cytopathic changes *in vitro* characterized by presence of intranuclear inclusions have been isolated from 3 infants during life. The first derived from a liver biopsy from a 3-months-old child (Davis) with microcephaly, persistent jaundice, and hepatosplenomegaly; cytomegalic cells were demonstrated in the liver specimen. From a second child, virus was isolated from a liver biopsy, and on three occasions between the 14th and 91st day of life from the urine. This infant had jaundice and hepatosplenomegaly. More recently, an agent was recovered from the urine of a third infant, who evidenced hepatosplenomegaly, cerebral calcification and chorioretinitis. The Davis strain has been propagated in human fibroblasts for 20 passages during an elapsed period of 494 days. Neutralization tests with the Davis agent have indicated that neutralizing antibodies occur frequently in children with cytomegalic disease but also are not uncommon in normal adults. The agents are apparently related to those recovered in other laboratories from a human salivary gland, from a fatal case of cytomegalic disease, and from spontaneously degenerating tissue cultures of human pharyngeal tonsillar tissue.

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Anticonvulsant Properties of *l*-Glutamine and *l*-Asparagine in Mice and Rats.* (22842)

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Because of the considerable experimental and clinical interest in *l*-glutamine and *l*-asparagine as potential therapeutic agents in epilepsy, based largely on the laboratory and clinical studies of Tower(1), it was thought important to screen these agents for anticonvulsant activity in mice and rats by a battery of laboratory tests. The results obtained provide the basis for this report.

Methods. Adult male mice (CF #1 strain) from the Carworth farm and adult male albino rats from the Sprague-Dawley farm were used as experimental animals. They were maintained on Purina Laboratory Chow and, except for the experiments in rats, were allowed free access to food and water until removed from their cages for testing. The *l*-glutamine and *l*-asparagine were administered either orally or intraperitoneally in aqueous solution or suspended in 10% acacia mucilage. Both compounds were tested *in mice* after *acute* administration by a battery of six anticonvulsant assay procedures: maximal electroshock seizure pattern (MES); maximal Metrazol seizure pattern (MMS); minimal electroshock seizure threshold (MET);

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hyponatremic electroshock seizure threshold (HET); "psychomotor" seizure threshold (PsM); and Metrazol seizure threshold (Met.). Except for the fact that a Grass Stimulator (Model S4B) is now used for the PsM test, the details of these tests and the special features of the electroshock apparatus have been described in detail elsewhere (2,3, 4,5,6). Both agents were tested *in mice* after *chronic* administration by the MET and HET tests and for ability to elevate the MET lowered by cortisone. In addition, they were tested *in rats* for ability to elevate the MET lowered by food restriction. The details of these procedures have been described elsewhere(3,6,7,8). In threshold studies, the minimum current necessary to elicit facial, lower jaw and/or forelimb clonus without loss of upright posture in 50% of mice (CC_{50}) was calculated for each group by the method of Litchfield and Wilcoxon(9). Finally, *l*-glutamine and *l*-asparagine were tested for ability to prevent methionine sulfoximine-induced seizures by a method similar to that described by Tower(1).

Results. In *acute experiments,* single oral doses up to 3000 mg/kg of either *l*-glutamine or *l*-asparagine were non-toxic and were ineffective by the four electroshock assay procedures: MES, MET, HET, and PsM tests. Although 3000 mg/kg of *l*-glutamine induced 10% and 20% protection by the Met. and MMS tests, respectively, a dose of 6000 mg/kg of either *l*-glutamine or *l*-asparagine was virtually devoid of anti-Metrazol activity.

To reveal more subtle anticonvulsant effects, *l*-glutamine and *l*-asparagine were tested for *any* ability to elevate the MET, HET, and the PsM threshold. The data shown in Table I indicate that, in the dose employed, these agents are incapable of elevating electroshock seizure threshold.

Sixty rats with a mean weight of 100 g were divided randomly into 3 groups, one control group (A) and 2 experimental groups (B and C). Group C was used as the experimental control. The initial minimal electroshock seizure threshold (CC_{50}) for groups A, B, and C were 15.2 (14.4-16.5), 16.2 (15.0-17.4), and 15.5 (14.0-17.1) mA, respectively.

TABLE I. Effect of *l*-Glutamine and *l*-Asparagine on Electroshock Seizure Threshold.

Threshold test	CC_{50}*		
	Control	*l*-glutamine†	*l*-asparagine†
MET	4.5 (4.3– 4.8)	4.4 (4.0– 4.8)	4.6 (4.2– 5.0)
HET	2.4 (2.2– 2.7)	2.5 (2.2– 2.9)	2.7 (2.4– 3.1)
PsM	14.4 (13.5–15.4)	16.3 (15.1–17.6)	15.4 (14.4–16.5)

* Expressed in mA for MET and HET; in volts for PsM.
† 3000 mg/kg orally.

The control group was allowed food *ad lib.,* whereas the food intake of the 2 experimental groups was limited to 6 g/animal/day for the first 27 days and then 8 g/animal/day for the remainder of the experiment; all groups were allowed water *ad lib.* After 63 days, the mean weights were 314, 130, and 143 g for groups A, B, and C, respectively. The mean CC_{50}s were 22.8 (21.8-23.9), 15.1 (14.0-16.6), and 13.8 (12.6-15.1) mA for groups A, B, and C, respectively. Neither acute nor chronic administration of 1000 mg/kg of *l*-glutamine or *l*-asparagine had any significant effect on the CC_{50} for group A or group B. In contrast, phenobarbital (20 mg/kg, i.p.) significantly increased the CC_{50} of both groups.

The LD_{50} and 95% confidence limits of methionine sulfoximine (i.p. in aqueous solution) were determined to be 218 (194-244) mg/kg. In order to produce convulsions in nearly all animals, 30 mice were injected intraperitoneally with the LD_{97} (350 mg/kg) and at the previously determined time of seizure onset (4 hours after methionine sulfoximine) 10 mice were injected i.p. with 10 mM/kg of *l*-glutamine and 10 with a similar dose of *l*-asparagine; the remaining 10 mice were injected with the requisite volume of saline and served as controls. All animals (test and control) exhibited seizures and, except for the survival of one *l*-asparagine-treated animal, all died within 48 hours. In another experiment, pretreatment with 10 mM/kg of *l*-glutamine or *l*-asparagine 30 minutes *prior* to the methionine sulfoximine injection and again at the anticipated time of seizure onset failed to alter seizure incidence

or to increase the survival rate. Finally, neither *l*-glutamine nor *l*-asparagine had any significant effect on the seizure incidence or the LD_{50} for methionine sulfoximine.

In *chronic experiments*, the 2 agents were given orally in a dose of 500 mg/kg twice daily and tested for ability to antagonize the electro-shock-threshold-lowering effects of cortisone and hyponatremia. In the *cortisone* experiments, the minimal electroshock seizure threshold (CC_{50}) of 120 mice was determined and the animals were randomly divided into 4 groups. Two groups served as controls, one to show the response of normal animals and the other the response to cortisone (0.5 mg/animal/day s.c.). The remaining 2 groups were also given cortisone, but one group was given intraperitoneally 250 mg/kg of *l*-glutamine twice daily and the other the same dose of *l*-asparagine. The CC_{50} was determined for each group after 2, 5, and 8 days of drug treatment. Cortisone significantly reduced the CC_{50} of both the treated and the control groups. Thus, at the end of the experiment, the CC_{50} in normal, cortisone-treated, *l*-glutamine-cortisone-treated, and *l*-asparagine-cortisone-treated mice were 7.7 (7.1-8.4), 5.6 (4.8-6.5), 4.8 (3.6-6.3), and 3.8 (2.7-5.3) mA, respectively. The chronic administration of *l*-glutamine and *l*-asparagine, therefore, does not antagonize the threshold-lowering effects of cortisone. In the *hyponatremia* experiments, the experimental design was identical to that employed for cortisone except that the amides were given for 12 days. The CC_{50}s in normal, hyponatremic, *l*-glutamine-hyponatremic, and *l*-asparagine-hyponatremic were 7.0 (5.6-8.7), 5.0 (3.7-6.7), 4.6 (3.8-5.5), and 4.9 (4.2-5.7) mA, respectively. Thus, *l*-glutamine and *l*-asparagine had no significant effect on minimal electroshock seizure threshold lowered by hyponatremia.

It is concluded that *l*-glutamine and *l*-asparagine, administered acutely or chronically in the doses indicated, are devoid of significant anticonvulsant activity as measured by a variety of procedures in mice and rats.

Discussion. Tabachnick and coworkers‡ reported that *l*-glutamine and *l*-asparagine exhibited "little or no anticonvulsant effect" as measured by the MES and Met. tests; Ginsberg(10) observed an increase in audiogenic seizure incidence in mice; and Woodbury† found that *l*-glutamine failed to prevent carbon dioxide-induced withdrawal seizures, although the dose of *l*-glutamine employed restored the brain glutamic acid level, previously lowered by carbon dioxide, to normal. Such observations and the data presented herein contrast sharply with the report by Tower(1) that these agents abolish methionine sulfoximine-induced seizures in an "unknown" strain of mice.

All clinically effective antiepileptic drugs can be shown to have anti-convulsant activity by one or more of the tests employed in this study. In addition, many metabolic, endocrine, and physiological factors known clinically to exert a restraining or precipitating effect on seizure susceptibility have been shown in our laboratory to influence brain excitability as measured by these same tests. For example, such clinically important factors as pH changes, ketosis, plasma pCO_2, starvation, pyridoxine, adrenocorticosteroids, water, and electrolytes—known to influence the occurrence of various types of seizures—have been shown by us to exert predictable changes in seizure susceptibility in laboratory animals by procedures similar to those used to study *l*-glutamine and *l*-asparagine. Hence, if controlled clinical studies eventually indicate any efficacy of these amides in the treatment of epilepsy, the mechanism of their action must be considerably different from that of known anticonvulsant drugs, metabolic factors, hormones, or nutrilites.

Summary. Two amides, *l*-glutamine and *l*-asparagine, were tested for anti-convulsant activity in mice after *acute* administration by a battery of 6 anticonvulsant assay procedures and after *chronic* administration by 3 tests. In addition, both agents were tested in mice for ability to prevent methionine sulfoximine-induced seizures and in rats for ability to elevate the minimal electroshock seizure threshold lowered by food restriction. The results indicate that both these agents are vir-

‡ Reported in *Fed. Proc.*, 1956, 490.

† Unpublished observations.

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D_2O Equilibration Rates in Thermally Injured Animals.* (22843)

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There have been numerous attempts to make *in vivo* dissections of the human body to understand better the phenomena which occur in both normal and pathologic states. Isotopes have facilitated this work; but no isotope is ideal since each has its own peculiar advantages and disadvantages. Most investigators consider deuterium oxide the isotope of choice in measuring total body water. The principle of isotope dilution is well known and accepted; and it is dependent upon knowing the amount of substance injected, amount of substance excreted prior to equilibrium, and concentration of this substance in the body at equilibrium. In the majority of cases, equilibrium of D_2O in the normal state will be attained in 1 to 2 hours. Some investigators have allowed 3 hours for D_2O to equilibrate in certain pathologic states; thus they imply some delay in equilibration(6). Others insist that time to equilibrium is even longer(5). This is particularly true with ascites.

It is most important to determine total body water in any study of profound alterations in fluids and electrolytes following thermal injury. Determinations of total body water are also used with determinations of extracellular fluid space, to measure intracellular water compartment. It has been questioned as to whether or not D_2O would equilibrate within a 2- or 3-hour period in subjects when there were large edematous areas as a result of thermal injury. For this reason, equilibration studies were made on thermally-traumatized animals to determine whether or not the time to equilibrium was prolonged. Each animal acted as its own control.

Materials and methods. Sixteen adult mongrel dogs were used, average weight 16.8 kg, ranging from 12.7 to 23.0 kg. Each animal was weighed 3 times a week until its weight had stabilized, usually in 4 to 6 weeks. Food was withheld from animals for 12 hours prior to experiment; however, all animals had free access to water until 4 hours prior to experiment. Dogs were anesthetized with intravenous phenobarbital anesthesia (30 mg/kg). Femoral vessels were exposed through small incision in the groin and both femoral artery and femoral vein were cannulated with polyethylene tubing. Sterile D_2O (purity greater than 99.5%) as isotonic saline was obtained in 50 g ampules (Abbott). Twenty cc of D_2O-saline solution were injected into femoral vein through a calibrated syringe. The polyethylene tubing was rinsed repeatedly with

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