with the net result of a more potent action.

Summary. The capacity and affinity of avian plasma proteins for triiodothyronine are less than those for thyroxine. The half-lives of thyroxine and of triiodothyronine in avian blood plasma are approximately equal; the half-life of triiodothyronine in cardiac tissue is consistently less than that of thyroxine.

1. Barker, S. B., *Annual Review Physiology,* 1955, p551, Annual Reviews, Inc., Stanford, Calif.
2. Shellabarger, C. J., *Poult. Sci.,* 1955, v34, 1437.
3. Newcomer, W. S., *Am. J. Physiol.,* 1957, v190, 413.
4. Mellen, W. J., Wentworth, B. C., *Poult. Sci.,* 1959, v38, 228.
5. Newcomer, W. S., Barrett, P. A., *Endocrinol.,* 1960, v66, 409.
6. Tata, J. B., Shellabarger, C. J., *Biochem. J.,* 1959, v72, 608.
7. Shellabarger, C. J., Tata, J. B., *Endocrinol.,* 1961, v68, 1056.
8. Balfour, W. E., Tunnicliffe, H. E., *J. Physiol.,* 1960, v153, 179.
9. Taurog, A., *Endocrinol.,* 1963, v73, 45.
10. Hamolsky, M. W., Stein, M., Freedberg, A. S., *J. Clin. Endocrinol.,* 1957, v17, 33.
11. Crispell, K. R., Kahana, S., Hyer, H., *J. Clin. Invest.,* 1956, v35, 121.
12. Crispell, K. R., Coleman, J., *ibid.,* 1956, v35, 475.
13. Friis, T., *Acta Endocrinol.,* 1960, v33, 134.
14. ———, *ibid.,* p117.
15. Tata, J. R., *Biochem. J.,* 1959, v72, 214.
16. Friis, T., *Dan. Med. Bull.,* 1960, v7, 89.
17. Christensen, L. K., Endocrinol., 1960, v66, 138.
18. Crispell, K. R., Coleman, J., Hyer, H., *J. Clin. Endocrinol. Metab.,* 1957, v17, 1305.
19. Ingbar, S. H., *Endocrinol.,* 1958, v63, 256.
20. Albert, A., Keating, F. R., *ibid.,* 1952, v51, 427.
21. Roche, J., Michel, R., *Ann. N. Y. Acad. Sci.,* 1960, v86, 454.
22. Engbring, N. H., Lennon, E. J., Engstrom, W. W., *J. Clin. Invest.,* 1960, v39, 983.

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Bacteria Induced Morphologic Changes.* (29324)

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The occurrence and characteristics of enlarged ceca in germfree mammals has been reviewed by Luckey(1). Enlargement of the cecum is not a constant phenomenon in all germfree animals: some germfree rats have no enlargement of the cecum and it does not occur in germfree chicks. Reduction in the size of the enlarged cecum when germfree mammals were contaminated or inoculated with normal intestinal flora has been reported repeatedly. However all bacteria do not contribute this factor since enlarged ceca were regularly seen in germfree rats inoculated with *Lactobacillus acidophilus* and their gntophoric offspring. Gordon(2) reported that the rate of weight reduction of the cecal wall was considerably slower than that of the cecal contents. This led to the assumption that the increased cecum size of germfree mammals was due to reduced muscle tone.

The poor development of the lymphatic system of germfree mammals, first noted by Glimsted(3), was also reviewed by Luckey (1). Since the lymphatic system is intimately involved in the cellular and humoral responses to infection, it is assumed that the absence of demonstrable flora is a prime factor in this underdevelopment. The classic laboratory rat has a mass of lymphatic tissue at the apex of the cecum about one centimeter in diameter. That of the classic mouse is about 2 mm in diameter. Grossly and microscopically this looks like a large Peyer's patch and was noted by Farris and Griffith(4). The apex is the only place in the cecum where a well defined patch of lymphatic tissue is seen in classic rats, mice, hamsters and guinea pigs. The appendix of man, which appears at the apex of the "residual" cecum, is a lymphatic organ. Rabbits have both structures: the distal one-

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third of the large cecum is a lymphatic organ.

The purpose of this study was to note gross morphologic changes induced in the cecum of gnotobiotic albino rats by monoinoculating germfree rats with an organism isolated from the feces of a healthy classic rat. The parameters reported are the size of the cecum and its associated lymphatic tissue. The development of the cervical lymph nodes was studied in germfree and monoinoculated mice.

Methods. Male germfree white rats† of the Lobund-Fischer strain and mice† of the NLICR strain were maintained in portable plastic isolators. The gnotobiotic status was confirmed periodically according to the method of Wagner(5). Some of the germfree mammals were autopsied and the remainder were monoinoculated orally with 2 drops of media containing 1,000,000 Streptococci.‡ The monoinoculated animals were maintained in an isolator in the same manner as were the germfree animals. Monoinoculated animals were autopsied at one, 3, 7, and 14 days after inoculation. Specific pathogen free (SPF) rats of the same strain were autopsied and weanling SPF rats were placed in our stock colonies to become "conventionalized." These were autopsied when they reached the age of the other rats in the study—4 months. All animals were fed autoclaved (120°C for 25 min) practical diet§ *ad libitum* until sacrifice. The animals were anesthetized with Na pentobarbital and exsanguinated from both the jugular and carotid vessels. The cecum was weighed full, emptied and weighed again. The area of the cecum was determined with a planimeter following a tracing of the open sac spread upon a paper. All values were expressed in the amount per 100 g body weight. Statistical comparisons were made with the method of least squares using a Student t test. The results with the rats were taken from 2 experiments; the first had 2 animals per group and the second had 3. The

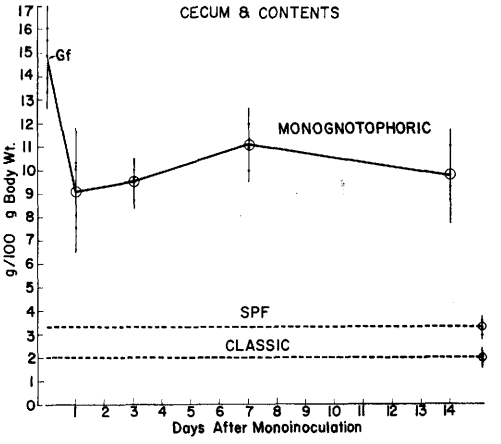

FIG. 1. Weight of cecum and contents per 100 g body weight in white rats. The monognotophoric rats were germfree rats inoculated at 0 days on abcissa scale. SPF and classic rats were uninoculated controls. Vertical bar extends 2 standard deviations from average. Dots give the actual values. Circle indicates statistical probability of differences from the average value obtained with germfree rats: quarter circle, p = 0.021-0.05; half circle, p = 0.011-0.2; three-quarter circle, p = 0.001-0.01; full circle, p = <0.001. GF = Germfree; SPF = Specific pathogen free.

groups taken at 7 and 14 days had only 4 rats each due to the death of 2 (out of 20) monoinoculated rats with bloat following inoculation. The number of mice were 4 in the germfree group, 4 were monognotrophoric one day and 3 in the other groups. All cervical lymph nodes of each mouse were extirpated, carefully cleaned and weighed as a unit.

Results. The weight of the cecum plus contents was found to drop sharply in the first 24 hours after inoculation to about 60% of the weight found in the germfree rats (Fig. 1). Over the remainder of the 2-week period no further reduction was noted. The mean value of the weights of the full ceca of SPF rats was only one-fifth as great as that of the germfree rats. This value was significantly higher than that of the classic rats. The weight and surface area of the cecal wall did not vary significantly during the 2-week period. The average weight of the empty cecum was twice as heavy in the germfree rats as in the clasic rats. Similar but less striking changes were noted in the ceca of the mice.

The cecal apical aggregate found in classic rats and mice was not grossly visible in the

† Obtained from Kelley National Animal Laboratory, Creve Coeur, Mo.

‡ Originally isolated as a *Streptococcus* species from the feces of a conventional rat, this organism could not be identified further by two laboratories.

§ Purina Laboratory Chow Special Formula—Code 5010, Ralston Purina Co., St. Louis, Mo.

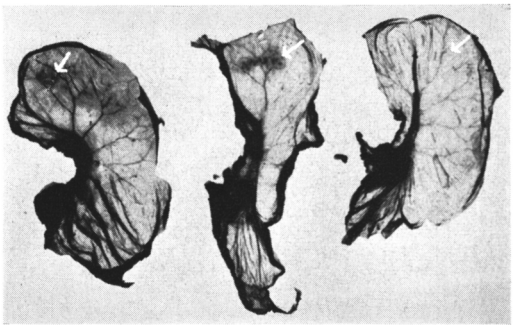

FIG. 2. Apical aggregate area of ceca. Ceca from classic, monoinoculated 1 day and germfree rats from left to right respectively. Cecum is opened and apex is spread to reveal dark aggregates of lymphatic tissue (or area where it would be found if it were present in the germfree rats). Magnification ⅜ ×.

cecum of the germfree rats (Fig. 2, Fig. 3-a), mice and some SPF rats. It was seen in monognotophoric mice 18 hours following inoculation. It was visible in 3 out of 5 of the gnotophoric rats monoinoculated 24 hours and in all animals monoinoculated for 72 hours or longer. At 24 and 72 hours it had a rather diffuse character, (Fig. 3-b) but within 7 days it had an appearance typical of that found in classic rats (Fig. 3-c). Microscopic examination revealed that this aggregate had the general features of a Peyer's patch.

The average weight of the combined cervical lymph nodes from each germfree mouse was 3-fold less than that of the inoculated mice (Fig. 4). The major increase occurred during the first 18 hours following inoculation.

Discussion. The expulsion of cecal contents without comparable change in the area or weight of the cecum wall as measured at autopsy is an apparent contradiction. The persistent decreased cecal contents (from 1 to 14 days in this study) without concomitant change in cecal wall would suggest that there must be frequent expulsions of the contents. If this be true, then the muscles of the cecal wall can contract and the apparent lack of muscle tonus in germfree mammals must be due to a lack of proper environment or stimulus. Fiber in the diet provides partial correction(1); the presence of certain bacteria is more effective. *Lactobacillus acidophilus* does not provide this stimulus(1); the streptococcus used herein did.

The rapid development of the cecal apical patch where none existed before is the most dramatic qualitative change in lymphatic tissue which has been found following the inoculation of germfree rats. Gordon and Wostmann(6) report an increased quantity of Peyer's patches within 7 hours, and almost

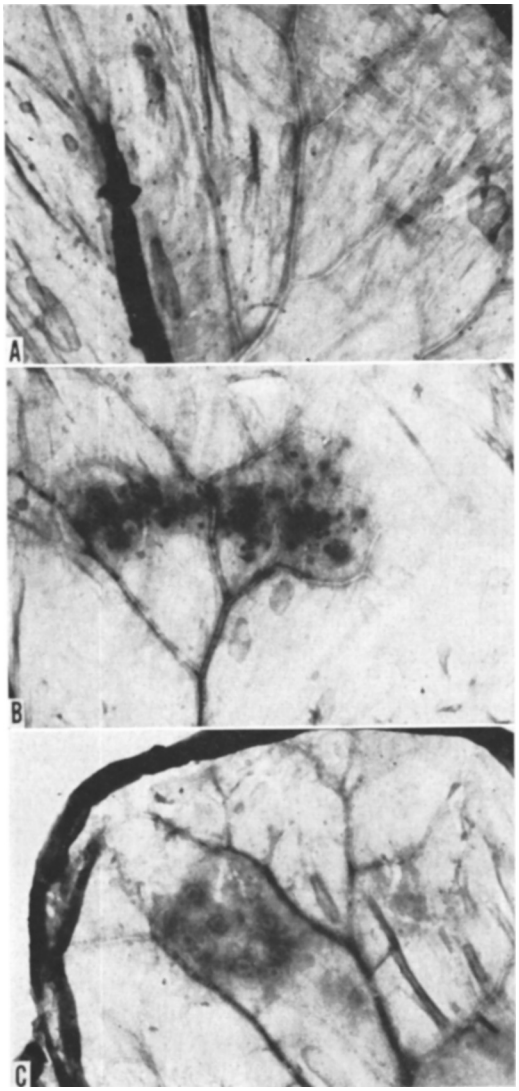

FIG. 3. 1.6 Enlargement of cecal apices. 3-a. Apex of cecum from a germfree rat. Note the clarity of the wall throughout the area which contains the apical patch in classic animals. 3-b. Somewhat diffuse aggregates are seen in gnotophoric rats which had been germfree until inoculated with a pure culture of *Streptococcus* one day before autopsy. 3-c. The more clearly defined aggregate of the apical patch found in classic rats. Some detail of the character is seen in this enlargement.

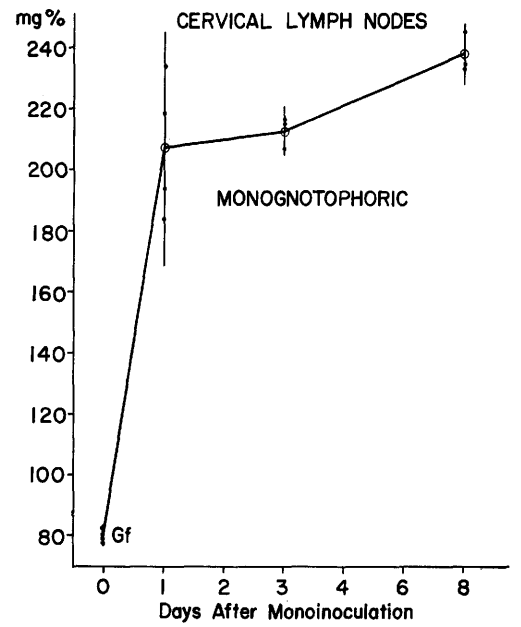

FIG. 4. Weight of combined cervical lymph nodes per 100 g body weight in white mice. The monognotophoric mice were germfree before being inoculated at 0 days on the abcissa. See Fig. 1 for explanation of the symbols.

5-fold increase of the size of ileocecal nodes one week after gross contamination.

Important questions are raised by the observation of the rapidly developing lymphatic tissue. What do the bacteria provide to evoke such dramatic response? How fast do these develop following inoculation? What other microorganisms will do this? What other defense mechanisms are stimulated? The ease with which answers to such questions may be approached illustrates the power of gnotophoric techniques(1) in the study of defense mechanisms.

Summary. Inoculation *of Streptococcus sp.* into germfree rats resulted in dramatic cecal changes within 24 hours. The weight of the cecum and its contents were reduced almost 50% and an apical aggregate of lymphatic tissue appears as a grossly visible entity, lacking only the more densely clumped nature of that found in classic rats. Exploratory work with mice confirmed this observation. Although the size (weight or surface area) of the cecal wall of the germfree rats is twice the size of that in classic rats, no change was noted when germfree rats were monoinoculated with *Streptococcus sp.* The cervical lymph nodes increased 3-fold following inoculation of germfree mice. This exploratory work illustrates the potential of gnotophoric animals in the study of defense mechanisms.

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1. Luckey, T. D., *Germfree Life and Gnotobiology,* Academic Press, N. Y., 512 pp., 1963.

2. Gordon, H. A., *Ann. N. Y. Acad. Sci.,* 1959, v78, 208.

3. Glimsted, G., *Anat. Anz.,* 1932, v75, 79.

4. Farris, E. J., Griffith, J. Q., *The Rat in Laboratory Investigation,* Lippincott, Philadelphia, Pa. 542 pp., 1949.

5. Wagner, M., *Ann. N. Y. Acad. Sci.,* 1959, v78, 89.

6. Gordon, H. A., Wostmann, B. S., *Recent Progress in Microbiology,* C. C Thomas, Springfield, Ill., 1959, p. 336.

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Factors Controlling Calcification *in vitro*: The Calcium Phosphate Ratio.* (29325)

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In a very early study of calcification processes, Robison, employing epiphyseal cartilage slices from rachitic rats, concluded that the calcium concentration was relatively more

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