Only trypsin had a marginal effect on glucose uptake, an effect that was much less than the effect of an equimolar concentration of insulin.

The authors wish to thank Mr. H. L. Neldon and Mr. F. E. Wright for valuable technical assistance. Trypsin and chymotrypsin were purchased from Worthington Biochemical Corp., Freehold, N. J.

1. Rieser, P., Rieser, C. H., Proc. Soc. Exp. Biol. and Med., 1964a, v116, 669.

2. ———, Biochem. Biophys. Res. Comm., 1964b, v17, 373.

3. Somogyi, M., J. Biol. Chem., 1952, v195, 19.

4. Edwards, Allen L., Statistical Methods for the Behavioral Sciences, Rinehart and Co., New York, 1960, p249.

Received April 23, 1965. P.S.E.B.M., 1965, v119.

A Defect in Cellular Immunity During the Incubation Period of Passage A Leukemia in C3H Mice.* (30323)

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A previous report has documented the reduced antibody-forming capacity to T_2 bacteriophage in C3H mice incubating Gross passage A leukemia(1). This study was undertaken to define further the extent of the immunologic deficiency subsequent to infection with this leukemogenic virus, by assaying the ability of mice infected at birth with this agent and destined to become leukemic at a later age to develop the capacity to reject allografts. Since the reduction in antibody-forming capacity was found to be quantitative rather than qualitative, we decided to graft across a relatively weak (non H-2) histocompatibility barrier.

Materials and methods. Animals. C3H/Bi strain mice obtained from the original colony of Dr. J. J. Bittner were used. They were weaned and grafted at 4 weeks of age. All mice were housed individually following grafting. Ce/J strain mice obtained from R. B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used as donors. Donors were of the same age and sex as recipients.

Passage A virus. Leukemic C3H mice, representing the 32nd passage of Gross' passage A leukemia were obtained from Dr. Ludwik Gross in June, 1962. The virus was passaged in C3H/Bi mice as reported by Gross(2) until used in the 42nd passage for the present experiment. The experimental mice were given 0.2 ml of the cell-free filtrate intraperitoneally on the third day of life.

Grafting technique. When the mice were 4 weeks of age, they received a 1.5 cm^2 graft onto the mid-dorsal area. Two such grafts were obtained from the same region of each donor mouse. After removal of the underlying fascia the grafts were turned 90° and were sutured in place with 16 interrupted 6-0 ophthalmic silk sutures. Recipient mice were anesthetized with nembutal given by the intraperitoneal route.

Grafts were inspected 3 times a week until rejection occurred or until the animals were about to expire from leukemia. A graft was considered to be rejected when the donor skin had sloughed completely. Partial rejection was represented by gross shrinkage of the graft with only minimal hair growth on the remaining part of the graft. Acceptance of the graft in the controls was characterized by luxuriant hair growth. In the experimental animals 2 types of acceptance were seen: a) full acceptance with hair growth as in the controls and b) maintenance of the graft

* Aided by grants from U.S.P.H.S., National Foundation, Am. Cancer Soc., and Am. Heart Assn.

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TABLE I. Effect of Neonatal Infection with Gross Passage A Leukemia Virus on Allograft Rejection.

	Rejection		Mean rejection time (days)	% Rejected
	Complete	Partial		
Noninfected control	11/20	2/20	23 (12–31)	65
Infected Gross leukemia virus	0/12	—	—*	0

* 12/12 animals accepted skin grafts until the time of their death with leukemia 68 (47-96) days after grafting.

without shrinkage or induration but with very scanty or no hair growth.

Animals were sacrificed when they displayed clinical evidence of leukemia, *viz.*, labored respirations, weight loss, loss of normal hair texture, prominence of the sternum, splenomegaly or lymphadenopathy. The presence of leukemia was verified by the finding of thymic and mesenteric tumors, lymphadenopathy, splenomegaly and hepatomegaly at autopsy.

Results. Among the controls, 7 of the 20 C3H mice accepted Ce skin grafts, a finding in keeping with the expected incidence of takes when grafting across this non-H-2 barrier. Eleven of 20 showed complete rejection in 12 to 31 days (mean 23) and 2 showed partial rejection. In contrast to the controls, 12 of 12 C3H animals infected with Gross passage A virus 4 weeks before grafting accepted the Ce strain skin and the latter remained intact for a mean time of 68 days (range 47-96 days). Eight of 12 grafts showed little or no hair growth and 4 of 12 showed luxuriant hair growth (Table I).

Discussion. The study of lymphoreticular malignancies has emerged as one of the avenues by which we have arrived at a better understanding of the immune system and of the role of its component parts. It has long been known that thymectomy will prevent the occurrence of spontaneous leukemia in Ak mice(3) and a number of other types of leukemia have been shown to be thymus dependent(4). More recently it has been shown that neonatal thymectomy in the mouse results in a profound impairment of immunologic competence(5,6). Although this impairment was thought at first to involve all the parameters of the immune system, it seems clear that those parameters which are most affected are those which come under the heading of cellular immunity, namely, graft rejection, delayed hypersensitivity and graft-*vs*-host reactivity. The impairment of humoral immunity appears to be more subtle, *i.e.*, the thymectomized animal has normal immunoglobulin levels but appears unable to produce antibody in response to stimulation with certain antigens(5,7,8).

Recent studies(9) in our laboratory have clarified experimentally the frequently proposed definition of the immune system in terms of 2 cell populations probably originating in 2 different organs and being responsible for the 2 types of immunity, cellular and humoral. The small lymphocytes originating from and/or being nurtured by the thymus appear to be responsible for cellular immunity and may also play some role in humoral immunity by means of an interaction with the large lymphocytes and plasma cells which originate from another central lymphoid source and which are responsible for immunoglobulin production.

The fact that thymectomy will prevent leukemia in mice injected neonatally with Gross passage A leukemia virus and the fact that histologic studies show that the first pathologic changes occur in the thymus(10), point to the thymus as the origin of this malignancy. One would, therefore, expect that involvement of the thymus by an oncogenic virus should be accompanied by some degree of functional impairment. We have clearly shown that C3H mice injected neonatally with Gross passage A leukemia virus are unable to reject allografts involving a weak histocompatibility barrier during the incubation period of the disease, that is before there is any histologic evidence of the leukemic process. In the preceding study a quantitative deficiency in humoral immunity was also demonstrated.

Thus our findings lend further support to present concepts of the role of the thymus in the immune response. Their implications as to the pathogenesis of leukemia are less clear but yet quite provocative. It may be that presentation of the leukemia-inducing virus to the susceptible cell, the immature thymocyte(11,12) leads to its incorporation into the genome of the cell. This incorporation in turn may compromise the normal development of the thymus cells and result in the observed functional impairment long before the appearance of obvious malignancy.

Further studies are being carried out to determine more precisely the degree of functional impairment of thymic function with regard to graft rejection across stronger histocompatibility barriers and graft-*vs*-host reactivity.

Summary. Six-week-old C3H mice injected neonatally with Gross passage A leukemia virus were found to be unable to reject skin grafts across a weak histocompatibility barrier. The similarity of the defects in the immune mechanisms of these mice to those of the neonatally thymectomized mice suggests that the establishment of the oncogenic agent in the neonatal thymus results in marked functional impairment long before histologic changes are apparent.

We gratefully acknowledge the editorial assistance of Miss Ann Gabrielsen.

1. Peterson, R. D. A., Hendrickson, R., Good, R. A., Proc. Soc. Exp. Biol. and Med., 1963, v114, 517.
2. Gross, L., ibid., 1957, v94, 767.
3. McEndy, D. P., Boon, M. C., Furth, J., Cancer Research, 1944, v4, 377.
4. Stansly, P. G., Progress in Exp. Tumor Research, 1963, v3, 216.
5. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., Papermaster, B. W., J. Exp. Med., 1962, v116, 773.
6. Miller, J. F. A. P., Proc. Roy. Soc. B., 1962, v156, 415.
7. Humphrey, J. H., Parrott, D. M. V., East, J., Immunology, 1964, v7, 419.
8. Arnason, B. G., de Vaux St. Cyr, C., Shaffner, J. B., J. Immunol., 1964, v93, 915.
9. Cooper, M. D., Peterson, R. D. A., Good, R. A., Nature, 1965, v205, 145.
10. Goodman, S. B., Block, M. H., Cancer Research, 1963, v23, 1634.
11. Kaplan, H. S., ibid., 1961, v21, 981.
12. Axelrad, A. A., van der Gaag, H. C., J. Nat. Cancer Inst., 1962, v28, 1065.

Received April 1, 1965. P.S.E.B.M., 1965, v119.

Micropuncture Study of Water Reabsorption and PAH Secretion in Urea Diuresis in Rats.* (30324)

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Platt(1) proposed that in advanced renal failure urine is formed by a reduced number of nephrons which function normally, but under the condition of osmotic diuresis due to an increased urea load. In evaluation of this hypothesis, it seemed important to examine directly water reabsorption during urea diuresis in the normal animal. The limited micropuncture studies available in urea diuresis(2) indicate lower fractional water reabsorption between glomerulus and distal puncture sites than in nondiuretic animals, and a small concentration gradient for sodium across the proximal tubular epithelium. In the present study an attempt was made to obtain more definitive data about fractional water and solute reabsorption in the different parts of the nephron in this type of osmotic diuresis. In addition, p-aminohippuric acid

* This investigation was supported by a grant-in-aid from Am. Heart Assn. and by U.S.P.H.S. Grant No. H-2334.

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