breast cancer, were examined with the aid of an electron microscope. Spherical particles, of a smooth surface and a high density† to the electron beam, varying in diameter from 20 to 200 mμ, in some instances grouped in pairs, or clusters, were found in all samples; they were particularly numerous, however, in 5 of the 10 samples examined. These spherical particles appeared to be similar to those previously observed in mouse milk known to contain the mouse mammary carcinoma agent.

2. Thirty-two control human milk samples, collected from young, healthy, nursing women having a family record apparently free from any malignant tumors for 2 preceding generations, were also examined with the aid of an electron microscope. Eleven of them were found to contain spherical particles essentially similar to those described above. Of the remaining 21 control milk samples, 17 were found to contain only occasional, isolated, single particles in some of the electron microscopic fields; the other 4 samples appeared to be free from spherical particles, but contained some unidentified debris.

3. No definite conclusions can be reached at this time as to the nature of the spherical particles revealed with the aid of the electron microscope in human milk.

Twenty-nine human milk samples were processed, and examined with an RCA electron microscope type EMB at the Research Laboratories of the Interchemical Corporation, New York; Mr. M. Parkinson, Mr. J. J. Kelsch, Mr. R. Bainbridge, and Miss Joan Bardet rendered valuable technical assistance in this part of our work. Thirteen human milk samples and several mouse milk samples were examined with an RCA electron microscope type EMU-2B, at the Research Laboratories of the Veterans Administration Hospital, Bronx, New York.

Most of the human milk samples were obtained from the New York Lying-In Hospital. Several samples were obtained through the courtesy of Dr. Milton J. Goodfriend, New York.

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Relative Susceptibility of Various Stocks of Mice to Experimental Disseminated Encephalomyelitis.* (18169)

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The present paper extends recent studies on experimental disseminated encephalomyelitis induced in white mice by means of injection of homologous brain plus Freund-type adjuvant(1) and offers further results of tests on the variation of susceptibility of certain stocks of mice. Certain aspects of the experimental affection will also be shown. A wide difference has been found(1) in the susceptibility of the W-Swiss(2) and the Rockefeller Institute(3) strains of mice, the former being susceptible, the latter resistant. Thus in an experiment in which the same method and materials were employed, 19 of 20 injected Swiss mice, while only 1 of 15 Rockefeller Institute mice developed encephalomyelitis(1).

The tests now to be described included a study of additional stocks of mice: (a) W-Swiss mice, H-line, of the same strain as was hitherto employed and found to be reactive to inoculation of homologous brain with development of encephalomyelitis. (b) Another stock was added, W-Swiss B-line, obtained from a source outside of the laboratories but

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propagated for more then ten years as an inbred strain deriving originally from W-Swiss H-line parents. This B-line stock was developed by selection so as to obtain certain uniform litters, and was nurtured under conditions different from those of the H-line. At present it may be regarded as a separate stock. (c) A third stock comprised a line developed from piebald house mice, a true breeding variety called white-faced(4), maintained as inbred for several years by Dr. H. Schneider.

Method and materials. Any of the following preparations of mouse brain-adjuvant mixtures was used without much difference in the outcome: (a) fresh mouse brain obtained from normal-appearing Rockefeller Institute or W-Swiss strain mice, 10 g; autoclaved tubercle bacilli H37Rv type(1), 20 mg; liquid petrolatum (Soconal), 50 cc; and 0.85% saline solution, 50 cc. (b) This preparation was similar to the aforementioned one except that the amount of tubercle bacilli was increased to 250 mg. (c) This preparation was similar to (b) except that light mineral oil and Falba 0.5 g was substituted for Soconal. The injections of 0.3 cc were given, as a rule, subcutaneously and rarely intramuscularly at 7-day intervals until either a total of 10 injections had been given or characteristic signs appeared earlier. Mice 1 to 2 months old were used; they were observed 2 to 3 months after the first introduction of the brain-adjuvant mixture. The picture presented by the reactors has already been described(1). On admission to the laboratory all animals were kept under the same environmental conditions and given the same diet of fox chow, bread and milk, and water.

Experimental. The tests which were made in addition to those already reported(1) are summarized and tabulated (Table I). It will be noted that among 65 mice of the W-Swiss H-line, 52 developed disseminated encephalomyelitis after multiple injections of homologous brain-adjuvant mixtures, the average number of inoculations being 4 in one experiment and 6 in two others. As already described this stock of mice has been shown to be susceptible: of 50 injected mice 46, or 92%, exhibited the experimental affection(1). In comparison with that reactive stock, the W-Swiss B-line showed a reverse effect, namely, a relative resistance to the development of the experimental disorder, *i.e.* of 45 injected mice only 9 were found to be reactors. Finally, of the 12 injected white-faced mice only one came down with the neurological syndrome. Thus the latter animals join the Rockefeller Institute strain(1) in the class of those that are refractory. It appears that there is a constitutional or endogenous factor in certain stocks which marks the animals thereof as susceptible or relatively resistant to the effects of the "antigen" that produces disseminated encephalomyelitis in mice.

It is also to be observed from the table that once an animal reacted to the injections, the time taken before the first sign was manifested, or the number of inoculations needed to produce the disorder, did not differ in animals that were susceptible or were relatively resistant. The only exception was the single white-faced mouse that reacted. Here the time taken for the first signs to become visible, and the number of injections given were both greater.

It should be stressed, moreover, that of the 62 animals which reacted 12, or 19%, exhibited a variation in degree and number of lesions in the nervous system characteristic of disseminated encephalomyelitis, even though none of the 12 revealed any objective sign of illness.

Discussion. Multiple sclerosis and other demyelinating diseases are brought into this discussion because investigators of the problem of experimental disseminated encephalomyelitis have drawn attention to the similarity—not, however, the identity—of the symptoms and pathological picture induced in lower animals to those found in the human affections. Moreover, in multiple sclerosis the prevailing theory of its origin includes two factors: (a) constitutional or familial or endogenous susceptibility which by itself is ineffective to bring about the disease and (b) an exogenous agent, or perhaps agents, which can induce the disorder especially when the

4. Dunn, L. C., and Durham, G. B., *Am. Nat.*, 1925, v59, 36.

TABLE I. Showing Variation in Susceptibility of Different Stocks of Mice.

	No. of mice used	Stocks of mice						Mice with no signs, only CNS lesions
		W-Swiss H-line		W-Swiss B-line		White-faced		
		Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	
Exp. I*	52	16	9	3	12	1	11	H-line, 3; B-line, 1
Avg days to 1st sign		42		41		64		
Avg No. inj.		6		5		9		
Exp. II	25	21	4					H-line, 4
Avg days to 1st signs		52						
Avg No. inj.		6						
Exp. III	30	15	0	0	15			
Avg days to 1st signs		29						
Avg No. inj.		4						
Exp. IV	15			6	9			B-line, 4
Avg days to 1st signs				32				
Avg No. inj.				5				
Totals	122	52	13	9	36	1	11	12
Approx. %		80	20	20	80	8	92	19 (of 62)

* In Exp. I, the W-Swiss H-line mice were maintained by others than those who supplied animals for Exp. II and III.

former, or endogenous factor, has first prepared the way(5). Any constitutional element influencing the production of encephalomyelitis has not been brought forward for the experimental animals hitherto studied by others. It was shown here, however, to exist in the mouse which served as a delicate indicator of the importance of the stock from which the animal is taken for the production of the neurological syndrome. The W-Swiss H-line was found to be susceptible and a separate variety, the W-Swiss B-line, relatively resistant, even though the latter was derived from the same original W-Swiss strain. Also resistant was the Rockefeller Institute strain, as previously shown(1) and the white-faced mice, now demonstrated.

One should not look here for a direct relation to the problem of human demyelinating diseases since it is yet to be proved that experimental disseminated encephalomyelitis induced in any species of animal is identical with them. It is, however, of interest that in the experimental encephalomyelitis which does bear certain resemblances to the human maladies an endogenous factor, as found in animals of certain stocks, should be correlated with susceptibility or resistance.

5. Merritt, H. H., Wortis, S. B., and Woltman, H. W., Foreword in Multiple Sclerosis and the Demyelinating Diseases, *Res. Publ. Assn. Nerv. Ment. Dis.*, 1950, v28, 1-675.

Another point of interest is the number of injected mice that showed no outward sign of illness yet at autopsy varied in the degree and extent of lesions in the central nervous system, chiefly vascular and perivascular, of a nature already described(1). These were noted most often in the white substance and granular layer of the cerebellum; in periventricular areas, especially the white matter about the IV ventricle; in the anterior thalamic regions, and in the dorsal spinal cerebellar tracts and fasciculi. It is clear, first, that considerable damage can exist in the nervous system of the mouse without observable, objective signs of illness. Moreover, since these histopathological changes resulted specifically from the injections of the brain tissue-adjuvant mixtures, it becomes necessary to examine animals apparently symptom-free for the presence of such lesions before they can be declared non-reactive. Finally, if what occurs in the mouse is a reflection of what may exist in other experimental animals in this type of work, *viz.*, monkeys, rabbits and guinea pigs, a clear indication arises to examine the central nervous system of all such as exhibit no symptoms before they can be considered negative.

Summary. 1. The stock from which the host is taken is apparently important for the outcome of attempts to induce in mice experimental disseminated encephalomyelitis,

for certain stocks of albino mice are susceptible and others, even though of related lines, are relatively resistant. 2. In 19% of the mice which reacted to homologous brain tissue-Freund type adjuvant mixtures, lesions characteristic of disseminated encephalomyelitis can be found in the central nervous system in the absence of any demonstrable objective symptoms of illness. 3. A diagnosis of the experimental encephalomyelitis should therefore be based on the results of histological examination of the nervous system as well as on typical symptomatology. The question is discussed whether reactors might not have been overlooked among animals of other species hitherto employed, when the basis for the diagnosis of the encephalomyelitis was only the presence of outward signs of illness.

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Role of the Erythrocyte in Inhibition by Allantoic Fluid of Mumps Virus Hemagglutination. (18170)

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It is known that virus hemagglutination is the result of a reaction between the virus and the red blood cell, and that this reaction may be specifically inhibited by sera containing antibodies for the virus. A variety of nonspecific substances may also interefere with this reaction(1). A substance of this sort present in allantoic fluid has been studied most intensively for its effect upon the agglutination of red blood cells by influenza viruses(2,3). From these investigations, it appears that normal allantoic fluid (NAF) contains an inhibitor which reacts with and is inactivated by these viruses. A filtrate of *Cl. welchii* and an enzyme from *Vibrio cholera* similarly inactivate this inhibitor. It has been postulated that a coenzyme exists for the hemagglutinating virus-inhibitor system. However, it has not been clear whether this hypothetical substance comes from the virus or from the red blood cell(2).

In the course of comparative studies on agglutination of chicken and human erythrocytes by a strain of mumps virus (Enders), observations were made which seem to furnish information regarding this question. These observations are the subject of the present communication.

Materials and methods. Viruses (a) *Mumps.* The Enders strain was used. For passage 0.1 ml of a 10^{-1} dilution of virus in 10% rabbit serum-saline was inoculated into the allantoic sac of 7-day-old chick embryos. These embryos were incubated further at 37°C for 4 days, chilled and the allantoic fluid collected. (b) *Influenza.* The PR-8 and Lee strains were used. For passage 0.1 ml of a 10^{-3} dilution of virus in 10% rabbit serum-saline was inoculated into the allantoic sac of 10- to 11-day-old chick embryos. The allantoic fluid was collected after 2 days at 37°C and a period of chilling.

Normal allantoic fluid (NAF) was obtained from chilled 11-day-old embryos. All the fluids were stored after collection in lusteroid tubes at approximately −72°C.

Chicken red blood cells were obtained by bleeding chickens from the wing vein and allowing the blood to flow into an excess of 2% sodium citrate or sodium oxalate.

Human group O cells were obtained from the Hospital Blood Bank where they had been collected in ACD (acid citrate dextrose) solution. The cells were washed in normal saline 3 times and used as 1% or 25% suspensions as indicated. In some experiments other anticoagulants were used without modifying the results. Cells were not used if

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