scarcely greater than at 20 days. Again, structures derived from the urogenital sinus distal to its juncture with the genital ducts were keratinized throughout their length; and metaplasia was always more pronounced on, and tended to be limited to, the dorsal aspect of these structures. In one newborn male an area of true metaplasia was observed in the vesico-urethral portion of the sinus at a point corresponding to the upper prostatic urethra. Otherwise, only prekeratinization changes were seen in this more cephalic portion of the sinus. Even in these newborns and more mature fetuses no histologic deviations from normal were seen in the epithelia in regions of the definitive bladder, ureters, kidneys, mesonephric ducts or Müllerian ducts.

Comment. These observations reveal that genito-urinary epithelia in fetal rats, like many other epithelia in older animals, require vitamin A for their morphologic integrity. It appears that this requirement is not manifested by keratinizing metaplasia until the fetal tissues have undergone a certain degree of differentiation. In the urogenital sinus this is attained by, or shortly before, the 18th day of gestation. The fact that keratinization did not affect other epithelia, which in older animals do respond to A-deficiency by undergoing metaplastic changes, does not prove that the unaffected epithelia were differentiated to a lesser degree than those of the urogenital sinus. Nevertheless, it is suggestive in this direction, and it undoubtedly reflects some unobserved morphologic or perhaps functional difference between those tissues that undergo metaplasia during the fetal period and those that react in this fashion to A-deficiency only at an older age.

The prevalence of congenital malformations in the genito-urinary tract of these fetuses is probably somehow related to the particular susceptibility of epithelia in this region to keratinizing metaplasia, although any attempt to evaluate such a relationship must await a more thorough study of the malformations.

Summary. Keratinizing metaplasia in epithelia of the genito-urinary tract was observed in fetal rats taken from mothers fed a vitamin A-deficient ration. Only structures derived from the fetal urogenital sinus were affected. The earliest metaplastic changes were seen in a fetus of 18 days gestational age; by the 20th day keratinization was seen in the dorsal wall of all organs distal to the termination of the genital ducts. Epithelia in other parts of the body were not affected by these changes.

15815 P

Immunization of Mice with Dysentery Antigen Administered by Gavage and by Voluntary Drinking.*

MERLIN L. COOPER AND HELEN M. KELLER.
From the Children's Hospital Research Foundation, and the Department of Pediatrics, College of Medicine, University of Cincinnati.

I. *Living and Killed Culture by Gavage.* In the course of attempts to infect white mice by gavage with living *Shigella sonnei,* we have observed the development of immunity. A high degree of immunity follows administration of large numbers of bacteria. Less immunity follows administration of fewer bacteria.

Fifteen mice were given 6 consecutive daily doses of 49 billion living bacteria with a syringe and blunt needle introduced into the stomach by way of the esophagus. Five days after the last dose, small groups of these mice were challenged intraperitoneally with 16 to

* This study was conducted under a grant-in-aid from the U. S. Public Health Service.

TABLE I.
Immunity in Mice Given Living and Killed *Shigella sonnei* by Gavage. Challenged Intraperitoneally with 0.5 to 1280 MLD of Homologous Culture.

Type of antigen	% of mice surviving No. of doses of antigen 3	4	5	6	7	8
Living culture	57*	55	72	89	73	92
Killed ''	70	50	71	76	85	83

* Each % is based on results with 30 mice.

TABLE II.
Immunity in Mice Drinking Killed *Shigella sonnei* in Place of Water for 21 Days.

No. of mice	Fluid consumed	Homologous challenge intraper. MLD	No. of mice surviving
36	Killed culture*	2 to 2048	36
30	Sterile broth	''	2
31	Water	''	2
36	''†	''	0

* 7.5 billion per day per mouse.
† Virulence control.

160,000 MLD of homologous culture suspended in sterile mucin.[1] All mice survived except one which received 160 MLD.

Smaller numbers of living or heat-killed bacteria were compared for their ability to stimulate active immunity when administered by gavage. Six groups of 30 mice were given 3 to 8 daily doses of 1.4 billion living *Shigella sonnei*. Six other groups of 30 mice were given the same number of doses of 1.1 billion killed organisms. Seven days after the last doses each group of 30 mice was divided into 6 equal groups and challenged intraperitoneally with 0.5 to 1280 MLD of homologous culture suspended in mucin. The percentages of mice surviving are found in Table I. The striking finding was that both living and killed culture yielded similar immunity which increased with increasing number of doses.

The majority of mice remained well during the time they were receiving living culture intragastrically. During this period and for 24 to 48 hours subsequently stool cultures were positive.

II. *Voluntary Drinking of Killed Culture in Place of Water.* A group of 36 white mice were given killed broth culture of *Shigella sonnei* in their water bottles in place of water over a period of 21 days. Another group of 30 mice were given sterile broth in place of water for the same length of time. A third group of 31 mice received only water to drink. Four days after the 21-day period each group of mice was challenged intraperitoneally with 2 to 2084 MLD of homologous culture in mucin. Data in Table II show that all mice survived which had ingested killed culture in place of water; that only 2 mice survived which had consumed sterile broth; and only 2 mice survived which had consumed water. The quantity of killed culture, sterile broth and water consumed daily by each mouse was very similar.

Conclusions. Six daily doses of 49 billion living *Shigella sonnei* administered by gavage to white mice stimulated complete immunity to intraperitoneal injection of 16 to 160,000 MLD of homologous culture suspended in sterile mucin. Killed bacteria in doses of 1.1 billion and living bacteria in doses of 1.4 billion stimulated immunity, the titer of which increased with the number of daily doses. There was no significant difference in the immunizing effects of living and killed culture. Mice which drank killed broth culture in place of water for 21 days were immune against 2 to 2048 MLD of homologous culture injected intraperitoneally.

1 Cooper, Merlin L., and Keller, Helen M., *J. Pediat.*, 1941, **18**, 451.