increasing and decreasing milk strontium-90 levels, appears to be a more valid estimate. The present data show that the maximum values for fetal strontium-90 at peak fallout nuclide concentrations in milk, reached an average value of 6.03 pC Sr-90/g Ca. The linear relationship between milk strontium-90 and the deposition of the nuclide in fetal calcified tissue may be useful to estimate fetal concentrations of nuclide from milk values. Conversely, a knowledge of the fetal strontium-90 content may lead to an estimation of the mother's dietary strontium-90 when milk or diet data are not available.

Summary. The strontium-90 content of mandibular bone and tooth buds of 73 fetuses aborted in the St. Louis metropolitan area between 1961 and 1966 has been correlated with the mother's dietary intake of the nuclide. The data obtained during increasing and decreasing periods of fallout may be expressed by a linear equation, $C_B = K\ C^M_{D-6}$, where C_B is fetal bone Sr-90/g Ca, C^M_{D-6} is the mother's dietary strontium-90 intake for the first 6 months of pregnancy and K is a constant equal to 0.21. The equation is useful to calculate the amount of strontium-90 accumulating in fetal calcified tissues from known milk strontium-90 values or conversely, to obtain data concerning the mother's dietary strontium-90 intake when fetal strontium-90 values are known.

1. Rosenthal, H. L., Austin, S., O'Neill, S., Takeuchi, K., Bird, J. T., Gilster, J. E., Nature, 1964, v203, 615.

2. Rosenthal, H. L., Gilster, J. E., Bird, J. T., Science, 1963, v140, 176.

3. Rivera, J., Nature, 1965, v207, 1330.

4. Federal Radiation Council Report 6, Oct., 1964.

5. Michelson, I., Thompson, J. C., Jr., Hess, B. W., Comar, C. L., J. Nutrition, 1962, v78, 371.

6. Comar, C. L., Whitney, I. R., Lengeman, F. W., Proc. Soc. Exp. Biol. & Med., 1955, v88, 232.

7. Kulp, J. L., Schulert, A. R., Hodges, E. J., Science, 1960, v132, 448.

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Endotoxin-Induced Wasting Disease in Mice: A Temporary Condition Explained by Endotoxin Tolerance.* (32129)

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(Introduced by M. F. La Via)
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Bacterial endotoxins acting either directly or indirectly cause a transient depletion in thymic weight and an alteration of the morphology of the thymus of the mouse and rabbit(1,2). The work reported here was undertaken to characterize further the effect of endotoxin on the mouse thymus. First, since the thymus changes morphologically and functionally with the age of the animal, experiments were designed to determine if the effect of endotoxin on the thymus also varied with age. Endotoxin was found to affect the thymus of mice of all ages equally. Second, serial injections of endotoxin were given to neonatal and young mice in an attempt to induce a wasting syndrome similar to that seen following neonatal thymectomy(3) or treatment with cortisol acetate (4). Third, when it was found that bacterial endotoxin induced only a temporary wasting syndrome, the ability of mice to become tolerant to the effect of endotoxin on the thymus was demonstrated.

Materials and methods. Bacterial endotoxin was prepared from *Salmonella typhosa* 0-901 by the phenol-water extraction method of Nowotny *et al*(5), and was injected in non-pyrogenic saline. The mice used through

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TABLE I. Mean Thymic Weights of Mice Given a Single Injection of Endotoxin or Saline.

Day after injection	Age of mouse	Wt (mg) after injection of: Saline	Endotoxin	P value*
3	Newborn†	(11) ‡ 4.3	(16) 2.6	<.02
10	"	(7) 16.3	(13) 13.2	<.02
3	4-8-month§	(5) 25.5	(6) 8.6	<.01
10	"	(6) 21.9	(6) 15.3	<.05

* P values were determined by Student's t test.
† Newborn mice were given 10 μg of endotoxin intraperitoneally.
‡ Numbers in parentheses indicate number of mice per group.
§ 4-8-month-old mice were given 100 μg of endotoxin intravenously.

out these experiments were CBA mice from Cumberland Farms. For studies on neonatal mice, young were raised in this laboratory. At time of sacrifice, the thymuses were carefully dissected from the mice and weighed immediately. Histological sections of the thymus stained with methyl green pyronin were examined.

Results and discussion. To examine the effect of endotoxin on the thymuses of neonatal mice, 10 μg endotoxin were injected intraperitoneally into newborn CBA mice within 24 hours of birth. Control littermates were injected with saline intraperitoneally. Animals from each group were sacrificed 3 and 10 days after injection and the thymuses were weighed and examined histologically. From the data reported in Table I, it is apparent that thymic weight loss occurred in the endotoxin treated animals within 3 days after endotoxin injection. Three days after endotoxin injection the histological appearance of the thymus was similar to that reported previously for 4- to 6-week-old endotoxin treated mice(1). The architecture of the thymus was destroyed; there were fewer lymphocytes, especially in the cortex; there was an increase in pyroninophilic cells in the cortex; and the distinction between cortex and medulla was not clear. Although the weight of the thymus was somewhat below normal 10 days after endotoxin injection, the histological appearance of the thymus had returned to normal by this time.

To compare the effect of endotoxin on the thymuses of newborn mice with the effect of endotoxin on the thymuses of old mice, a similar experiment was performed on mice that were 4 to 8 months old. These animals were given either 100 μg endotoxin or saline intravenously. After endotoxin injection thymic weight loss and recovery were observed as shown in Table I. Although histologic sections showed that the thymuses of normal mice in this age group were partially involuted and contained fewer lymphocytes than the thymuses of the neonatal and the young adult mice, endotoxin nevertheless caused a decrease in thymic lymphocytes as well as a striking loss in weight. The pattern of thymic weight loss following injection of bacterial endotoxin was similar in the newborn mice and in the 4- to 8-month-old mice, and paralleled that seen in the 4- to 6-week-old mice as reported earlier(1). The conclusion seems warranted, therefore, that endotoxin exerts a similar deleterious effect on the mouse thymus regardless of the age of the animal.

Neonatal thymectomy predisposes mice to a wasting syndrome which is characterized by stunted growth, loss of weight, and often death(3). A similar syndrome has been reported following treatment with cortisol acetate(4), reserpine(6), and killed staphylococci or streptococci(7). It was of interest to know if a series of endotoxin injections in young mice could result in drug-induced thymectomy and wasting. Accordingly, neonatal mice were given 10 or 15 μg endotoxin intraperitoneally within 24 hours of birth. Littermate controls were given saline. Since the deleterious effect of endotoxin was maximal 3 days after injection, treatment with endotoxin or saline was repeated every 3 days. All mice were weighed daily and examined for gross physical changes. The mice that received 10 μg endotoxin every third day developed normally. However, the

TABLE II. Mean Thymic Weights of Mice Given 10 Daily Injections of Endotoxin or Saline and Challenged with Endotoxin or Saline.

Materials injected	No. of mice	Avg thymic wt (mg) †
Endotoxin daily, endotoxin challenge*	8	21.45
Endotoxin daily, saline challenge	10	41.0
Saline daily, endotoxin challenge	10	14.8

* Challenge injection was 3 days after last endotoxin injection.
† The differences in mean thymic weights between all groups were found to be significant by Student's t test (P values <0.01).

growth and development of those that received 15 μg endotoxin every third day were delayed. The average body weight of these animals and their littermate controls is plotted in Fig. 1. Each point represents an average of at least 10 mice. It is apparent that the endotoxin treated animals did not gain weight as rapidly as the controls. Body weight was significantly different ($P<0.01$) 1 day after the first injection of endotoxin and continued to be significantly different ($P<0.02$) until day 45 ($P>0.05$). These results are essentially similar to those reported by Salvin *et al*(8). In addition, until about day 25 these mice showed delayed hair growth, they were less active, and they had peculiarly humped postures. As the experiment progressed, these mice began to gain weight at the same rate as the controls and became more normal in appearance. At termination of the experiment all mice were autopsied. With the exception of one animal, the thymuses and spleens of these mice were normal in weight and histological appearance.

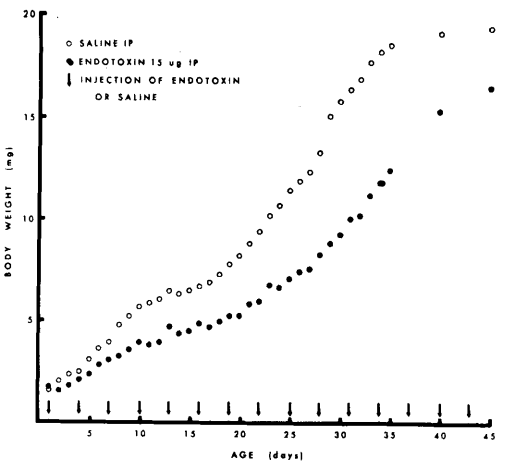

FIG. 1. Mean body weights of mice given 15 μg endotoxin or saline intraperitoneally every third day since birth.

Since, as seen in Fig. 1, this wasting condition was temporary and mice began to gain weight normally about the 25th day of life, it was quite possible that they had become tolerant to the toxic effects of endotoxin. Thus an experiment was performed to determine whether or not mice could be rendered tolerant to the effect of endotoxin on the thymus. Four- to 6-week-old CBA mice were given daily injections of 100 μg endotoxin intraperitoneally for 10 days. After a 3-day rest, half were given a challenge injection of endotoxin and half were given saline. Controls included normal mice given either endotoxin or saline. Three days after the challenge injection all mice were sacrificed and their thymuses examined. As seen in Table II the thymuses of tolerant mice given endotoxin weighed less than those of tolerant mice given saline, but more than those of normal mice treated with endotoxin. Thus we conclude that mice can develop tolerance to the effect of endotoxin on the thymus. Therefore, chronic treatment with endotoxin would not be expected to result in a permanent and fatal wasting syndrome, but rather would cause a temporary delay in growth as seen in Fig. 1.

Summary. 1. Bacterial endotoxin causes a loss of thymic lymphocytes and a change in thymic morphology regardless of the age or stage of development of the mouse. 2. Injection of endotoxin into neonatal mice every 3 days causes a temporarily stunted growth rate, as manifested by slower gain in total body weight than that seen in saline injected controls. 3. That the wasting-like syndrome induced by endotoxin is temporary appears to be due to the development of tolerance to the effect of endotoxin on the thymus.

1. Rowlands, D. T., Jr., Claman, H. N., Kind, P. D., Am. J. Path., 1965, v46, 165.

2. Landy, M., Sanderson, R. P., Bernstein, M. T., Lerner, E. M., II, Science, 1965, v147, 1591.
3. Miller, J. F. A. P., ibid., 1964, v144, 1544.
4. Schlesinger, M., Mark, R., ibid., 1964, v143, 965.
5. Nowotny, A. M., Thomas, S., Duron, O. S., Nowotny, A., J. Bact., 1963, v85, 418.
6. Draskoci, M., Jankovic, B., Nature, 1964, v202, 408.
7. Ekstedt, R. D., Nishimura, E. T., J. Exp. Med., 1964, v120, 795.
8. Salvin, S. B., Peterson, R. D. A., Good, R. A., J. Lab. Clin. Med., 1965, v65, 1004.

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Recent Adenovirus Isolates Exhibiting Broad Intratypic and Intertypic Antigenicity.* (32130)

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Typing of human adenovirus isolates is routinely accomplished by demonstrating a serological relationship with one of the prototype viruses using either serum neutralization (Nt) or hemagglutination-inhibition (HI), or preferably both(1-3). Recent reports have described strains which demonstrate intertypic crossings when tested by either technique or by both(4-10). At present, the significance of this antigenic overlap and variation within the adenovirus group is not clear, but practically it poses important problems for investigators concerned with the identification of field isolates. In a recent study of infantile diarrhea(11), several adenovirus strains were recovered which behaved as variants of prototype strains. The present report describes those strains which were found to be serologically related to, but certainly not identical with, prototype strains, and further demonstrates the importance of the reactivity of the antisera used in typing adenovirus isolates.

Materials and methods. Viruses and sera. Prototype human adenovirus types 1-18 were received from Dr. W. Dowdle, Communicable Disease Center (CDC), Atlanta, Georgia, who also kindly performed some serological tests with the strains described in this report. Additional strains of type 14 were obtained from Dr. M. Green, St. Louis and Dr. R. Chanock, Bethesda. Types 19-30 were obtained from the American Type Culture Collection. The isolates referred to as Karachi strains were recovered from rectal swabs collected during a study of infantile diarrhea in Pakistan(11). Isolates were propagated in monolayer cultures of human fetal kidney cells maintained with Eagle's medium containing 2% fetal bovine serum and 0.225% sodium bicarbonate. For hemagglutination studies, virus stocks were prepared in KB monolayer cell cultures maintained with medium 199 containing inactivated chicken serum and 0.225% sodium bicarbonate. Twenty-four hours after the infection had detached all the cells from the glass (usually 48-72 hours post-inoculation), culture fluids were frozen and thawed, clarified by centrifugation at 1000 $\times$ *g* for 15 minutes, and frozen in aliquots at —90°C.

Antisera to prototype adenovirus types 1-18 prepared in horses were kindly made available by the CDC, Atlanta(12). Antisera to types 19-30 were reference reagents prepared in rabbits and distributed by the National Institute of Allergy and Infectious Diseases. Other rabbit antisera (London) against prototype adenoviruses were prepared by injecting rabbits with 2 weekly intramuscular doses of virus in complete Freund's adjuvant, followed a week later by one intraperitoneal dose of

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