serum Neutralization Tests for Measles Virus.
(107 Patients and 96 Controls)

Reciprocal of serum dilution	% MS patients		% Controls	
<4	14		19	
4	18	64%	27	86%
8	15		18	
16	17		22	
32	19		9	
64	7	36%	2	14%
128	9		3	
>128	1		0	
Total		100%		100%

Materials and methods. Serum and spinal fluid samples from patients with MS were tested for measles neutralizing and complement-fixing (CF) antibodies. Neutralization tests were carried out in HeLa cell cultures employing 100 TCD_{50} of HeLa adapted Edmonston strain measles virus and observed for 3 weeks. Complement-fixation tests were carried out with measles antigen prepared from HeLa cell cultures. Established negative and positive serum samples were tested simultaneously.

Control serum and spinal fluid samples from hospitalized individuals with no evidence of MS were similarly tested. A diagnosis was available on 48 of the control subjects from whom spinal fluids were obtained for study. Eighteen or 37% had disorders involving the nervous system. Eight of these had epilepsy or a convulsive disorder; 3 had a diagnosis of brain tumor, and the others were made up of the following: meningitis, paraplegia, hemicephaly, cerebral ataxia, Guillain-Barré syndrome and cerebral vascular accident. The remainder of the control subjects had no evidence of organic brain disease. Cerebral spinal fluids from patients and control subjects were also tested by the complement-fixation method for antibodies to mumps and influenza. All samples were heated at 56°C for ½ hour before testing.

Results. The results of neutralization tests are recorded in Table I, which shows that 36% of multiple sclerosis patients had serum titer levels of 1:32 or greater for measles antibodies in contrast to 14% of the control subjects. In Table II the results of the CF tests are recorded and show that 31% of MS patients had serum levels of 1:128 or greater, whereas 12% of controls had similar antibody titers. Percentage differences at the higher titer levels which were selected arbitrarily show that MS patients have slightly higher levels than control subjects.

These findings led to a serologic study of the cerebral spinal fluid (CSF) of patients with MS and control subjects. The results recorded in Table III show that of 35 patients with MS tested 25 have antibody titers by the CF method in their spinal fluid against measles antigen. One sample was anticomplementary and 9 were negative. Fifty-six control subjects were tested; 50 showed no titer against measles antigen, and 6 specimens were anticomplementary. Neutralization results on CSF are also shown in Table III.

TABLE II. Serum Complement-Fixation Tests for Measles Virus.
(109 Patients and 94 Controls)

Reciprocal of serum dilution	% MS patients		% Controls	
<32	13		35	
32	16	52%	21	71%
64	23		15	
128	20		8	
256	10	31%	2	12%
>256	1		2	
AC		17%		17%
Total		100%		100%

TABLE III. Measles Virus Neutralization and Complement-Fixation Tests on Cerebral Spinal Fluid.

	CF tests			Neutralization tests	
	Pos.	Neg.	AC	Pos.	Neg.
Multiple sclerosis	25*	9	1	18†	17
Control subjects	0	50	6	0	48

* 12 at titer undiluted, 11 at 1:2, and 2 at 1:4.
† 5 at titer undiluted, 7 at 1:2, 2 at 1:4, and 4 at >1·4.
P = <.001 for both CF and neutralization tests.

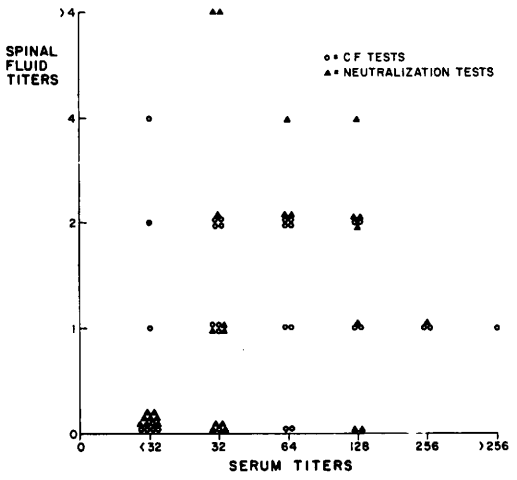

FIG. 1. Results of CF tests for measles antibodies on sera and spinal fluid of patients with multiple sclerosis are shown as reciprocal of dilution. Also shown are comparative results on measles neutralization tests on sera and spinal fluids from the same patients. (Each point represents titers on serum and spinal fluid of a single patient.)

Eighteen patients with MS neutralized measles virus and 17 showed no evidence of antibodies. All 48 specimens from control subjects failed to neutralize measles virus. Most of the specimens were retested with commercial measles CF antigen and results on the whole were parallel to those reported in the Tables. Serum and CSF titers were compared in individual cases and no apparent correlation was evident (Fig. 1).

One patient whose spinal fluid was anticomplementary neutralized measles virus and another patient who showed no CF antibodies also neutralized measles virus in tissue culture, bringing total with evidence of measles antibodies to 27 of 35 patients.

Spinal fluids were tested from 29 patients with MS and 40 controls with mumps antigen for complement-fixing antibodies. Nine patients had evidence of antibodies in undiluted fluid. Ten of the control subjects also had evidence of antibodies in undiluted spinal fluid. There was no correlation between measles and mumps antibody titers in the spinal fluids tested of either patients or control subjects. The cerebral spinal fluid of 12 patients with MS and 22 control subjects was tested against Influenza A virus by CF method and all were recorded as negative.

Discussion. Any possible association with a predominantly childhood disease, such as measles, and a disease which characteristically manifests itself in early or middle adulthood would appear to be extremely remote. The pathologic processes of demyelination suggest a possible relationship; the frequent association of optic neuritis as a feature of both diseases also invites closer inspection. The pathogenesis of measles encephalitis and multiple sclerosis is little understood; the etiology of both diseases is still unproved(2-5). Shaffer, Rake and Hodes (6) reported the recovery of measles virus from the brain of a patient with fatal encephalitis. They concluded that this agent is not only implicated in causation of the condition, but further that it indicates that active virus may persist in the human body for as long as 9 days after onset of the rash.

The marked differences in protein levels and particularly the gamma globulin fractions of blood and spinal fluid suggest that antibodies might be difficult to detect in even undiluted spinal fluid of normal subjects or patients with MS. Ruckle and Rogers(5) tested by the CF method the spinal fluid of 9 patients with ME, all within 48 hours of the development of CNS symptoms, and all were found to be negative for measles antibodies.

The excess globulin in the spinal fluid of many patients with MS(7,8) does not appear to be correlated with an excess in the serum and hence indicates the possibility of local globulin formation within the central nervous system. When the relative levels of protein are compared in the serum and spinal fluid of MS patients the ratio is found to be approximately 200 (serum) to 1 (CSF). Any measurable titers of antibody in undiluted spinal fluid therefore would suggest that they might be there in relatively greater amounts than in the serum. The possibility exists that the antibodies recorded in spinal fluid are related to serum antibodies and may be elevated because of the increased globulins. This relationship was not evident in the CF tests to Influenza A or mumps viruses. However, serum antibody titers for these 2 agents tend to be lower than in measles. Lennartz

(Hamburg) stated that spinal fluid antibodies were noted only in those cases in which the presence of virus was conclusively demonstrated in CSF (for example, mumps) (9).

It is possible that viruses or their nucleic acids may continue to infect tissue even in the presence of antibody(10). Rustigian(11) recently reported an experiment with measles virus which, after 39 serial passages in the presence of measles antibody contained in human gamma globulin, continued to be infectious for normal HeLa cell cultures. The persistent infection was characterized by viral inclusions and immunofluorescence. He suggests that the results may be due to the fact that the carrier line is associated primarily with immature virus. A study of measles virus infected HeLa cells by electron microscopy has revealed 2 particulate structures, one of which appears to be the fully developed mature virus and the other may represent immature virus or viral nucleic acid (12).

Herriott(10) points out that infectious nucleic acid provides a possible explanation for permanent immunity. A very low level infection, maintaining a stimulus for antibody formation, is possible if even a small proportion of virus is released as viral nucleic acid. In this connection Dulbecco and associates(13) reported that excess antibody failed to reduce below a minimum the infectivity titer of either western equine encephalomyelitis or poliomyelitis virus.

The pathologic demyelinating process of multiple sclerosis is considered by McAlpine and associates(2) and by Greenfield and associates(14) to be a continuously active one. McAlpine states that the plaques are hardly ever completely quiescent. "There is evidence of continued activity at the periphery of even the most sclerotic plaques." Greenfield(14) states, "The process may continue active for days or weeks and in the later stages of the disease sometimes appears to be continuously, although weakly, active." The probability that infectious or viral nucleic acid may continue to be infectious even at an extremely low level of activity suggests a possible explanation of the pathogenic processes involved in multiple sclerosis.

The relationship of retrobulbar or optic neuritis to measles and its common occurrence in MS may be relevant. Kennedy and Carter(15) recently reported 8 children with optic neuritis who developed multiple sclerosis in variable periods after the episode of visual failure; 6 developed evidence of dissemination within 2½ years. In the other 2, dissemination did not occur until 12 and 16 years later. McAlpine and associates(2) state that "the long interval" which may occur between the initial attack of retrobulbar neuritis and the appearance of subsequent manifestations may account for varying opinions regarding the frequency of optic nerve involvement in MS. The prevalence of retrobular neuritis as an initial sign has ranged from 14 to 46%, as recorded in the literature (2,16,17). However, Benedict(18) in a study of 225 cases of retrobulbar neuritis found evidence of MS in 155 (68.9%). McAlpine and associates(2) state that "there is only one common cause of unilateral retrobulbar neuritis—namely multiple sclerosis."

Ford(19) divided the manifestations of measles as they affected the nervous system into the following main groups: a) diffuse cerebral symptoms of brief duration, b) multiple focal cerebral symptoms, c) hemiplegia and aphasias, d) cerebellar syndromes, e) paraplegias and spinal cord syndromes and f) other incidental complications such as mental disturbances and optic neuritis. Capolongo(20), reporting on optic neuritis after measles, states that most cases occur during convalescence but occasionally it manifests itself during the course of the illness itself; it may be bilateral as well as monolateral.

Summary. Examination of the sera of patients with multiple sclerosis by neutralization and complement-fixation tests revealed slightly higher antibody titers to measles virus than control subjects. The cerebral spinal fluid of patients with multiple sclerosis showed measurable antibody titers to measles virus in over 75% of the cases tested, whereas control subjects have shown no evidence of antibodies. The finding of elevated measles antibodies in the serum and spinal fluid of

patients with MS does not imply or indicate a direct relationship but warrants continuing investigation.

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Metabolism of Cl^{36}-Dichloromethotrexate by Transplantable Liver Tumors. (27856)

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Dichloromethotrexate* (DCM), a folic acid antimetabolite, has been reported to be metabolized in several animal species(1). Recently such a metabolic change was also observed to occur *in vitro* by an enzyme system localized in the soluble (72,000 × *g* supernatant) fraction of liver(2). The metabolite† of DCM is about 1000 times less active in inhibiting folic reductase than the parent compound(3).

An ideal agent for treatment of hepatic tumors might be a carcinostatic compound which (i) accumulates in liver, (ii) is metabolized rapidly by normal liver to a less toxic form and (iii) is not metabolized by the tumor(4). Because DCM is bound to liver tissue(1), is rapidly metabolized by normal liver, and probably "detoxified" by this mechanism it was thought desirable to study the *in vitro* metabolism of DCM by a variety of transplantable liver tumors to determine if the above requisites were fulfilled. Another objective of the present study was further to characterize these tumors as to the extent of enzymatic deviation from normal liver.

Methods. The liver neoplasms used in this study were carried intramuscularly or subcutaneously in male rats in our laboratories. Transplantable tumors studied included the Novikoff hepatoma, tumor 5123, 5123 TC, hepatoma 3683, hepatoma 7800 and the Reuber (H-35) hepatoma. The Novikoff tumor was carried in Holtzman rats; tumors 5123, 5123 TC and 7800 were carried in Buffalo rats; and the 3683 and H-35 tumors were car-

*N-{3,5-dichloro-4-[(2,4-diamino-6-pteridinylmethyl)-methylamino] benzoyl}-glutamic acid is the chemical name. The abbreviated generic name, DCM, is used throughout the text.

† The metabolite has tentatively been identified as 4-deamino-4,7 dihydroxy DCM (generic name); N-{3,5-dichloro-4[2-amino-4,7 dihydroxy-6-pteridinylmethyl)-methylamino]benzoyl}-glutamic acid is the chemical name.