pyelonephritic rabbits were saved for further investigation. Cultures of the urine and kidneys were sterile and no gross or microscopic renal abnormalities were observed in the control rabbits.

Discussion. Although it has been well recognized that tolerance to endotoxin may be readily induced it had not previously been observed to result from actual bacterial infection(2,3). The present study indicates that infection of the kidney by Gram negative bacteria is capable of inducing tolerance to heterologous endotoxin. A previous study failed to demonstrate pyrogen tolerance following experimental *E. coli* peritonitis in rabbits(2), but these apparently conflicting observations may reflect only a difference in the 2 types of infection. The experimental peritoneal infection was acute and the animals either succumbed rapidly or recovered without evidence of persistent chronic infection. Pyelonephritis, on the other hand, was associated with bacterial proliferation which continued to the time of endotoxin challenge. The latter infection allowed more prolonged exposure of the reticuloendothelial system to endotoxin. This agrees with previous observations that repeated prolonged endotoxin challenge produces tolerance more effectively than a brief exposure.

1. Beeson, P. B., *J. Exp. Med.*, 1947, v86, 29.
2. Bennett, I. L., *ibid.*, 1948, v88, 267.
3. Heyman, A., Beeson, P. B., *J. Lab. & Clin. Med.*, 1949, v34, 1400.

Received April 17, 1961. P.S.E.B.M., 1961, v107.

Abnormal Palate Morphogenesis in Mouse Embryos Induced by Riboflavin Deficiency.* (26640)

BRUCE E. WALKER AND BURTON CRAIN, JR.
Department of Anatomy, Univ. of Texas Medical Branch, Galvestion

Kalter and Warkany(1) produced a variety of anomalies in the offspring of pregnant mice by feeding a riboflavin-deficient diet containing galactoflavin (a riboflavin antagonist). The frequency of the various anomalies differed among the several inbred strains used. Cleft palates occurred in 76% of the offspring from treated mice of the DBA strain. Embryonic development was not investigated by these authors, so the following study was undertaken to search for a critical deviation in palate morphogenesis during induction of riboflavin deficiency.

Materials and methods. Pregnant mice of the DBA and C57BL strains were fed a riboflavin-deficient diet containing 12 mg galactoflavin† per g of diet, starting at 10⅓ days postconception and transferring to regular diet at 14⅓ days. Uteri were collected at 14 to 16 days postconception or at 18⅓ days, fixed in Bouin's fluid, and the embryos dissected out for palate examination and for estimates of "developmental age" according to their stage of morphological development (2).

Results. Treated litters of the DBA strain at 14 to 16 days postconception differed grossly from untreated litters in 2 respects. First, the treated embryos were quite edematous; second, the long axis of the snout was usually at right angles to the body axis instead of being pressed down on the chest as in the control embryos.

Table I shows the condition of palate morphology(3) relative to the embryo's morphological rating, the latter calculation being based on the developmental state of several external features(2). The palates of all control embryos had moved to the horizontal plane and started to fuse by morphological rating 11 in this experiment and by 12 in a previous experiment(3). Some of the

* Supported by a research grant from Nat. Inst. of Dental Research and a Traineeship from USPHS.

† Supplied by Merck and Co., Inc., Rahway, N. J. through the courtesy of Dr. N. S. Ritter.

TABLE I. Relation of Palate Stage to Morphological Rating in Embryos from Riboflavin-Deficient (R) and Control (C) Pregnant Mice of the DBA Strain.

Morphological rating	Palate stage 1		2		3		4		5		6		7	
	R	C	R	C	R	C	R	C	R	C	R	C	R	C
−2	3													
−1	2	1												
0	5	1												
1	2	1												
2	1													
3	4													
4	5	4												
5	6	3												
6	3	1												
7	12	3												
8	3						3	2	2	2				
9	5					1			1	2	1			
10	3					1	1		1	1	3	3	1	1
11			1				1				6	3	1	1
12	7						2		1		2	2	2	2
13					2		1				3		5	5
14	2		1				1						2	1
15	2				2								3	1
16	2						1						4	2
17							2						3	3
18	2				2		1						4	4
19							1				1		3	3
20													1	
21											1			
22					1									
23	1				2									
24					1								1	
25														
26														
27														
28													1	
29														

treated embryos had also commenced palate fusion by morphological rating 11, but most of them showed some degree of retardation in movement of the palatine shelves from a vertical to a horizontal position (Table I). However, aside from this alteration in shelf position, no pronounced morphological changes of the palatine shelves were seen grossly or in sections.

At 18 days postconception, the morphology of the fetal palates was as follows: both shelves vertical in 6 fetuses; one shelf vertical and the other horizontal in 7 fetuses; both shelves horizontal in 10 shelves horizontal and fused (normal) in 31 fetuses. This constitutes an average palate stage(2) of 0.80 and a cleft palate frequency of 42%.

There were no cleft palates among 67 fetuses collected at day 18 from 11 treated C57BL female mice, so no litters were collected at the time of palate closure in this strain.

Discussion. The observations on effects of riboflavin deficiency in this experiment support the conclusions of Kalter and Warkany(1) that there are strain differences in the effects of this treatment and that a high frequency of cleft palate can be induced in offspring of the DBA strain. In the embryos from treated DBA mice, the only observed deviation in palate morphogenesis of sufficient magnitude to cause cleft palate was retardation of palatine shelf movement. At the time of normal palate closure, no difference was seen in the morphology of palatine shelves in treated embryos which achieved shelf movement within the normal period of time and those with delayed shelf movement. Subsequent alterations in the morphology of the palate region were apparently secondary

to failure of shelf fusion. In untreated embryos of 3 strains previously investigated(3), palatine shelves had reached the horizontal position and started to fuse before a morphological rating of 13 in all cases. It is probable that embryos in which shelf fusion had not started by morphological rating 15 would develop cleft palate. In Table I, 20 embryos had unfused palates and 22 had fused palates at morphological rating 15 or later. This frequency of delayed shelf movement is similar to the frequency of cleft palate in the late (day 18) fetus. The delay in shelf movement was also reflected in the various shelf positions in the fetus.

Thus, the sensitive phase of palate morphogenesis is again the process of shelf movement, as it was with cortisone-induced cleft palate(4), hypervitaminosis A-induced cleft palate(2), the spontaneous cleft lip-cleft palate of the A/Jax strain(5) and cleft palate produced by amniotic sac puncture(6). The cause of shelf movement retardation was not pressure against the lower jaw and tongue due to the head being forced against the chest as in the case of amniotic sac puncture(6), since the lower jaw was not in contact with the chest. However, the possibility of pressure effects cannot be ruled out since the edematous condition of the embryo may have altered normal pressure relationships in the amniotic cavity and embryo.

Summary. Retardation of palatine shelf movement was found to be the morphogenetic basis for the cleft palates induced in DBA strain mouse embryos by a riboflavin-deficient, galactoflavin-containing diet.

1. Kalter, H., Warkany, J., *J. Exp. Zool.*, 1957, v136, 531.
2. Walker, B. E., Crain, B., Jr., *Am. J. Anat.*, 1960, v107, 49.
3. Walker, B. E., Fraser, F. C., *J. Embryol. Exp. Morph.*, 1956, v4, 176.
4. ———, *ibid.*, 1957, v5, 201.
5. Walker, B. E., Crain, B., Jr., *Tex. Rep. Biol. Med.*, 1959, v17, 637.
6. Walker, B. E., *Science*, 1959, v130, 981.

Received April 17, 1961. P.S.E.B.M., 1961, v107.

Quantitative Changes of a Human Salivary Bactericidin for Lactobacilli Associated with Age.* (26641)

LLOYD B. AUSTIN AND BERNARD J. ZELDOW (Introduced by J. J. Holland)
School of Dentistry, University of Washington, Seattle

It has been established that the oral microbial flora are relatively characteristic for an individual. Antibacterial systems present in the saliva are among several factors postulated to contribute to selection of the resident oral flora. Since selection of the oral microbiota usually commences shortly after birth, the influences exerted by such systems would be expected early. The salivary glands of human infants, however, are regarded as physiologically immature(1) and it is possible, therefore, that the level of any salivary antibacterial system would also reflect this immaturity.

A bactericidin for various species of lactobacilli has been described in mixed, parotid and submaxillary saliva of human adults(2). Since this is not a product of bacteria and is lacking in the serum, it appears to be an *endogenous* product of the salivary glands. The present report gives results of an investigation of the titers of this salivary lactobacillus bactericidin as a function of age.

Methods. The mixed salivas were collected from humans ranging in age from birth to 56 years. Individual salivas were grouped together for study as follows:

A. Premature newborns: delivered prior to expected gestation and weighing 5½ lb or less
B. Full-term newborns: age 1 to 5 days
C. Infants: age 5 days to 1 year
D. Preschool children: age 1 to 6 years

* Supported by grant from N.I.H., U.S.P.H.S.