for pancreatic enzyme activity. On this basis, and from our histologic evidence, it would appear that a quantitative indicator may be applied to measure pancreatic damage. The ethionine-treated rat provides a convenient laboratory model particularly when physical limitations prevent use of larger animals.

Summary. Rats fed dl-ethionine for 25 days showed a significant decrease in small intestinal trypsin, chymotrypsin and lipase activity when compared to controls. The results suggest that levels of intestinal enzyme activity may be used as a quantitative indicator of pancreatic exocrine damage.

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Effect of Anoxic-Ischemic Procedure on Experimental Epilepsy in the Rat. (29277)

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Surgical eradication of spontaneous epileptogenic foci in human brains and induced epileptogenic foci in animal brains has proved curative(1-3). The present study is an attempt to alter or destroy an epileptogenic focus in the rat brain by anoxia-ischemia and/or infarction, rather than surgical excision. The results indicate that the anoxic-ischemic procedure raises the low convulsive threshold of most epileptic rats.

Materials and methods. One hundred and twenty female Wistar rats (Hemlock Hollow Farms) with a mean initial body weight of 145 g (range 130-160 g) were divided into 4 groups and treated as indicated in Table I. On the first day of experiment the convulsive threshold of each rat of all groups was determined by the average number of intraperitoneal injections of pentamethylentetrazol (Metrazol), 15 mg/kg at 15 minute intervals, required to produce generalized convulsions (4). This initial convulsive threshold was used as a base line for comparison with the convulsive thresholds obtained after subsequent experimental interventions. Rats of Group A were kept as non-epileptic controls. On the second day of experiment, Groups B, C and D were made epileptic by application of 2 mm pellets of 325 mesh cobalt powder in the right frontal cortex, with the aid of a stereotaxic apparatus(4-6). A second Metrazol test was performed on all animals on the 16th day of experiment to determine the effectiveness of cobalt-induced epilepsy. On day 20, an attempt was made to produce an infarct by the anoxic-ischemic procedure in the left cerebral hemisphere of Group C and in the right cerebral hemisphere of Group D. This was accomplished by ligation of the common carotid artery of the desired side, followed immediately by exposure of the animal to nitrous oxide(7). Only 6 animals of Group C (60%) and 31 animals of Group D (32%) survived the anoxic-ischemic procedure. The

TABLE I. Effect of Anoxic-Ischemic Procedure on Cobalt Epilepsy.

Group	Metrazol threshold* (day 1)	Cobalt implant (day 2)	Metrazol threshold (day 16)	Anoxic ischemic procedure (day 20)	Metrazol threshold (day 30)	No. of survivors
A	5.6	—	4.6	—	3	5
B	4.7	Right	2.0	—	1.8	10
C	4.5	"	2.3	Left	1.8	6
D	4.4	"	1.9	Right	3.2	31

* Group average of number of injections required to produce generalized convulsions.

non-surviving animals of the 2 groups were eliminated from the experiment. Therefore Table I represents the experimental results obtained from the animals of all groups that survived to the end of experiment. On day 30, all surviving rats were tested with Metrazol for the third time to determine the effect of the infarct on the epileptogenic focus. Following this, the animals were sacrificed with chloroform and the brains were fixed in 10% formalin for subsequent embedding in paraffin and staining with hematoxylin-eosin (general structure) and Luxol fast blue (myelin stain).

Results and discussion. A progressive decrease in Metrazol threshold was noted in all the groups (Table I). This is perhaps related to repeated tests which made the animals more sensitive to Metrazol. However, the cobalt-treated groups (B, C and D) showed a much greater decrease in the Metrazol threshold as compared to normal control animals (Group A).

The anoxic-ischemic procedure on the contralateral side of the brain (Group C) had no effect on the Metrazol threshold. However, when the anoxic-ischemic procedure was on the same side as cobalt (Group D), a definite reversal of Metrazol threshold towards normal was noted. The anoxic-ischemic procedure had produced a brain infarct, proven histologically, in 16 of the 31 rats of Group D and 2 out of 6 rats in Group C. The Metrazol threshold had reverted towards normal in all but 2 of the 16 animals with homolateral infarcts. It is reasonable to assume that the reversal of Metrazol threshold in these 16 animals with infarct was due to damage to epileptogenic areas, or interruption of associated tracts. However, an increased Metrazol threshold was also observed in the remaining 15 animals in which brain lesions were of minimal severity, or questionable, as determined by light microscopy. The mechanism for reversal of Metrazol threshold in these animals has not been elucidated.

Statistical analysis. Table I is an unbalanced, 3-way classification design with unequal class frequencies of Metrazol thresholds on 52 rats. Observations (thresholds) are classified as follows:

1. No cobalt, no anoxic-ischemic procedure (all tests on Group A, plus tests on Groups B, C and D on day 1).
2. Right cobalt, no anoxic-ischemic procedure (tests on Group B on days 16 and 30, plus tests on Groups C and D on day 16).
3. Right cobalt, left anoxic-ischemic procedure (tests on Group C on day 30).
4. Right cobalt, right anoxic-ischemic procedure (tests on Group D on day 30).

Tests (t) on differences in means were performed. There were significant differences, with 0.1% probability of false positive, between categories 4 and 3 and between categories 4 and 2. There was no significant difference between categories 2 and 3; probability of such a value of t, assuming no average difference, is about 100% (zero sample difference).

Summary. Rats with cobalt epileptogenic foci had a reduced convulsive threshold which could be increased toward normal by producing homolateral brain infarction. The alleviation of the epileptic state is assumed to be due to damage to epileptogenic areas or associated tracts in some, but not all, rats.

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Comparative Immunology. Lymph Nodes in the Amphibian, *Bufo marinus** (29278)

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There are several statements that lymph nodes are not found in animals which are phylogenetically more primitive than birds (1-4). During comparative studies of antibody formation in poikilothermic vertebrates (5-7), structures resembling the lymph nodes of mammals were observed in the marine toad, *Bufo marinus*. Because of the potential importance of this finding to studies on the phylogeny of the immune response, experimental observations on the lymph nodes of *B. marinus* are presented here.

Methods and materials. Six adult marine toads were injected in the dorsal lymph sac with 0.25 to 1 ml of India ink 24 hours before sacrifice. The body cavities, neck, axilla, and inguinal areas were dissected. Samples of tissue that contained India ink on gross examination were fixed in neutral buffered formalin and subsequently embedded in paraffin.

Forty-nine additional adult animals being used in the study of antibody formation to bovine serum albumin (BSA) were also dissected. Twenty-six of these animals received a single injection of 10 mg of BSA in the dorsal lymph sac 4 to 24 days before sacrifice. Seventeen animals had received three 10 mg injections of BSA at intervals of one month or longer. They were sacrificed 2 to 18 days after the final injection. Six animals were non-injected controls. Samples of the liver, spleen, kidney and lymph nodes were fixed in cold 95% alcohol after the method of Sainte Marie(8). Using the fluorescent antibody technique, the tissues were examined for cells forming antibody to BSA(9). Fluorescein labelled BSA (0.5 mg/ml) was used for the direct demonstration of antibody forming cells. As controls, alternate sections were exposed to fluorescein labelled *B. marinus* serum protein (0.5 mg/ml). Tissue from non-injected toads as well as tissue from toads receiving BSA for the first time shortly before sacrifice were used as additional controls. Sections of all of the tissues were stained with hematoxylin and eosin and with methyl green pyronin(10). Sections were also stained with Masson's Trichrome stain, with Giemsa stain and by Gridley's reticulum stain(10,11).

Results. On gross examination 24 hours after the injection of India ink the liver, spleen, kidneys and lungs were black. In addition circumscribed black nodes of lymphoid tissues were noted along the great vessels at the base of the heart and their branches in the neck and axilla (Fig. 1). These masses of lymphoid tissue were associated with the arteries and veins but could be dissected readily from these large vessels. Four to ten nodes measuring from 0.5 mm to 8.0 mm in greatest diameter were noted on either side of the mid line in the upper thorax, axilla, and neck. Most of the nodules were from 1 to 4 mm in diameter. They were rounded, usually slightly flattened and sometimes were "bean" shaped. Similar nodules were found in all 55 animals studied. In

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