Summary. In toads (*Bufo bufo (L.)*) with deficient pars distalis function due to extirpation of median eminence, subcutaneous injection of synthetic lysine-vasopressin causes shedding of the hyperkeratinized stratum corneum. In hypophysectomized toads lysine-vasopressin is without effect. Lysine-vasopressin is thus able to stimulate pars distalis activity in toads, indicating a chemical relationship between pars nervosa and median eminence (adenohypophysiotropic) hormones.

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Histopathology of Lymphocytic Choriomeningitis in Mice Spared by Amethopterin. (24055)

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Amethopterin, a folic acid antagonist, spares the lives of mice infected with normally fatal lymphocytic choriomeningitis (LCM), but does not prevent virus replication in spared mice. Folic acid-deficient diet produces a similar effect(1,2). These alterations resemble those obtained earlier with whole body X-irradiation; in mice spared by this treatment, decreased tissue reactivity or diminished inflammatory cellular exudate were suggested as the explanation for survival (3). The present report indicates that mice spared by amethopterin tend to develop inflammatory reaction more slowly than untreated mice, most of them showing great modification of the natural disease process, but that a significant number survive even though extensive inflammatory reaction is present.

Materials and methods. The LCM virus used was a murine strain maintained through more than 400 serial intracerebral (i.c.) passages(4). Preparations were mouse brain emulsions (ground by hand in a mortar and uncentrifuged) made to 10% suspensions and decimally diluted in 0.85% buffered saline. The i.c. dose was 0.03 ml for all animals. Mice were N.I.H. Swiss "general purpose," of both sexes, weighing 16-18 g when inoculated. They were fed a stock diet consisting of whole

TABLE I. Incidence of Lesions and Virus Recovery in Untreated Mice Given LCM Virus Intracerebrally.

No. mice	Log dilution virus given	Days post inoc.	Lesions in: Central nervous system	Lymph nodes & spleen	Viscera	Log highest dilution virus recovered*
1	-5	5	+	+	—	-5
4	-5	6	+	+	+	-7
	-4	6	+	+	+	-6
	-5	6	+	±	—	-7
	"	6	+	+	—	-7
3	-4	7	+	+	—	-5
	-5	7	+	+	—	-7
	"	7	+	+	±	-6
Total 8			8 (100%)	7	2	

+, typical of acute LCM. ±, modified or equivocal. —, minimal or negative.

* Highest dilution which killed 3 or more out of 5 mice inj. intracer. Titrations as performed did not establish end-points.

wheat bread (fortified with thiamine, riboflavin, niacin, and iron) soaked in powdered whole milk reconstituted with tap water. Amethopterin, 0.06 mg in 0.2 ml of 2% $NaHCO_3$, was given intraperitoneally on days 3, 5, 7, and 9; virus having been given on day 0. No mouse received amethopterin on the day it was killed; *e.g.*, an animal killed on day 7 received amethopterin on days 3 and 5 only. All studies were on killed mice. The brain of each was divided sagitally *in situ*, and half removed for virus titration, the remainder being left in the cranium for fixation, along with the body of the mouse, in 10% buffered formalin. (The cerebral hemisphere which had been injected with virus was the one left for study.) Brain, meninges, choroid plexus, spinal cord, all viscera, lymph nodes, and bone marrow were stained with buffered azure-eosin and examined histologically. Virus titrations employed 5 mice for each log dilution.

Results. 1. *Untreated Mice.* Eight untreated mice killed from 5 to 7 days after i.c. injection of virus (2 with 10^{-4} dilution; 6 with 10^{-5}) showed typical lesions of severe, acute lymphocytic choriomeningitis in the central nervous system. Seven of these mice had changes in lymph nodes and spleen, and 2 in viscera. In 7 mice, virus titrations failed to reach end-points at 10^{-6}; in one, titration was carried only to 10^{-5}, which killed all mice. Results are summarized in Table I. No untreated mice survived beyond 7 days. Lesions in mice after intracerebral inoculation of LCM virus have been described by Lillie and Armstrong (5,6), and the changes observed are noted here only briefly, for purposes of comparison with those in treated animals. Infiltration by large numbers of lymphocytes accompanied by a few macrophages and very few polymorphonuclear leukocytes was intense in the meninges over the superior surface and base of the brain and frequently in the sheaths of penetrating submeningeal blood-vessels, and to a lesser degree in the spinal meninges. The choroid plexus in all locations contained varying amounts of lymphocytic exudate, as did the ependymal lining of the ventricles. Lesions in lymph nodes, spleen, and viscera were less consistent in frequency and severity. Involved lymph nodes, most frequently those of the cervical group, showed hyperplastic follicles, numerous phagocytic cells, considerable reticulo-endothelial hyperplasia which occasionally was so extensive as to obliterate follicular architecture, and occasionally perinodal infiltration by lymphocytes. The spleen when involved showed changes similar to those in lymph nodes, as well as occasional congestion of the pulp, or hemorrhage with necrosis and destruction of follicles. Other viscera much less frequently showed focal and interstitial lymphocytic infiltration; of these, the liver and kidney were most often involved. This was in accord with the previous observations of considerable variability in tissues and

TABLE II. Incidence of Lesions and Virus Recovery in Amethopterin-Treated Mice Given LCM Virus Intracerebrally. Acute stage.

No. mice	Log dilution virus given	No. doses drug given	Days post inoc.	Lesions in: Central nervous system	Lymph nodes & spleen	Viscera	Log highest dilution virus recovered*
1	−5	1	5	—	—	—	−5
4	−5	2	6	—	—	—	−6.5
	−4	2	6	—	—	—	−6
	−5	2	6	—	±	—	−5
	−5	2	6	—	—	—	−6
4	−5	2	7	—	+	—	−6.5
	−4	2	7	—	—	—	−7
	−5	2	7	—	+	±	−7
	−5	2	7	—	±	±	−7
3	−4	3	8	—	±	±	−6
	−5	3	8	+	+	—	−7
	−5	3	8	+	+	—	−7
2	−5	3	9	±	±	—	−7
	−5	3	9	—	+	—	−6
3	−4	4	10	+	±	—	−7
	−5	4	10	—	—	—	−7
	−5	4	10	—	±	±	−7
2	−5	4	13	—	—	±	−7
	−5	4	13	+	—	±	−7
1	−4	4	14	—	+	±	−7
1	−5	4	17	—	+	±	−6
1	−4	4	20	—	—	+	−7
Total 22				4 (18%)	7	1	

+, typical of acute LCM. ±, modified or equivocal. —, minimal or negative.

* Highest dilution which killed 3 or more out of 5 mice inj. intracer. Titrations as performed did not establish end-points.

organs involved from mouse to mouse.

2. *Treated mice: acute stage.* Twenty-two mice were sacrificed from 5 to 20 days after LCM virus injection (6 given 10^{-4} dilution; 16 given 10^{-5}). Only 4 mice showed intense lymphocytic infiltration of the meninges and choroid similar to that seen in the untreated control animals, and were considered to have severe active lymphocytic choriomeningitis. The changes in lymph nodes, spleen, and viscera in these 4 mice were less consistent and less frequent than those observed in the control animals (Table II). The remaining 18 animals showed extraordinary modification of the natural disease. Of this group, some were completely devoid of histological evidence of lymphocytic choriomeningitis. Others showed changes varying from extremely slight traces of LCM to greatly modified disease suggesting healed or healing LCM. These changes were manifested by minimal infiltrations by a very few lymphocytes to slight amounts of lymphocytic infiltration of meninges or of choroid, and similar slight infiltrates in the linings of the lateral, 3rd, or 4th ventricles. Lesions in the lymph nodes, spleen, and viscera were infrequent, generally less severe than in untreated animals, and were not consistently associated with the presence of modified central nervous system lesions when the latter occurred.

Since the central nervous system was the only consistent site of characteristic pathological changes in control animals, and since the virus had been introduced by the intracerebral route, the presence or absence of lesions in this location was considered the most reliable index of active, modified, or absent cellular response.

In 20 of these mice, virus titrations at 10^{-6} failed to reach end-points; in the other 2, tests carried out to 10^{-5} killed all mice. This

TABLE III. Incidence of Lesions and Virus Recovery in Amethopterin-Treated Mice Given LCM Virus Intracerebrally. Chronic stage.

	No. mice	Log dilution virus given	No. doses drug given	Days post inoc.	Lesions in: Central nervous system	Lymph nodes & spleen	Viscera	Log highest dilution virus recovered*
	2	-5	4	21	+	+	—	-7
		-5	4	21	+	—	—	-6
	1	-4	4	26	+	+	+	-5
	2	-5	4	27	+	±	±	-7
		-5	4	27	+	+	—	-5
	1	-5	4	28	±	—	+	-5
	1	-4	4	29	+	+	+	-5
	1	-4	4	32	—	+	±	-5
	1	-4	4	42	±	±	±	<-3†
	1	-4	4	43	+	+	±	-7
	1	-4	4	56	+	+	+	-7
	1	-5	4	57	—	+	—	<-3†
Total	12				8 (67%)	8	3	

+, typical of acute LCM. ±, modified or equivocal. —, minimal or negative.

* Highest dilution which killed 3 or more out of 5 mice inj. intracer. Titrations as performed did not establish end-points.

† Brains from mice killed on days 42 and 57 killed 1 out of 5 and 2 out of 5 respectively at 10^{-3} dilution.

is taken to indicate virus replication in all the animals studied in this group. Results are shown in Table II.

3. *Treated mice: chronic stage.* Twelve mice were sacrificed from 21 to 57 days after LCM virus injection (6 given 10^{-4} dilution; 6 given 10^{-5}). Of these, 8 showed the characteristic heavy lymphocytic infiltration of meninges and choroid similar to that seen in control animals. Changes in lymph nodes, spleen, and viscera in these 12 mice were similar in severity and frequency to those seen in the untreated controls. (Table III).

Four mice showed modification of the natural disease, varying from extremely slight traces of lymphocytic choriomeningitis to greatly modified disease, similar to the 18 mice described in Section 2. Despite the length of time after inoculation, those animals showing evidence of active disease had extensive histological lesions even as late as 56 days post injection, similar and comparable in some cases to the extent and severity of the lesions observed in untreated control animals at the acute stage.

Virus recoveries in titration tests, in dilutions of 10^{-5} to 10^{-7}, without reaching end-points, indicated virus replication in these mice. Results are given in Table III. The two instances where virus recovery is shown as less than 10^{-3} (42 and 57 days post infection) do not prove virus replication, but do not exclude it.

Discussion. The characteristic pathological picture of lymphocytic choriomeningitis was completely eradicated or markedly suppressed in mice given amethopterin after intracerebral inoculation with normally lethal doses of virus, in 82% of animals in the acute stage, and in 33% in the chronic stage. The difference in incidence of lesions of active LCM at these 2 stages tends to indicate that this treatment actually may have delayed rather than suppressed the inflammatory reaction, to a large degree. If the reaction which occurred in 67% of mice in the chronic stage was delayed by amethopterin, it is possible that the early effect of amethopterin had abated, but that some degree of immunity had been acquired from exposure to the virus. This alteration of response may be presumed to occur with folic acid-deficient diet, and has been shown to follow whole body X-irradiation, both of which have similar life-sparing effects. In the studies reported here, amelioration of the pathological process was not essential for

survival, since a significant number of spared mice showed an active cellular response. The sparing effect was not attributable to eradication or diminution of virus, since spared mice with and without active lesions showed evidence of virus multiplication (Tables I, II, III).

The host-parasite relationship becomes somewhat obscured by these findings, since it has been generally assumed that an inflammatory cellular response to an invading microorganism is beneficial to the host, by providing under some circumstances a defensive mechanism for the arrest, destruction, or elimination of the invader. Rowe has suggested that in lymphocytic choriomeningitis the cellular response may play an opposite role, being responsible for mortality, and that its suppression therefore allows survival(3). In the present study, resistance to normally lethal doses of virus occurred whether or not a cellular response developed, and in the presence of consistently active virus multiplication in the host.

Since amethopterin is one of a class of compounds which are capable of interfering with various aspects of metabolism and growth, alteration of the metabolic activity of the virus as well as its synthesis or destruction must be considered as possible determining factors in the outcome of LCM infection in treated mice. It is conceivable that modification of viral activity might in some fashion affect its lethal capacity, but if such a factor were operative it could be effective only while the virus was in the amethopterin-treated mouse. However, it seems likely that the virus itself was not affected, since its multiplication in the host was not impeded and its infectivity and lethality for new mice appeared unimpaired, in spite of the alteration of the pathological picture and suppression of the lethal effect.

Another possibility is that interference with the cellular metabolism or cellular production of the mouse could alter the outcome of the infection. The influence of humoral responses of the host is suggested by the observations that spared mice are refractory to subsequent challenge with the virus(1,2). In order to determine the significance of modification of the cellular response on the course of this infection, it would be necessary to prevent cellular response by procedures which could impede cellular production or the exudation of pre-existing cells, and yet not interfere with viral metabolism or growth, or with other responses (conceivably humoral) of the host.

Summary. Mice infected with lymphocytic choriomeningitis virus in doses which were otherwise uniformly fatal were spared by administration of amethopterin. Histological examination of spared animals at intervals ranging from 5 to 57 days revealed greatly modified objective evidence of disease in the majority of these animals. In the acute stage, 82% of the mice showed no disease or extremely modified lesions of LCM. In the chronic stage, 33% showed similarly modified LCM. The remaining mice in both groups showed lesions of active LCM, even as late as 56 days after inoculation. Practically all spared mice gave evidence of multiplication of the virus in the central nervous system.

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