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.

E. Primary school children: age 6 to 12 years
F. Adults: age 20 to 56 years

All subjects beyond one year of age were placed into their respective group by calculating to the nearest birthday.

Saliva from the adult and primary school age children was collected by paraffin stimulation and expectorating the accumulated saliva into a sterile jar. In the case of preschool age children, infants and the full-term newborns, a sterile ball of cotton firmly attached with a string was inserted into the oral cavity with the aid of a tongue blade, permitting the secretions to collect in the cotton. When saturated, it was removed by the string and the saliva was expressed with a sterile hand press into a sterile receptacle. It was found that this method of collection had no deleterious effect upon the antibacterial system investigated.

The bactericidal activity of the individual salivas was measured using the assay organism, *Lactobacillus acidophilus* ATCC 4357. All specimens with the exception of those collected from the premature newborn group were assayed by the 2-fold dilution method in Lactobacillus Selective broth pH 5.5(3), to which 1/5 volume of a suspension of the assay organism containing approximately 3×10^7 organisms per ml was added. The mixtures were incubated at 37°C for 18 hours and examined for turbidity as evidence of growth. For quantitative comparison, bactericidal activity is expressed in units representing double the reciprocal of the maximum number of times 0.5 ml of saliva could be diluted and still inhibit the growth of the assay organism. Four units of activity per ml is the minimum activity discerned by this method.

Saliva from the premature newborn was collected by inserting a pledget of cotton into the mouth with a hemostat and absorbing the salivary secretions. The antilactobacillus activity of these specimens was immediately tested by placing the saturated cotton pledget on a pour plate seeded with the assay organism. After 18 hours incubation at 37°C, activity by this qualitative agar diffusion method was discerned by a clear zone representing inhibition of the organism.

TABLE I. Number of Subjects in Varying Age Groups Showing Indicated Levels of a Human Salivary Lactobacillus Bactericidin.

Levels of bactericidin activity (units/ml)	Groups					
	A*	B	C	D	E	F
	No. of subjects					
0	4					
<4†		15	0	0	0	0
4		4	11	2	0	0
8		2	10	16	2	1
16		0	5	12	18	11
32		0	0	9	10	7
64		0	0	0	1	2

* Agar diffusion method applicable to Group A only.
† <4 indicates no activity by quantitative bactericidin test.

Results. The data are presented in Table I. One hundred forty-two specimens of mixed saliva were studied. Although limited in number, the results strongly suggest that the lactobacillus bactericidin system is lacking in the saliva of the premature infants. Moreover, during the first 4 days of life the majority of full-term newborns showed no salivary antilactobacillus activity as discerned by the quantitative lactobacillus bactericidin test. However, the appearance of the lactobacillus bactericidin is rapid. After 4 days of life the salivas of all of the full-term infants demonstrated activity. With increasing age, there was an increase in amount of the lactobacillus bactericidin in the saliva. A chi square test of the frequency distributions of subjects according to levels of lactobacillus bactericidin shows significant differences at the .001 level in Groups B, C, D and E. The pattern of distributions reveals systematic shift of increasing activity with increasing age until the adult level is reached. Apparently, it is not until 6 to 12 years of age that the adult level is approached, there being no significant difference in the frequency distribution of subjects in Groups E and F.

The lactobacillus bactericidin of the saliva of infants and children possessed all the known qualities of the adult system(2,4); namely, it required a dialyzable heat stable anionic cofactor, and the non-dialyzable fraction was destroyed at 75°C for 5 minutes at

pH values in the range of 7.2 to 11.0 but was unaffected by such treatment at pH 5.5. Moreover, the bactericidin is not adsorbed by the homologous organisms but is adsorbed on bentonite. Comparison of the various groups indicated that the same system appeared at the various age levels.

Discussion. The correlation between increase of age and increasing levels of salivary lactobacillus bactericidin in infants and children does not differ from that which has been demonstrated with other biological systems. For example, the amylase content of saliva has been shown to increase with age (5,6). Moreover, it has been reported that the elevated sodium(7,8) and chloride ion(9) levels of infants decrease as the individual passes to adulthood. Humans have long been considered to be deficient in immunologic capacity during the neonatal period (10,11,12). The explanation for these findings is open to speculation, but maturation of the tissues involved should be considered.

A survey of the literature indicates that there may be several salivary antilactobacillus systems(2,13). Because of this, it was necessary to characterize the system under investigation. The data presented regarding pH range, heat stability, dialysis and adsorption suggest that this antilactobacillus system is markedly similar to the one previously described by Zeldow(2,4) and Kerr and Wedderburn(14). A comparison between the various groups also indicates that the same system appeared at the various age levels.

Because of the time of first appearance and the quantitative increases in the lactobacillus bactericidin with increasing age, it is evident that the role played by this system is probably initiated at a very early age.

Summary. 1. The results strongly suggest that the lactobacillus bactericidin system is not present in saliva of premature newborns. 2. Within the first 4 days of life the majority of full-term newborns show no salivary antilactobacillus activity. 3. There is a systematic increase of lactobacillus bactericidin titers until the adult group's range of activity is approximated, which occurs at about the first decade of life. 4. With the exception of the premature newborns, all age groups show a range of activity. 5. The lactobacillus bactericidin studied was characterized with respect to pH range, dialysis, heat stability and adsorption.

1. Burgen, A. S.V., *Canad. Dent. A. J.,* 1960, v26, 258.

2. Zeldow, B. J., *J. Dent. Res.,* 1959, v38, 798.

3. Rogosa, M., Mitchell, J. A., Wiseman, R. F., *J. Bact.,* 1951, v62, 132.

4. Zeldow, B. J., *J. Dent. Res.,* 1961, in press.

5. Nicory, C., *Biochem. J.,* 1922, v16, 387.

6. Mayer, W. B., *Bull. Johns Hopkins Hosp.,* 1929, v44, 246.

7. Prader, A., Gauteir, E., Gauteir, R., Naef, D., *Helv. paediat. Acta,* 1955, v10, 29.

8. Hungerland, H., Quenzlern, J., Weber, H., *Z. Kinderheilk,* 1957, v80, 179.

9. Dances, J., Grobow, E., Boyer, A., *J. Pediat.,* 1957, v50, 459.

10. Baumgartner, L., *Yale J. Biol. Med.,* 1934, v6, 403.

11. Osborn, J. J., Dances, J., Julia, J. F., *Pediatrics,* 1952, v9, 736.

12. Bridges, R. A., Condie, R. M., Zak, S. J., Good, R. A., *J. Lab. Clin. Med.,* 1959, v53, 331.

13. Green, G. E., *J. Dent. Res.,* 1959, v38, 262.

14. Kerr, A. C., Wedderburn, D. L., *Brit. Dent. J.,* 1958, v105, 321.

Received April 20, 1961. P.S.E.B.M., 1961, v107.