weights were increased, and ovarian weight was decreased. Histological examination of the ovaries of the constant light treated rats showed large numbers of well-developed follicles with few small corpora lutea. The uteri of these rats showed a marked augmentation in vascularity, muscle mass, number of glands in the endometrium, and increased height of the epithelium. These results suggest that constant light stimulates increased release of FSH from the pituitary by increasing hypothalamic production of FSH-RF.

*Addendum.* We have recently observed that FSH-RF content of the hypothalamus of female rats is high during diestrus and in the morning of proestrus, and low in the afternoon of proestrus and during estrus (Negro-Vilar, Sar and Meites, unpublished observations). This suggests that if all the control rats had been in the estrus phase of the cycle, their hypothalamic FSH-RF content would have been even lower than reported here, and the difference from the rats under constant light would have been greater.

1. Browman, L. G., J. Exptl. Zool. **75**, 375 (1937).
2. Hemmingsen, A. M. and Krarup, N. B., Kgl. Danske Videnskab. Selskab, Biol. Medd. **13**, 1 (1937).
3. Fiske, V. M., Proc. Soc. Exptl. Biol. Med. **40**, 189 (1939).
4. Fiske, V. M., Endocrinology **29**, 187 (1941)
5. Maric, D. K., Matsuyama, E., and Lloyd, C. W., Endocrinology **77**, 529 (1965).
6. Lawton, I. E. and Schwartz, N. B., Endocrinology **77**, 1140 (1965).
7. Bradshaw, M. and Critchlow, V., Endocrinology **78**, 1007 (1966).
8. Mittler, J. C. and Meites, J., Endocrinology **78**, 500 (1966).
9. Steelman, S. L. and Pohley, F. M. Endocrinology **53**, 604 (1953).
10. Parlow, A. F. and Reichert, L. E., Jr., Endocrinology **73**, 740 (1963).
11. Bliss, C. I., Biometrics **12**, 491 (1956).
12. Negro-Vilar, A. and Meites, J., Program 49th Ann. Meeting Endocrine Soc., Miami, Florida, p. 119, 1967.
13. Piacsek, B. E. and Meites, J., Neuroendocrinology **2**, 129 (1967).
14. Singh, K. B. and Greenwald, G. S., J. Endocrinol. **38**, 389 (1967).

Received October 9, 1967. P.S.E.B.M., 1968, Vol. 127.

## Congenital Lymphocytic Choriomeningitis Virus Infection in Gnotobiotic Mice* (32793)

MORRIS POLLARD, NEHAMA SHARON, AND B. A. TEAH
*Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556*

Lymphocytic choriomeningitis (LCM) virus is unique in that it is disseminated congenitally to progeny, in which it resides for long periods without distinct symptoms (1,2). The LCM virus inoculated in mice at birth remained in the circulatory system; and the animals manifested as part of the disease, what appeared to be an accelerated aging process (3,4).

When mature mice were inoculated with LCM virus the appearance of disease and death was related to the immunological status of the hosts: they were spared if pretreated by X-rays (5), by amethopterin (6), or by neonatal thymectomy (7), otherwise they died.

Thus far, evidence of two types of viral agents have been reported in gnotobiotic Lobund mice: leukemia virus has been detected in the thymic tissues of six mouse strains, and lymphatic leukemia appeared in significant numbers of them following whole-body exposures to small doses of X-rays (8). In addition, strain AKR mice carry leukemia virus and have manifested the same pattern of leukemic disease as the conventional stock from which they were derived (9). During 6 years, seven gnotobiotic Lobund mice (Balb/c, C3H/f and Swiss-Webster strains) developed spontaneous breast carcinomas, and B-type virus particles were detected in two

* Supported by funds from the U.S. Public Health Service Grant #CA 7271 and the Office of Naval Research.

of them (10). It was of interest to determine if congenital LCM infection and disease would occur in bacteria-free gnotobiotic mice, and if the symptoms and lesions induced thereby would be more distinct and virus-related than those observed in conventional counterpart mice. In conventional mice, lesions associated with persistent LCM infection may actually be due to other elements in the microbial flora.

*Materials and Methods.* White Swiss mice with congenital LCM infection were provided by Dr. Wallace P. Rowe of the National Institutes of Health. Neonatal mice were derived from them by caesarian section into the germfree isolator where they were foster-nursed on gnotobiotic Lobund C3H/f mice (11). When mature they were propagated by natural means up to third generation, at which time they were subjected to a number of examinations. They were maintained at all times under germfree conditions; and monitored for bacterial content at weekly intervals by techniques which have been described (12).

Fifty of the mice mentioned above (to be designated from now on as LCM mice), representing age groups from newborn to 8 months of age were removed from the germfree isolator. They were immediately sacrificed with ether; and, where possible, each was exsanguinated from the heart, and each serum specimen was stored at −20°C. Three-fourths of each spleen was extracted in about 10× (w/v) Mixture 199 (balanced salt solution), centrifuged, and the cleared supernatant fluid was inoculated intracerebrally or subcutaneously into mature conventional mice of ICR strain which was found to be susceptible to standard LCM virus when tested 2 years previously. The inoculated animals were maintained in plastic isolators in order to prevent spread of LCM to the environment. Serums from 11 mice which had been inoculated subcutaneously 3 weeks previously were examined by the complement fixation test for LCM antibody.

Fourteen serum specimens from gnotobiotic LCM mice of different age levels were examined by the technique of acrylamide gel disc electrophoresis and the levels of separated protein components were traced and analyzed with a Chromoscan densitometer.

A variety of tissues from each of the LCM mice were fixed in Bouin's fluid, embedded in paraffin, and processed for histological examinations.

Control mice for all of the above examinations were of the gnotobiotic Lobund ICR strain, which provided pertinent specimen material for comparative purposes. Although mice of the gnotobiotic ICR strain carried leukemia virus, they did not develop spontaneous leukemia, or other virus-related disease.

*Results.* Gnotobiotic ICR control mice produced large litters of vigorous young. Their tissues revealed no gross or microscopic evidence of disease: the lymphoreticular organs were small and relatively inactive. The lymph nodes and spleens showed very occasional small germinal zones and rare plasma cells. The Peyer's patches were very small and flat. Their thymus glands showed age-related changes of enlargement and shrinkage which were comparable to those of conventional counterpart mice (13); in all cases they had well defined zones of cortical thymocytes. The kidneys were free of lymphoid cell aggregations, and their glomeruli and ducts appeared to be within normal morphological limits. The livers, pancreas, and brains were notably free of lymphoid cell aggregations. The characteristics of ICR mice were thus similar to those observed among four other strains of gnotobiotic Lobund mice: CFW, C3H/f, Swiss-Webster, and Balb/c strains.

Gnotobiotic LCM mice produced large litters of young with a growth pattern similar to that observed with six other strains of gnotobiotic mice. They have manifested no overt sign of disease up to at least 8 months of age. None of the early "runting" or stunting, as reported in conventional LCM-infected mice (14), has been observed.

All the gnotobiotic LCM mice carried virulent LCM virus in the spleens, and virus was also detected in extracts of LCM embryos. Adult mice which were inoculated intracerebrally with spleen extracts from the LCM mice, became sick and died within 6–8 days after inoculation. All mice which survived subcutaneous inoculation of extracts from gno-

tobiotic LCM mice, developed complement-fixing antibodies for LCM. Extracts of spleens from control gnotobiotic ICR mice were not pathogenic for LCM-susceptible mice by the intracerebral route; and serums collected from the latter mice were negative for LCM by complement fixation test. Gross lesions were not observed in LCM mice of age-range from newborn to 2 months. Thereafter, with increasing frequency, mice over 2 months of age showed splenomegaly (as much as 3–5× enlarged), marked enlargement of lymph nodes and Peyer's patches, and reduction in size of the thymus glands. The kidneys were swollen, discolored, rough-surfaced, and some had small petechiae.

Tissue changes were noted by light microscopy in all LCM mice which were older than 1 month of age. The swollen spleen tissues contained extensive areas of white pulp with very large germinal zones. The red pulp contained many mononuclear cells (reticulum cells, lymphocytes, and plasma cells). The lymph nodes contained numerous large germinal zones in the cortical follicles, and cords of plasma cells in the medullary regions. The Peyer's patches were large focal accumulations of lymphoid cells which in many instances extended almost around the intestine. Some of them contained well defined germinal zones. The laminae propria of the intestinal villi were relatively cell-free, similar to that associated with the disease-free gnotobiotic mouse (15). All kidneys contained perivascular accumulations of lymphoid and plasma cell-like cells (3) (Fig. 1), and significant numbers of the cells were in mitosis. The glomeruli of young mice were enlarged, hypercellular, and had small areas of hyalinization. In mice over 6 months of age, the Bowman's capsules were thickened and increasingly altered with fibrotic changes which eventually changed the entire glomerular area into a hyalinized mass (Fig. 2). Perivascular areas were infiltrated to varying extent with lymphoid and plasma cells. In older mice the tubular epithelium was degenerated, the tubules were dilated, and many of them contained hyaline casts in the lumens.

The thymic glands of older LCM mice were small: In 18 out of 19 mice over 6 months of age, the thymic glands showed depletion of cortical thymocytes, which ranged in extent from local segments to extensive and generalized depletion (Fig. 3). Local areas of cell depletion in the thymic cortex were noted in 9 of 15 five-months old mice, in 3 of 8 three-months old mice, and in none of 8 which were under 2 months of age. Germinal zones were noted in the thymic medullary region of 7 mice in this group. Some of the depleted thymus glands contained multiple large cysts (Fig. 3).

In all of 19 mice over 6 months old, perivascular aggregations of lymphoid and plasma cells were noted in the pancreas. The lymphomatous aggregations in the pancreas, as in the kidneys, were very prominent; they displaced much of the parenchymatous tissues excepting the islets of Langerhans, and contained numerous mitotic cells (Fig. 4). Necrotic cells were not noted in this organ. Significant aggregations of lymphoid cells were noted around the choroid plexus in two of four brains, in the periportal regions of two of four livers, and in the peribronchial areas of three lungs which were examined.

The tissue changes which were observed in the gnotobiotic LCM mice were not observed in the ICR control mice.

As determined by acrylamide gel electrophoresis, the serums from the control gnotobiotic ICR mice contained low levels of globulin, i.e., 2.6% of gamma globulin. On the other hand, the serums from gnotobiotic LCM mice contained higher levels of globulin; and the rise was increasingly apparent in those over 5 or 6 months old which showed more extensive lymphoid infiltrations in the organs and glands, and sclerosing glomerulonephritis (Table I): levels of gamma globulin increased from 2.7% in 2-months old mice, to 49.7% in 8-months old mice.

*Discussion.* It is apparent that LCM is disseminated by congenital route(s), as originally reported by Traub (1–3). LCM is the third virus which has thus far been detected in gnotobiotic mice. Doubtless, we can anticipate the occurrence of all congenitally-disseminated viruses in gnotobiotic mice if they were derived from infected stock.

Since we did not have virus-free control

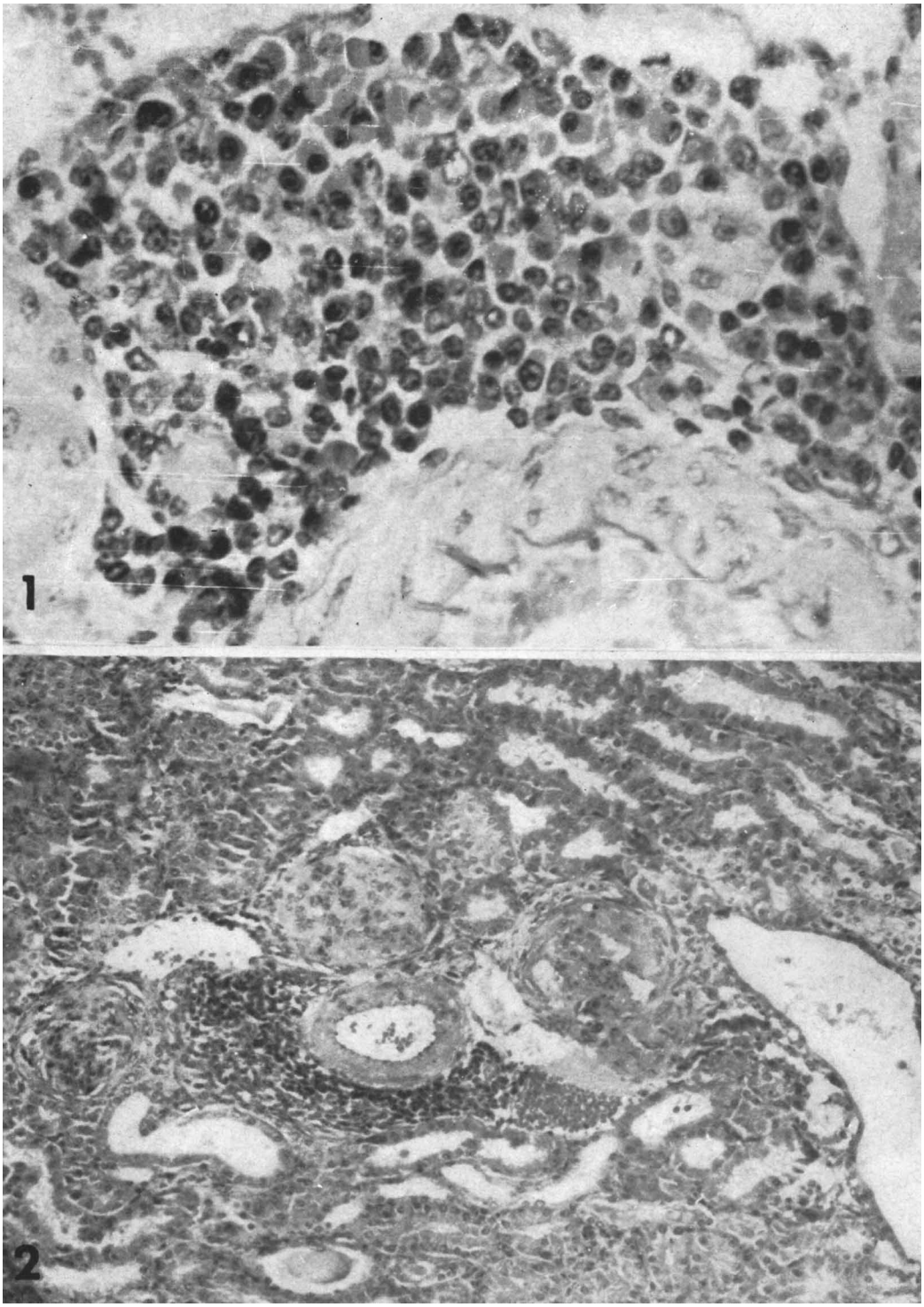


FIG. 1. Lymphoid perivascular infiltration in kidney of LCM mice. Note plasma-like cells. H & E; × 160.

FIG. 2. Kidney of 5-months old LCM mouse. Note glomerular degeneration, tubular degeneration, and lymphoid cells in perivascular region. H & E; ×40.

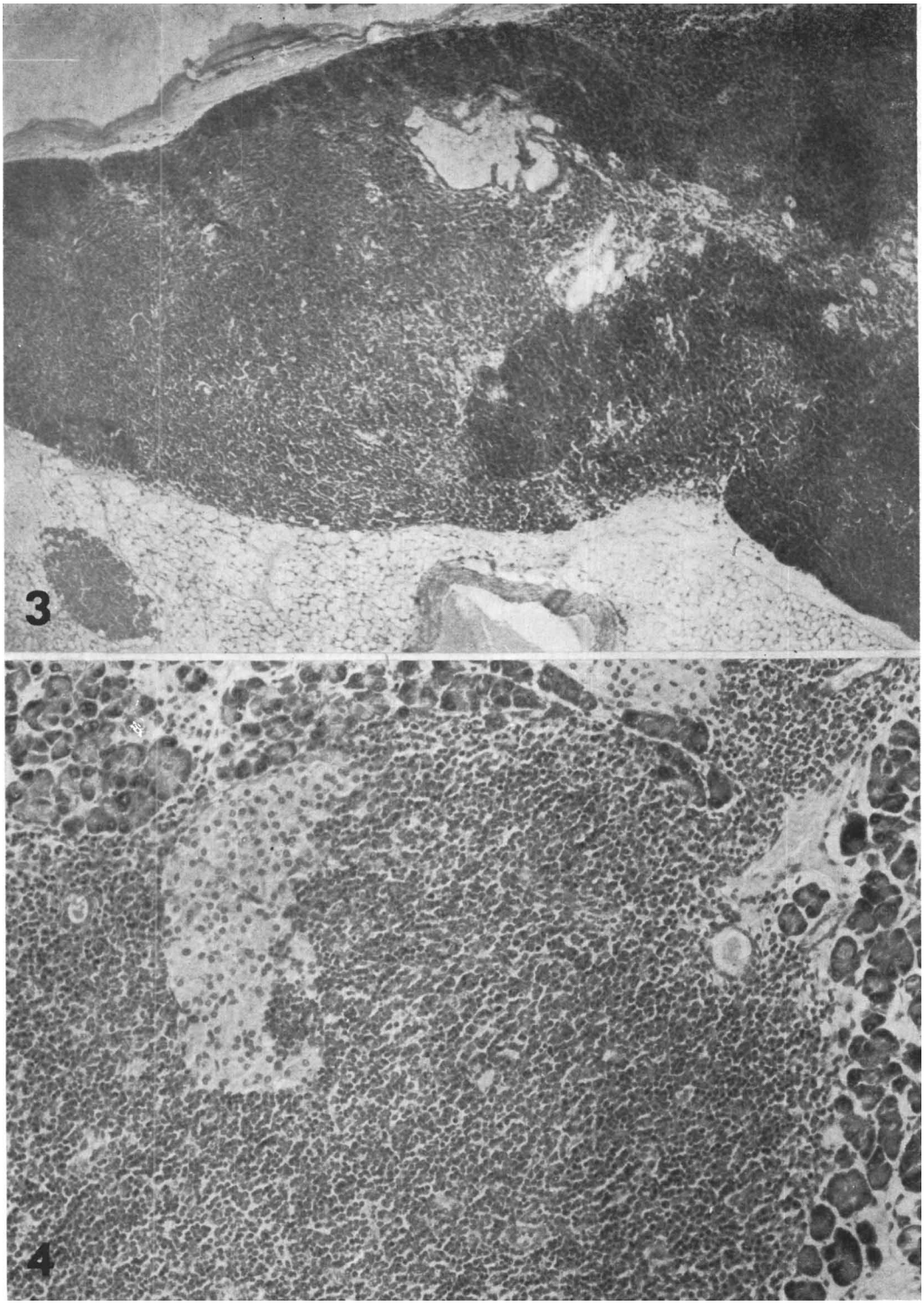


FIG. 3. Thymus of 4-months old LCM mouse. Note cortical depletion of thymocytes and cyst in medulla. H & E; × 16.

FIG. 4. Pancreas of 6-months old LCM mouse. Note extensive accumulation of lymphoid cells. H & E; × 40.

TABLE I. Serum Components of Gnotobiotic Mice as Determined by Acrylamide Gel Electrophoresis.

| Strain | Age (months) | Protein (mg/ml) | Serum components (%) | | |
|---|---|---|---|---|---|
| | | | Albumin | Total globulin | γ Globulin |
| ICR (control) | 6 | 59.6 | 72.0 | 28 | 2.6 |
| | 6 | ND | 74.5 | 25.5 | 2.7 |
| LCM | 2 | 82.5 | 74.9 | 25.1 | 2.7 |
| | 3 | 82.6 | 51.9 | 48.1 | 15.9 |
| | 3 | ND | 46.2 | 53.8 | 13.8 |
| | 5 | ND | 35.2 | 64.8 | 17.7 |
| | 5 | 115.8 | 34.4 | 65.6 | 23.6 |
| | 6 | ND | 38.9 | 61.1 | 22.3 |
| | 6 | ND | 35.6 | 64.4 | 23.7 |
| | 8 | ND | 26.2 | 73.8 | 49.3 |
| | 8 | ND | 25.8 | 74.2 | 49.7 |
| | 8 | 676 | 30.5 | 69.5 | 44.05 |
| | 8 | 594 | 33.7 | 66.3 | 37.2 |
| | 8 | 702 | 42.8 | 57.2 | 47.9 |
| | 8 | 648 | 37.03 | 62.9 | 30.5 |
| | 8 | 702 | 33.1 | 66.9 | 34.7 |

mice of the same strain as that from which the LCM mice were derived, we had to depend on gnotobiotic mice of another strain, i.e., ICR, for comparative studies. The lymph nodes, spleens, and thymuses of the latter representative strain were notably lacking in the virus-related changes which were so prominent in the gnotobiotic LCM mice. A causal relationship between LCM virus infection and the observed immunopathological lesions seems to be a justified interpretation. Lesions were noted with increasing frequency in the older mice: All of the kidneys and pancreases contained infiltrations of lymphoid cells and plasma cells. Similar cellular aggregations were noted in brain, liver, and lung tissues. The lymph nodes and spleens of the LCM mice had very large germinal zones and extensive aggregations of plasma cells. With age, the kidneys showed increasing evidence of glomerulonephritis and tubular damage. All of the above changes, plus the hyperglobulinemia noted in Table I, would indicate that the LCM virus induced an immunopathologic dyscrasia which resembled some aspects of autoimmune disease in mice (16,17), of lupus erythematosis in man (18), and of Aleutian disease in mink (19). The latter disease appears to be of viral etiology as is that of lymphocytic choriomeningitis.

The host–virus interaction would indicate that a state of immunologic tolerance prevailed in the congenitally-infected mice. The changes noted in the lymphoreticular tissues reflected immunogenic activity: the lymphatic tissues contained prominent germinal zones, elsewhere there were significant accumulations of plasma cells, and the serums had levels of globulin far above those in the control animals. Since all of them contained active virus, then the virus–antibody relationship must differ from that in an immunized animal. The gnotobiotic LCM mice showed neither symptoms of disease nor antibody. How this "tolerant" status relates to the depleted thymic cortex is uncertain. It may reflect exhaustion of the factor(s) contributed by the thymus gland to the immunologic competency of the host. Stress, as exemplified by cortisone injection and by whole-body X-irradiation usually results in cell depletion of the lymphoreticular system (thymus, lymph nodes, and spleen). In LCM mice the latter two tissues appeared hyperactive while the thymus was depleted. Thymic depletion has been noted in preleukemic germfree AKR mice (20), and this

change may be antecedent to a threatened "break" in the tolerant status of the host. Obviously, more information is needed on thymic function in immunological deviations.

*Summary.* The LCM virus penetrated the "germfree barrier," thus reaffirming the congenital dissemination of the virus. The lesions associated with LCM infection in the gnotobiotic mice were clearly related to a hyperreactive immunologic mechanism, which induced degenerative kidney changes, depletion of cortical thymocytes, lymphoid cell infiltrations of many organs, and hypergammaglobulinemia.

We appreciate the valuable assistance and council of Dr. Wallace P. Rowe and Dr. William A. Antopol, in this investigation.

1. Traub, E., J. Exptl. Med. 63, 533 (1936).
2. Traub, E., J. Exptl. Med. 63, 847 (1936).
3. Traub, E., J. Exptl. Med. 68, 229 (1938).
4. Hotchin, J., Public Health Service Publ. No. 1378, 341 (1965).
5. Rowe, W. P., Naval Med. Res. Inst., Res. Report Project NM 005-048-14-01 12, 167 (1954).
6. Haas, V. H. and Stewart, S. E., Virology 2, 511 (1956).
7. Levey, R. H., Trainin, N., Law, L. W., Black, P. H., and Rowe, W. P., Science 142, 483 (1963).
8. Pollard, M. and Matsuzawa, T., Proc. Soc. Exptl. Biol. Med. 116, 967 (1964).
9. Pollard, M., Kajima, M., and Teah, B. A., Proc. Soc. Exptl. Biol. Med. 120, 72 (1965).
10. Kajima, M. and Pollard, M., J. Bacteriol. 90, 1448 (1965).
11. Reyniers, J. A. and Trexler, P. C., "Micrurgical and Germfree Methods," p. 114. Thomas, Springfield, Illinois, 1943.
12. Wagner, M., Ann. N. Y. Acad. Sci. 78, 89 (1959).
13. Bealmear, M. and Wilson, R., Anat. Record 154, 2 (1966).
14. Hotchin, J. and Weigand, H., J. Immunol. 86, 392 (1961).
15. Gordon, H. A. and Wostmann, B. S., Anat. Record 137, 65 (1960).
16. Helyer, B. J. and Howie, J. B., Brit. J. Haematol. 9, 119 (1963).
17. East, J. and deSousa, M. A. B., Natl. Cancer Inst. Monograph No. 22, (1965).
18. Klemperer, P., Pollack, A. D., and Baehr, G., Arch. Pathol. 32, 569 (1941).
19. Hartsough, G. R. and Gorham, J. R., Natl. Fur News 28, 10 (1956).
20. Pollard, M., Nature 213, 142 (1967).

Received Oct. 17, 1967. P.S.E.B.M., 1968, Vol. 127.

## The Effect of Glucagon and Insulin on the Prothrombin Response to Coumarin Anticoagulants* (32794)

MURRAY WEINER AND DORIS MOSES
*New York University Research Service, Goldwater Memorial Hospital, Welfare Island, New York, New York 10017*

The clinical observation that poor nutrition may exaggerate the prothrombin response to coumarin anticoagulants has been readily reproduced in controlled experiments with short periods of starvation in guinea pigs (1). Further studies have indicated that deficiency in protein rather than caloric intake was a responsible factor (2). The effect is not related to an altered fate of the coumarin drug as reflected in its blood levels (1).

Recent studies by Greengard and Baker (3) point out that the administration of glucagon mimics the effect of starvation on several enzymes which play a role in tryptophan and tyrosine metabolism. The present report presents data indicating that glucagon also mimics starvation in its effect on the prothrombin response to the anticoagulant, acenocoumarin. Insulin has no significant effect on sensitivity to acenocoumarin, but blocks the hypersensitivity induced by glucagon.

*Methods.* Guinea pigs weighing 279–580 gm were fed with Purina guinea pig chow *ad libitum,* supplemented by fresh lettuce before

* Supported in part by USPHS Grant No. HE-04347 and in part by the Health Research Council of the City of New York under Contract U-1579.