Discussion. The protective effect of the KB-95 against ionizing radiation differs from that against HN_2(1) in at least one important respect, namely that its protective effects appear limited to pre-irradiation treatment, whereas in the case of HN_2, the KB-95 actually appears more protective if given *after* the HN_2 than if administered prior to the toxic agent.

The experiment reported in Table III suggests that KB-95, an established antagonist of serotonin in the usual tests,† also somewhat antagonizes the radioprotective ability of the serotonin. This is in interesting contrast to the experiments of van den Brenk and Elliott (2), who found that several antagonists and anti-metabolites of serotonin were not themselves at all radioprotective and, furthermore, completely negated the radioprotective ability of the serotonin when given together with that agent.

Summary. The serotonin antagonist 1-(N-methyl-piperidyl-4')-3-phenyl-4-benzyl-pyrazolone-5 (KB-95) shows mildly protective effects against 600 r whole-body X-irradiation in mice, if administered prior to irradiation. The compound exhibits a dose-reduction factor of approximately 1.1 in both rats and mice.

† Unpublished data from laboratories of Sandoz, Inc.

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Protective Effect of Neonatal Thymectomy on Mouse LCM Infection. (28643)

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(Introduced by R. J. Huebner)
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A number of features of lymphocytic choriomeningitis (LCM) infection of mice indicate that the mechanism by which the cerebral form of infection induces fatal convulsions involves some form of tissue hyperreactivity(1,2). Symptomatology is closely correlated with lymphocytic infiltration of the meninges and choroid plexus, which is probably elicited by a hypersensitivity reaction. It is not clear whether the stimulus for induction of convulsions is the inflammatory infiltrate itself or some other hyperreactivity response such as cerebral vasospasm or edema. Agents which produce both lymphopenia and suppression of cell-mediated immune responses, such as delayed hypersensitivity and homograft rejection, protect against the disease manifestations of infection, although not suppressing growth of virus. These include total body x-irradiation(3), cortisone(4), and various antileukemic drugs, particularly folic acid antagonists(5,6).

Intraperitoneal (i.p.) infection of mice with a viscerotropic LCM strain rarely produces disease of the central nervous system, but produces illness and death primarily by massive pleural effusion(1). The pleural reaction is completely prevented in irradiated mice (3), suggesting a hypersensitivity component in the pathogenesis of this form of the disease as well. The prime importance of lymphocytic reaction is also indicated by the uniform association of the pleural effusion with lymphocytic infiltration of the mediastinal and pulmonary pleura(7).

The precise form of tissue hyperreactivity involved is not clear, although delayed-type hypersensitivity and the Shwartzman reaction have been considered. However, present understanding of the fundamental pathogenetic mechanisms of the diverse hyperreactivity syndromes is too incomplete to warrant equating the LCM mechanism with any particular one.

Since thymectomy of newborn mice results in marked suppression of both humoral and certain cell-mediated immune responses(8,9), experiments were conducted to test the reactivity of neonatally thymectomized mice to the two forms of LCM infection.

Materials and methods. Mice of the non-inbred "NIH" Webster Swiss strain, obtained from the Animal Production Section of NIH, were thymectomized within 24 hours after birth. Each litter contained thymectomized and sham-operated animals. Experimental mice and normal non-operated controls of the same age were maintained under identical conditions. Thymectomized mice showed none of the characteristics of the wasting syndrome during the period of study.

Two strains of LCM virus were used. The CA 1371 strain, a mouse-brain passage strain, was obtained from Dr. Charles Armstrong. The WCP strain, which had been carried for over 200 serial intraperitoneal passages in mice, was obtained from Dr. Victor Haas.

Mice were inoculated at 21 to 28 days of age. Intracerebrally (i.c.) challenged mice received 0.03 ml of a 10^{-1} dilution of a 10% suspension of CA 1371 infected brain. As determined by titrations, carried out with each experiment, this dosage corresponded to 100 to 1000 LD_{50}. The mice were examined once or twice daily from the 5th to 15th days after inoculation, by spinning by the tail.

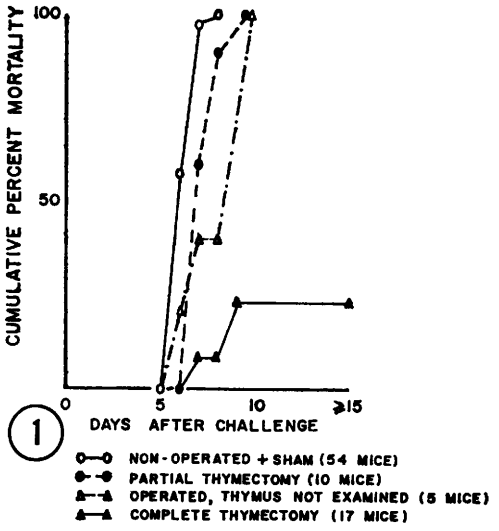
Intraperitoneal challenge consisted of 0.1 ml of a 10^{-1} dilution of a 10% extract of WCP infected liver, spleen, and lung tissue. I.p. titrations with this strain were too erratic to permit calculation of LD_{50} titers; however, the majority of mice inoculated with virus dilutions through 10^{-5} developed labored respiration (pumping), and died. The i.p. challenged mice were observed daily for development of pumping, and dead mice were autopsied for confirmation of the presence of pleural effusion.

The mediastinum of operated mice that died was examined grossly for presence of thymic tissue; histologic sections of mediastinal tissue of mice with questionable small remnants confirmed the adequacy of the gross observations. However, no attempt was made to detect ectopic thymus tissue. At termination of experiments, surviving mice were similarly examined for adequacy of the thymectomies.

Results. Three i.c. challenge, and 2 i.p. challenge experiments were done; since the results of repeat experiments were highly comparable, they are combined for presentation here. In the NIH mice, the thymus adheres firmly to the pericardium, and a number of thymectomies in the early i.c. experiments were incomplete, with one-fourth to one-half of the organ remaining. Operative technique (10) was then modified to free the thymus from the heart by sharp dissection. The modified procedure was used for the groups receiving i.p. challenge, and resulted in complete thymic extirpation in all but one of the 14 attempts. The mouse incompletely thymectomized was included with the controls in the analysis of the i.p. experiments.

The mortality of mice challenged i.c. is summarized in Fig. 1. Control and sham-operated mice showed the classical mortality curve, with all mice dying by the 8th day after inoculation. The challenge was also uniformly lethal for mice with partial thymic extirpation, but death was delayed by a day. Five operated mice that for various reasons could not be examined for thymic remnants died at the same time as the animals with partial removal, and are presumed to have had incomplete operations. In contrast, only 4 of 17 completely thymectomized mice died during the 15- to 57-day observation periods. Of the 13 survivors, 4 had shown tremors or mild convulsions for several days, generally between the 9th and 12th days after challenge. Two of these 4 mice were sacrificed on the 15th day, and histologic sections made of the brains and meninges. These sections showed mild or moderate round cell infiltration of the basal meninges and in cerebral fissures; one brain showed thickening of the adventitia of parenchymal vessels. Sections of 4 asymptomatic mice sacrificed on the 15th day were negative or showed minimal infiltration of basal meninges.

The brains of the other 7 surviving thymectomized mice were tested for virus at 46 to 57 days by inoculating 10^{0} and 10^{-3} dilutions of 10 per cent extracts into weanling NIH strain mice by the i.c. route. All 7 ex-



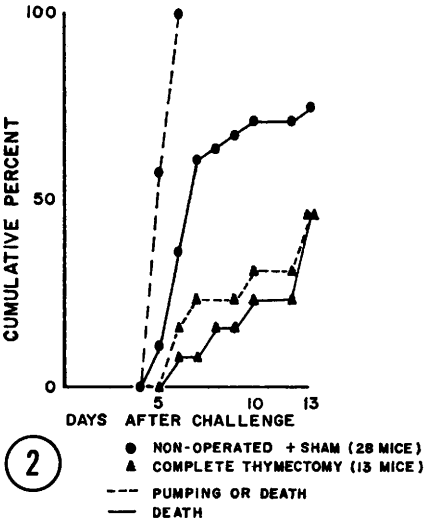


FIG. 1. Mortality curves of control and thymectomized mice challenged intracerebrally with LCM virus.

FIG. 2. Morbidity and mortality of control and thymectomized mice challenged intraperitoneally with LCM virus.

tracts were infectious at 10^0 dilution, and 6 at 10^{-3}. Sera of these 7 mice were tested at a dilution of 1:5 for complement fixing antibody to LCM-infected guinea pig spleen antigen; 5 of the sera were positive.

The intraperitoneal experiments showed similar protection in the thymectomized mice (Fig. 2). All control mice showed pumping respirations, and 75% died. The thymectomized mice showed later onset of disease, and significantly lower frequency of pumping and of death. In both groups, mice that died by the 10th day showed massive pleural effusion; after the 10th day the mice became ruffled and undernourished, and the late deaths were not accompanied by pleural effusion.

For comparison with the LCM findings, a similar test was done using i.c. challenge with $10^{2.5}$ LD_{50} of vesicular stomatitis virus (Indiana strain). Control and thymectomized groups showed identical mortality curves.

Discussion. The protective effect of neonatal thymectomy against both forms of LCM infection provides further evidence that the pathogenetic mechanism involves an immune response. However, this cannot be considered as ruling out other forms of tissue hyperreactivity until the effect of neonatal thymectomy on these is established. The concept that classical delayed hypersensitivity alone is the triggering mechanism is difficult to reconcile with the recent observation by Hotchin(2) that i.c. infected mice may die in typical convulsions as early as 30 hours after virus inoculation if challenged by intravenous injection of a normally sublethal dose of endotoxin; this suggests that there is an early change in vascular reactivity to nonspecific stimuli, such as seen in the Shwartzman phenomenon. The animals in the present study showed complement fixing antibody response, which diminishes the likelihood that a simple humoral antibody-antigen reaction is the disease mechanism. The protection closely resembled that produced by x-irradiation and folic acid deficiency, in that virus multiplication was not inhibited, but lymphocytic infiltration of the meninges was prevented or ameliorated. The thymectomy system differs from the other protective systems in that it does not induce marked lymphopenia in the NIH strain mice (unpublished data).

The present findings also substantiate the similarity in disease mechanism of the two forms of LCM. The histologic features of the i.p. disease resemble classical infectious disease processes somewhat more closely than does the i.c. disease, in that there is lymphocytic infiltration of the parenchyma of viscera,

particularly lung and liver. This suggests that the participation of hyperergic reactions in other viral infections should be more thoroughly investigated, and that the thymectomized mice may provide a useful approach. The only other virus studied here, vesicular stomatitis, produces severe necrotizing encephalitis, and would not be considered at all likely to involve a hypersensitive reaction.

It is possible that the failure of neonatal thymectomy to protect all mice may be due to ectopic thymic tissue, or to variation between mice of this non-inbred strain with respect to degree of thymic activity at time of operation.

Summary. Mice thymectomized at birth were protected against the lethality of intracerebral and intraperitoneal infection with LCM virus given 3 to 4 weeks later. Although virus multiplication was not prevented, as evidenced by high infectivity titers in the brain 6 to 8 weeks after infection, the meningeal inflammatory reaction was suppressed. These observations provide further evidence for the importance of tissue hyperreactivity and lymphocytic inflammatory response in production of symptomatology.

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Starch Gel Electrophoresis of Rat Hypophysis in Relation to Prolactin Activity.* (28644)

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The starch gel method of electrophoresis (1) is used commonly for the separation of proteins and has been applied to homogenates of the adenohypophysis(2,3). However, the localization of pituitary hormones in the bands revealed by staining of such columns is unknown except that human gonadotropin occurs in more slowly migrating fractions but cannot be associated with any individual protein bands(3). This report deals with (a) modification of protein bands in starch gel columns by ovariectomy, thyroidectomy, and replacement therapy with estrogen and (b) distribution of prolactin activity in these columns.

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Methods. Eleven days after ovariectomy of young adult female Sprague-Dawley rats their thyroid and parathyroid glands were excised. These rats are to be referred to subsequently as being ovariectomized and thyroidectomized. Two weeks later 100 μc of I^{131} were injected subcutaneously to destroy remaining fragments of the thyroid gland. The rats were divided into the following groups: (a) ovariectomized, vehicle-treated, (b) ovariectomized, thyroidectomized, vehicle-treated, (c) ovariectomized, estrogen-treated, and (d) ovariectomized, thyroidectomized, estrogen-treated (Table I). Beginning 64 days after ovariectomy in Experiment I and 6½ months later in Experiment II, 2 μg of α-estradiol dipropionate were ad-