at substantially similar caloric levels, and that the factor of inanition was therefore not a major one in lessening the tumor incidence in the *Lithospermum*-fed mice.

A peculiarity in our data is the fact that only 38% of the controls developed tumors, whereas the strain of C_3H mice used by us has been reported as having as high as a 97% incidence of spontaneous mammary tumors(6). Our low control incidence is probably explicable on the grounds that keeping 5-10 mice in a cage constituted crowding and competitive feeding, factors which have been shown repeatedly to lower the incidence of tumors in this strain(7). On the other hand, these factors would not seem to have vitiated the experiment, for crowding was identical in both groups. The relative rather than the absolute tumor incidence in the control and *Lithospermum*-fed groups would seem to be the figures of significance.

As to the comparative death rates in the two groups, Cranston, *et al.* unfortunately do not give survival curves for their control and *Lithospermum*-fed mice, and hence their statement that the average non-tumor death age was 483 days for both groups cannot be fully appraised. Our own finding of an earlier susceptibility of non-tumor death in the *Lithospermum*-fed mice than in the controls, as plotted in Fig. 4, is a disturbing one and suggests the need for caution in interpreting the fact that the *Lithospermum*-fed group eventually developed only about half as many tumors as the controls. Whether this decrease in tumor incidence is directly related to the *Lithospermum*-fed mice being in a state of more-or-less sustained diestrus, we are not, on the basis of the present data, prepared to say.

As pointed out by the Cranston group, experiments of this sort could lead to more decisive results if pure extracts of the *Lithospermum* anti-estrous factor were available, thus minimizing possible side reactions of unknown identity.

Summary. About 400 6-month-old virgin female C_3H mice were divided into two groups: one was maintained on a normal diet; the other on an anti-estrous diet of 10-15% *Lithospermum*. Eighteen months later the latter group had developed half as many spontaneous mammary tumors as the former; however, if the differential mortality observed throughout the experiment is taken into statistical account, the tumor incidence ratio for the total period is 3 for the control, 2 for the experimental group. This reduction in tumor incidence was possibly connected with the *Lithospermum*-diestrus, but inanition and differential survival factors remain to be more fully evaluated.

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Neuropathology of Teschen Disease (Virus Encephalomyelitis of Swine).* (18660)

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(Introduced by Francis G. Blake)

From the 98th General Hospital, European Command, U. S. Army in Germany. Work done under auspices of Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, and the Section of Preventive Medicine, Yale University School of Medicine.

Teschen disease of swine is a virus encephalomyelitis which although its occurrence

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is unknown on the American continent, has been enzootic and epizootic in eastern Europe for at least 20 years. The disease is one which to date has been seen only in swine, and aside from the wild boar, no other susceptible host is known. It has been studied chiefly in Czechoslovakia where it has been most prevalent(1), and in Germany(2). Its virus etiology was demonstrated by Klobouk, who did the first and much of the subsequent experimental work with the disease(1,3). An extensive pathological study has been reported by Dobberstein(4), who mentioned the similarity between Teschen disease and poliomyelitis. Others(1,5,6) have also laid stress on this supposed similarity, and it is our purpose to consider the validity of the resemblance. In particular, we have attempted to determine whether the term poliomyelitis-like can be accurately applied to Teschen disease, and in this we have been guided by a recently published definition of poliomyelitis virus in which the differentiation is based in some measure on the distribution of lesions in the CNS(7). In the present study therefore, our interest was focussed on the type and distribution of CNS lesions in experimental Teschen disease, as compared with poliomyelitis and certain other neurotropic virus diseases.‡ At a later date a more detailed report including a comparison of the pattern of distribution of these lesions after various routes of inoculation, will be published.

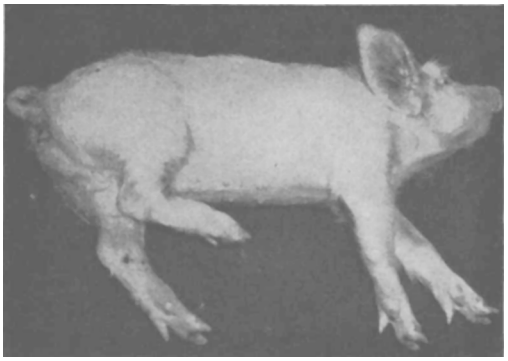

FIG. 1.
A pig showing the spasticity characteristic of the early stages of the disease. The animal was having almost continual convulsions.

The material studied consisted of brains and cords of 6 young pigs (*aet* 4-6 weeks) infected intracerebrally with 10% suspensions of several strains of virus.§ After an average incubation period of 6 days, the animals all showed the typical clinical course for this infection in severe form. The onset of the experimental disease was heralded by fever, followed, in order, by stiffness of the extremities (Fig. 1), ataxia, tremors, spastic convulsions, nystagmus, cranial nerve palsies, weakness or paralysis of the extremities and in the majority of cases, death. Not every animal showed all of these signs, and in the most severe cases coma, subnormal temperature and death supervened before the paralytic phase was clinically apparent. The duration of illness from onset to death or sacrifice thus varied between 24 hours and 4 days. At autopsy, the brain and cord were fixed in formalin, cut serially, and stained

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‡ We are greatly indebted to Professor W. Scholz, Director of the German Research Institute for Psychiatry, Munich, and Consultant in Neuropathology to the 98th General Hospital, for his continuous help and advice in this study.

§ We are indebted to the late Dr. Frantisek Gallia, National Institute of Health, Prague, for several strains of virus, and to Dr. Sven Gard, State Bacteriological Institute, Stockholm, and Dr. Pierre Lépine, Pasteur Institute, Paris, for kindly supplying us with their strains. The experimental work was all carried out in Germany because for obvious reasons it is not wise to import this virus into the United States.

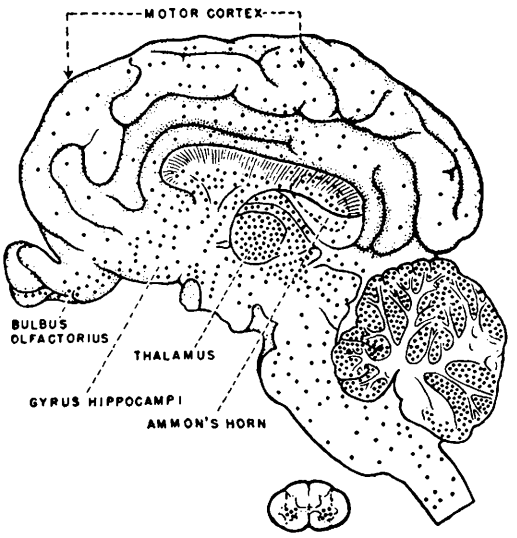

FIG. 2.
Diagram illustrating the distribution of lesions in the pig brain and medulla following intracerebral inoculation. (Projection of the lesions on the median surface of the brain).

with Nissl, or gallocyanin stains as indicated. Zenker fixed material was stained by the Giemsa method in a search for inclusion bodies.

On gross examination of the brain and cord, mild hyperemia was commonly noted, but edema was minimal even in the most severe cases. No abnormalities other than some congestion of the viscera were apparent in other organs. Histologically, the lesions were found to be virtually limited to the grey matter of the CNS. Perivascular cuffing and discrete nodules surrounding damaged neurons and blood vessels were characteristic. In some animals, rare perivascular cuffs were found in the white matter, and there was also occasional involvement of the undifferentiated nerve cells which are normally found in the matrix around the ventricles in very young animals such as those we used.

One of the most striking features of the pathological picture was the widespread distribution of cerebral and cerebellar lesions (Fig. 2). There was no part of the brain which was not at one time or another involved in the animals studied. However, the usual pattern was one in which the cerebellum was most heavily infiltrated, the Purkinje layer most severely, next the molecular, and least the granular layer (Fig. 3). At times there was disappearance of almost all the Purkinje cells over large areas. Next in density of lesions was the thalamus, then the basal nuclei (especially the striatum) and in general, the base of the brain. The olfactory apparatus which forms a relatively large part of the pig brain was severely involved: the olfactory bulbs, hippocampal gyrus, and Ammon's horn, —the entire rhinencephalon. The pons and the nuclei of the medulla showed usually a less severe reaction.

The motor cortex, which in the pig runs parallel to the median fissure and extends from the middle of the frontal lobe to the parietal lobe, was slightly more affected than the rest of the cortex, but the number of lesions here, although considerable, was far less than was found in the cerebellum or thalamus. It was characteristic for the massive involvement to be confined to the base and central parts of the brain, with a progressive diminution in the extent of lesions laterally and toward the periphery. The occipital lobes were almost spared.

In the spinal cord, the lesions were almost entirely in the anterior horns, with occasionally a few in the lateral and posterior horns (Fig. 4). In this respect the lesions resemble those of poliomyelitis and could be easily mistaken for them if examination of the brain were omitted. In Teschen disease, all levels of the cord tended to be equally affected. Two additional pigs killed 24 hours after onset of encephalitic signs, showed no cord lesions at any level, although the brain lesions were typical. Another animal, however, which had an unusually mild course clinically, showed very few lesions in the brain, but typical ones in the cord.

The meningeal reaction was usually mild, but occasionally more severe, and always most marked over the cerebellum. Lymphocytes and plasma cells composed the inflammatory exudate; polymorphonuclear leukocytes—as elsewhere—were strikingly rare. The choroid plexuses were similarly, but rarely affected.

Histologically, the primary attack of the virus appeared to be on the neurons. Although perivascular cuffing,—with lympho-

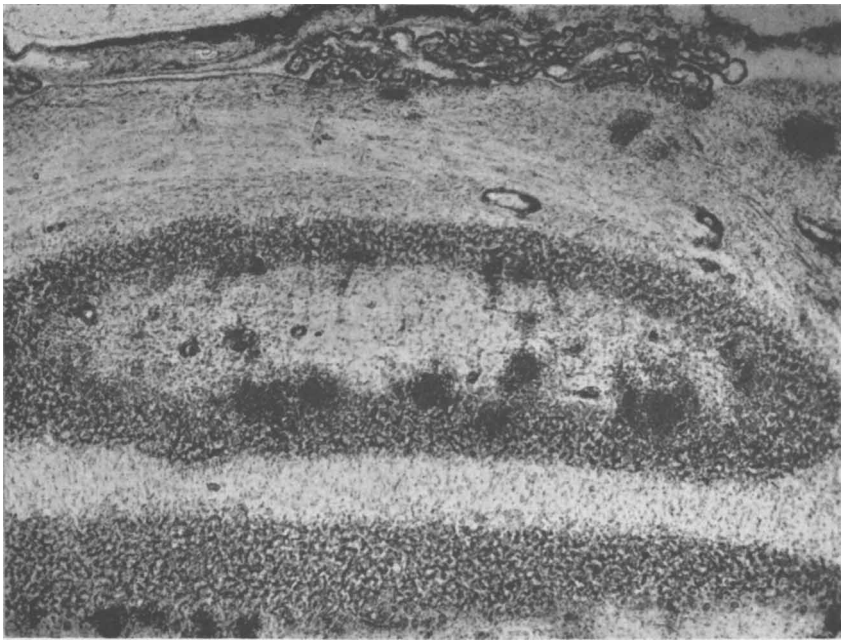

FIG. 3.
A section of the cerebellum underlying the choroid plexus of the 4th ventricle. Numerous nodules in the Purkinje layer are seen, and there is moderate perivascular cuffing. ×60.

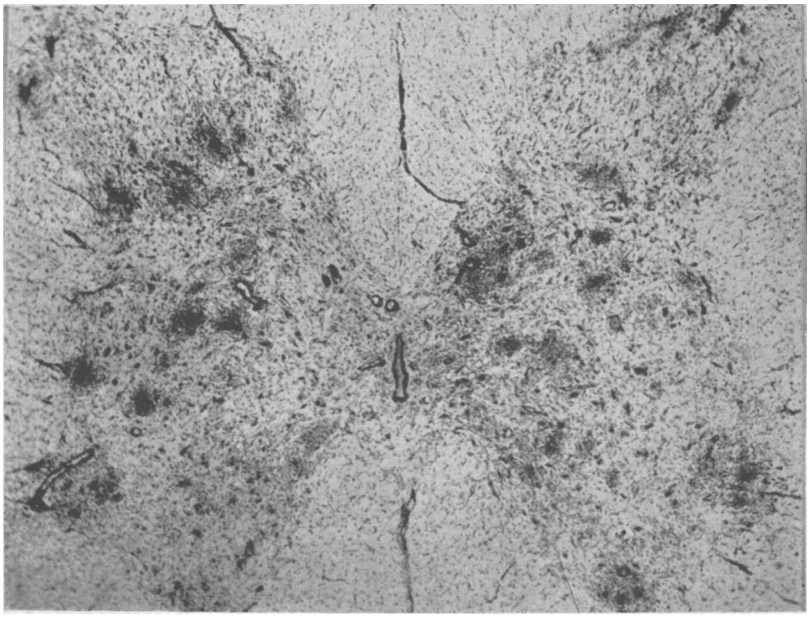

FIG. 4.
Section of the central area of the spinal cord, showing multiple neuronophagic nodules and perivascular cuffing. ×16.

cytes, polyblasts, and plasma cells—was characteristic, there was no evidence of necrosis of blood vessels. The damaged neurons showed first,—decrease in size of the Nissl

bodies, then chromatolysis and disappearance of the Nissl substance, which often began at the periphery of the cell. Peripheral vacuolation sometimes accompanied this. Chromatolysis may progress to the point of complete dissolution of the Nissl substance so that the whole cell takes on a foggy reddish blue color with Nissl stain. Nuclear changes proceeded from pyknotic alterations in the chromatin, to karyorrhexis, karyolysis, and disappearance of the nuclear membrane, leaving a distorted and shriveled nucleus in a homogenized cytoplasm. Phagocytosis of dead cells seemed to be carried out chiefly by progressive forms of microglia; lymphocytes and occasional plasma cells were seen, but polymorphonuclear leukocytes were extremely rare in areas where phagocytosis was going on. Nerve cells may also undergo cytolysis, or vacuolation and absorption without neuronophagic reaction. Using Giesma stained sections, a thorough search for inclusion bodies was made, but none was found at any stage of neuronal damage.

The characteristic lesion in the brain and anterior horns of the cord was a collection of cells forming a nodule, which may either be found around a blood vessel, or around a damaged neuron. Nodules of varying size were composed of gliomesenchymal elements: micro and macroglia, lymphocytes, polyblasts and plasma cells. Here too, polymorphonuclear leukocytes were conspicuously absent.

In comparing the pathology of Teschen disease with other virus encephalomyelitides, it seems, Japanese B, and louping ill came particularly to mind for consideration. Although a similarity to poliomyelitis has been previously stressed, actually we were much struck by the differences, the most obvious being the widespread distribution of lesions in the brain in Teschen disease, in contrast to the highly selective distribution of lesions in the brain in poliomyelitis(8). It is true that certain areas of the brain and cord are severely affected in both diseases, but the pattern in Teschen—the extensive involvement of the cerebellum and basal nuclei, and the numerous lesions in all parts of the cortex—is completely unlike poliomyelitis in man or in the monkey. The similarity between the two diseases pathologically must rest on the spinal cord lesions. There is also some difference in the cellular reaction in that in Teschen disease, polymorphonuclear leukocytes are very rarely seen, while in acute lesions of poliomyelitis, they often form a part of the inflammatory reaction. But in an individual slide of a spinal cord it might be difficult to distinguish between the two. The lesions in Teschen disease in the pig brain and cord resemble strikingly those found in Japanese B encephalitis in man(9) both as to type and distribution. In fact, pathologically, Teschen disease is far more like Japanese B than like poliomyelitis. Widespread neuronophagic nodules and nodules associated with blood vessels are characteristic of both, but in Japanese B, the distribution of nodules tends to be more uniform throughout the brain, and the entire cerebral cortex is more densely involved, especially at the periphery, which is relatively spared in Teschen disease. There is also a more striking leptomeningitis in Japanese B, and although polymorphonuclear leukocytes are not prominent, they are not so rare as in Teschen disease. There are also close similarities between the pathological picture of Teschen disease in the pig and of louping ill(10), particularly experimental infections in the monkey(11), and in the pig(10,12).¶ The character and distribution of lesions in the cerebellum may be almost identical, although there tends to be a more pronounced cellular reaction in Teschen disease than in louping ill. The cortical and midbrain lesions in Teschen disease are more constant than in louping ill in the monkey,

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¶ We are grateful to Dr. J. Russell Greig, and Dr. D. R. Wilson of the Moredun Institute, Edinburgh, for making available to us sections and blocks of tissue from pigs which they infected with louping ill virus in 1930.

where, for instance the thalamus may be completely spared and the cortical lesions very scant(11).

Summary. A study of the neuropathology of Teschen disease reveals it to be a diffuse encephalomyelitis with characteristic lesions both as to type and distribution. The lesions are, with minor exceptions, confined to the grey matter of the brain and cord. Their widespread distribution in the CNS, and particularly their striking density in the cerebellum serve to distinguish Teschen disease from poliomyelitis, although spinal cord lesions may be similar in the 2 diseases.

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Methylation of Homocystine by Chicks Deficient in Vitamin B_{12}.* (18661)

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Gillis and Norris(1,2) and Schaefer, Salmon and Strength(3) have shown that chicks partially depleted of their reserves of vit B_{12} have an increased requirement for compounds which furnish labile methyl groups. Jukes, Stokstad and Broquist(4) have presented evidence which suggests that chicks deficient in vit B_{12} are unable to utilize homocystine plus betaine for the synthesis of methionine. Oginsky(5) concluded that the livers from vit B_{12}-deficient rats exhibit a lower ability to form methionine from homocystine and either choline or betaine as compared with livers from animals dosed with vit B_{12}. The work of Jukes *et al.*(4) and Oginsky(5) appeared to be in disagreement with the unpublished results of an earlier experiment conducted at this laboratory which showed that the methylation of homocystine by means of choline or betaine was not hampered by a deficiency of vit B_{12}. In view of this discrepancy the original study has been repeated and extended. The results of both experiments are presented in this report.

Experimental. The chicks used in this investigation were hatched from eggs laid by White Leghorn hens fed a diet containing no animal products or vit B_{12} and housed in pens with wire-mesh floors. The hatchability of the eggs was low, and the chicks were found in a preliminary experiment to be markedly responsive to vit B_{12} administration. The chicks were fed a vit B_{12} deficient diet containing 25% vegetable protein for a pre-experimental period of 10 days. They were then uniformly distributed on the basis of weight among the experimental lots. Thereafter they were weighed daily at the same hour. The basal diet furnished approximately 0.2% methionine and was deficient in vit B_{12} and choline. In Exp. 1 it contained corn starch 65.55; isolated soybean protein 20; gelatin 4; L-cystine 0.15; soybean oil 2; ground cellophane 3; dicalcium phosphate 2; calcium carbonate 1; sodium chloride 0.5; dibasic potassium phosphate 0.5; magnesium sulfate 0.25; trace mineral mixture 0.05; and vitamin-starch mixture 1%. In Exp. 2 the basal diet was the same except that it contained 22% water extracted, isolated soybean protein and no gelatin, and hydrogenated vegetable fat was used in place of soybean oil. In Exp. 1 each lot contained 6 chicks and the experimental treatment lasted 5 days. In Exp. 2 each lot contained 5 chicks and the experimental period was of 9 days' duration.

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† Research Associate.

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