infection of the mouse with a single intraperitoneal dose of 0.02 g/kg, while the maximum tolerated dose, intraperitoneally, was found to be 0.12 g/kg, corresponding to a therapeutic index of 6. The immediate spirochetocidal effect of (II) was screened in rabbit syphilis: 16 animals with fully developed, dark-field positive chancres were treated with a single subcutaneous injection of (II) dissolved in propylene glycol in doses ranging from 2.5-40 mg (.0008-.013 g/kg). In all animals treated with doses equal to or greater than .006/kg the spirochetes were not demonstrable 5 days after the treatment. As to toxicity, a single intramuscular dose of .05 g/kg is well tolerated while .08 g/kg is lethal.

It follows from these experiments that the condensation product of a chemotherapeutically active arsenical with BAL may result in a new compound combining relatively low toxicity with a significant trypanocidal and spirochetocidal activity.

Similar results, to be reported in a forthcoming publication, have been obtained with analogous BAL derivatives of other aromatic arsenicals as well as with aromatic antimony compounds, including arsanilic acid, tryparsamide, carbarsone, stibanilic and acyl stibanilic acids.

Hence, in a general manner, inclusion of a phenyl substituted arsenic or antimony residue in a 5-membered sulfur-containing ring as in (II) is not incompatible with a significant chemotherapeutic activity.

The new BAL compounds seem of more than theoretical interest in view of their stability and solubility properties. As a group they are more soluble in nonaqueous solvents than the parent organometallics, a fact which may well have repercussions on their chemotherapeutic spectrum. The solubility, and consequently, the distribution within the organism, may readily be modified by replacing the BAL radical in the above-mentioned compounds by other dithiols, such as 2,3-dimercaptopropionic acid, BAL ethers, BAL glucoside, 1,2-dimercaptobenzene, etc.

The mechanism of the therapeutic effect of the BAL derivatives remains to be elucidated. There are at least 2 possibilities: (1) the molecule may act as a whole, which would imply a mechanism not foreseen by the "Ehrlich-Voegtlin SH-arsenoreceptor theory," (2) the BAL compounds are dissociated in the organism and act essentially like the parent compounds.

15814

Epithelial Keratinization as Evidence of Fetal Vitamin A Deficiency.*

JAMES G. WILSON AND JOSEF WARKANY.

From the Department of Anatomy, School of Medicine and Dentistry, University of Rochester and from the Children's Hospital Research Foundation, Department of Pediatrics, College of Medicine, University of Cincinnati.

The appearance of stratified keratinized epithelium in places where such epithelium is not ordinarily found is the cardinal sign of vitamin A deficiency. It has been stated that this histologic change, referred to as keratinizing metaplasia, occurs in all vertebrates regardless of age.[1] However, it has not been described in fetal or newborn animals whose mothers were in a state of vitamin A deficiency during pregnancy. This is noteworthy, since Hale,[2] using the pig, and Warkany and Schraffenberger,[3] using the rat, obtained fetuses that bore severe morphologic

* This work was aided in part by a grant from the Nutrition Foundation, Inc., N. Y.

1 Wolbach, S. B., and Bessey, O. A., *Physiol. Rev.*, 1942, **22**, 233.

2 Hale, F., *Am. J. Ophth.*, 1935, **18**, 1087.

3 Warkany, J., and Schraffenberger, E., *Arch. Ophth.*, 1946, **35**, 150.

anomalies when the mothers were deprived of vitamin A during pregnancy.

The present communication is a report of the finding of keratinizing metaplasia in the genito-urinary tract of fetal rats taken from mothers that were deficient in vitamin A during pregnancy. It is based on a study of serial sections of 14 fetuses and newborns from as many different litters, the gestational age of which ranged from the 16th through the 22nd day. Maternal deficiency was induced and maintained by the same diets and experimental procedures as were used by Warkany and Schraffenberger.[3] Fetuses of comparable ages and newborn rats from mothers fed an adequate diet were sectioned and studied as controls in order to rule out the possibility of keratinization of genito-urinary epithelia by maternal estrogens during the latter part of pregnancy. The histology of the normal epithelia will not be described in this paper, and only those histologic features which represent distinct deviations from the normal will be described for the A-deficient animals. Nevertheless, it should be stated that keratinization normally does not occur in any part of the genito-urinary tract of fetal and newborn rats except in the most distal portion of the urethra, *i.e.*, the external orifice and a short segment contiguous with it.

Neither the control fetuses nor those subjected to maternal vitamin A deficiency exhibited keratinization in any part of the body prior to the 18th day of gestation. All fetuses older than 18 days whose mothers were fed the A-deficient diet, however, showed keratinizing metaplasia in at least some of the genito-urinary epithelia which constituted, or were derived from, the urogenital sinus. A precise localization of the affected epithelia in terms of definitive organs is not feasible in the present report, since the differentiation of the entire urogenital sinus was conspicuously retarded and this retardation, in turn, complicated by the occurrence of several frank malformations. Until a larger series of animals has been studied and plastic reconstructions prepared, no attempt will be made to analyze the malformations, and the location of areas of metaplasia will be described only in a general way.

The 18th day of gestation was the earliest time at which metaplastic keratinization appeared in the offspring of A-deficient mothers. A male fetus of this age was found to possess a small area of truly keratinized epithelium within the urethral plate and another area in the pelvic part of the urogenital sinus where, although no completely keratinized cells were seen, the cytoplasm of superficial cells was hyalinized and contained keratohyalin granules. These changes might be designated "prekeratinization." The findings seem particularly interesting in view of the fact that the epidermis and other ultimately cornified epithelia had not undergone keratinization in either this animal or control fetuses of the same age.

True keratinization, typified by cornification and desquamation of the surface-cells and by hyalinization and keratohyalin granulation in the superficial layers of cells, was consistently found in fetuses of a gestational age of 20 days. It was limited, however, to the distal two-thirds of the urogenital sinus, specifically to the segment lying between the point of juncture of the genital ducts with the sinus, cephalically, and the exterior of the body, caudally. Metaplasia occurred as a continuous strip along the dorsal wall of this segment but, curiously, had affected to a much less striking degree, or not at all, the lateral and ventral walls of this region of the sinus. The point at which keratinization was most pronounced, both in degree and in circumferential extent, was where the genital ducts joined the sinus. This was also the most cephalic part of the affected area. This fact speaks against the possibility that keratinizing changes spread into and along the sinus from the already keratinized epidermis on the exterior, and seems to indicate instead that an *in situ* transformation to a keratinized type of epithelium had occurred. No true cornification was seen in 20-day fetuses above the point of entry of the genital ducts, but prekeratinization changes, beginning hyalinization of the cytoplasm and the appearance of keratohyalin granules in the

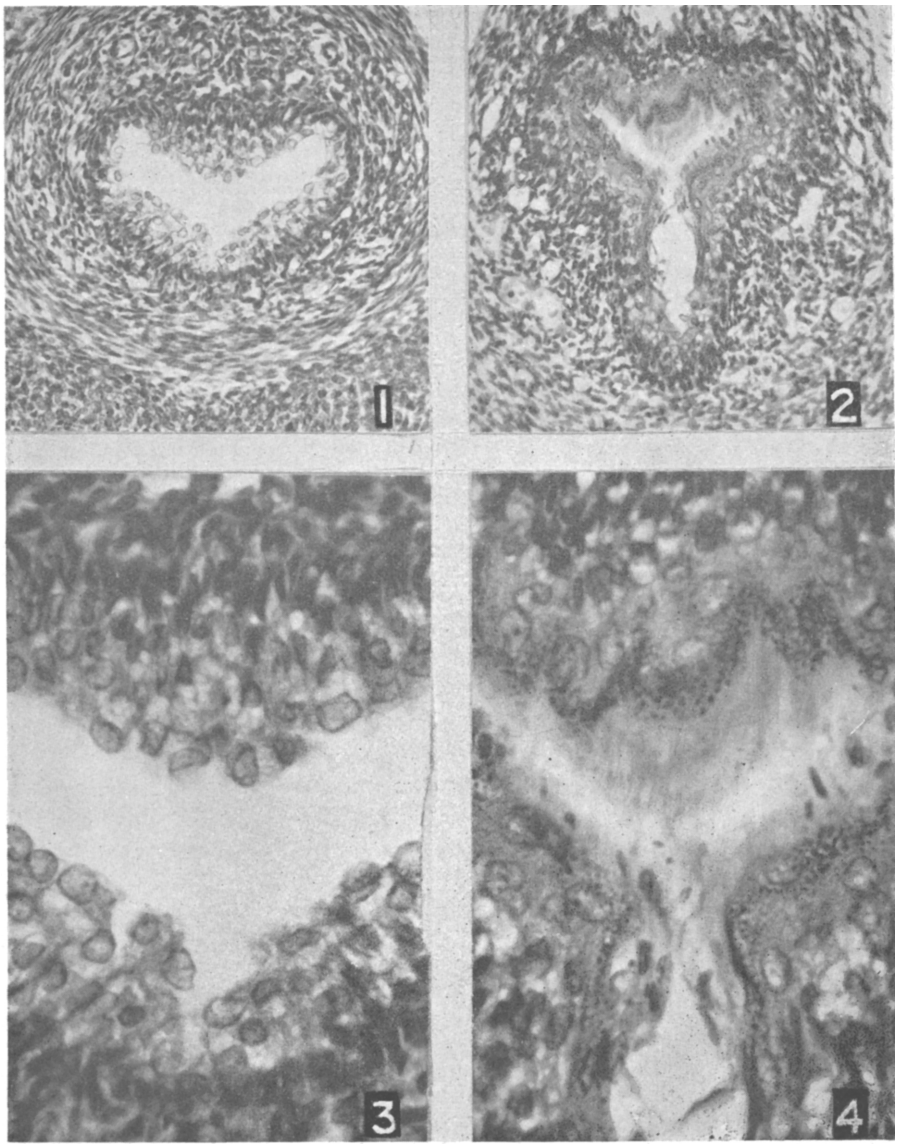

Fig. 1-4.

Fig. 1. Urethra at the level of the urethral sinus of a newborn rat, offspring of a mother fed an adequate diet. (×210)

Fig. 2. Urethra at the level of the urethral sinus of a newborn rat, offspring of a mother fed a vitamin A-deficient diet. (×210)

Fig. 3. Higher magnification of the urethra shown in Fig. 1. (×650)

Fig. 4. Higher magnification of the urethra shown in Fig. 2. (×650)

These photomicrographs are oriented with the "dorsal" wall toward the top of the page. As used here, the term "dorsal" refers to that wall of the urethra derived from the dorsal wall of the embryonic urogenital sinus, and does not accurately describe the relative position of this wall in the newborn rat.

surface-cells, were observed in the region of the upper sinus that roughly corresponded to the neck of the bladder.

Fetuses taken on the 21st and newborns taken on the 22nd day showed perhaps a more advanced degree of cornification (Fig. 1-4), with considerable desquamation at some points, but the extent of the affected area was

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.