to the bladder. Elongation of the canal plus active ingrowth readily explain the presence of the invading tissue at the horn level in older stages (and later still in the lateral vaginal canals).

Recently Zuckerman,[6] reviewing an extensive literature, has reached the same conclusions with regard to a common origin from the urinogenital sinus for tissues sensitive to estrogens; and Raynaud's results on the cat[7] deserve particular mention since he reports cornification of urethra and trigone. Zuckerman also raises, but does not decide the question of ectodermal contribution to the sinus. This is an old problem, never conclusively attacked, but now fairly clearly answered in the opossum.

Summary and Conclusions. 1. Those epithelia in the urinogenital tract of the opossum which respond to estrogens by squamous metaplasia and cornification, have a common origin from the lining of the urinogenital sinus. 2. The sinus epithelium itself is apparently derived at an early stage of development from the ectodermal cloaca or uro-proctodeum, and perhaps in part from the urethral plate. 3. The primitive cloacal endoderm is eventually restricted to the bladder proper. 4. A common ectodermal origin best explains the histological and physiological likenesses of the tissues involved, and the occurrence of stratified squamous epithelium in the lower urinogenital tract of mammals generally. Early development in higher mammals needs reinvestigation with respect to this point. 5. The use of hormones (particularly estrogens) in the immature organism is a valuable method of precociously differentiating tissues not so readily delimited in normal development of the urinogenital tract.

13054

Decomposition of Urea by Proteus.

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At the Third International Congress for Microbiology St. John-Brooks and Rhodes[1] suggested a simplification of the taxonomy of the Proteus genus. On the basis of maltose fermentation and indol

6 Zuckerman, S., *Biol. Rev.*, 1940, **15**, 231.

7 Raynaud, A., *C. R. Soc. Biol.*, 1937, **126**, 215.

1 St. John-Brooks, R., and Rhodes, M., *Third International Congress for Microbiology, Report of Proceedings*, 1939, 167.

production 3 species were set up in the genus, *indologenes (vulgaris), morganii,* and *anindologenes (mirabilis). Proteus americanus* and *Proteus ammoniæ* were considered variants of *anindologenes. Proteus hydrophilus, Proteus pseudovaleriei,* and *Proteus icthyosmius* were excluded from the genus on the basis of their ability to ferment mannitol.

The decomposition of urea with formation of ammonia is one of the major criteria for the determination of the Proteus group (Bergey).[2] This phenomenon, however has received relatively little attention, particularly when recent additions have been made to this genus. From a review of the literature it appears that the action on urea of *Proteus morganii, Proteus hydrophilus, Proteus icthyosmius,* and *Proteus bombycis* is unknown.

Investigators, with few exceptions, have used sterile urine as a culture medium to test urea decomposition by Proteus. Complications arising from the use of such a variable medium are obvious. Moltke[3] and Yacob[4] employed a medium consisting of 2% urea in 0.85% saline. These investigators point out that substances in the inoculum initiate growth and we have found that serial transplants in their medium fail to grow. After several attempts a medium of known composition has been devised, in which small inocula grow and in which serial transplants can be made.

Materials and Methods. The medium finally employed contained 2% urea (Merck), 0.01% yeast extract (Difco), and M/15 primary and secondary phosphate buffers (Sorensen) in distilled water to give a final pH of 6.8. Sterilization was accomplished by filtration through Berkefeld filters (Type N). The test medium was inoculated from 24-hour agar cultures and incubated at 37°C. Nessler's reagent was employed to determine the presence of ammonia by adding a loopful of the culture to a drop of the reagent on porcelain plates. In order to follow the pH changes, a portion of the medium was made up with phenol red in a concentration of 1-100,000/ml, and another portion with thymol blue in a concentration of 1-50,000/ml, thus covering the pH range from 6.8 to 9.8. A portion of the medium without urea, but containing yeast extract, buffer, and indicator, controlled pH changes in the basic medium while another portion with yeast extract and buffer alone controlled the production of ammonia from the basic medium.

[2] *Bergey's Manual of Determinative Bacteriology,* 1939, Fifth Edition, Williams and Wilkins Co., Baltimore.

[3] Moltke, O., *Contributions to the Characterization and Systemic Classification of Bac. proteus vulgaris* (Hauser), 1927, Levin and Munksgaard, Copenhagen.

[4] Yacob, M., *Indian J. Med. Research,* 1932, **19**, 787.

A total of 160 known Proteus strains were tested, including *Proteus vulgaris, Proteus mirabilis, Proteus morganii,* the X strains, and one strain each of *Proteus americanus, Proteus ammoniæ, Proteus icthyosmius, Proteus bombycis,* 2 strains of *Proteus pseudovaleriei,* and 4 strains of *Proteus hydrophilus.* In addition 31 cultures isolated in other laboratories and tentatively classed as Proteus strains were also included. Tests for ammonia were made and pH changes observed at 4, 8, 24, and 48 hours after inoculation. Cultures negative after 48 hours were held an additional 72 hours.

The results are summarized in Table I. All members of the genus except *Proteus hydrophilus, Proteus icthyosmius,* and *Proteus bombycis* were capable of attacking urea. At 4 hours color reactions of yellow, yellow orange, and orange were obtained with the Nessler test for *Proteus vulgaris, Proteus mirabilis, Proteus ammoniæ, Proteus pseudovaleriei, Proteus americanus,* and with one exception the X strains. The exception, which will be discussed in a subsequent paper, was a non-motile variant of a motile culture. The pH reactions varied from 6.9 to 7.2 at 4 hours and from 7.2 to 8.0 at 8 hours, whereas at 24 hours all were practically the same, 9.2 to 9.4. In 2 days the limit of the thymol blue indicator was reached with a majority of these strains. Variation in the pH reaction might result from differences in the size of the inoculum or from slightly different rates of activity of the various strains.

Although the *Proteus morganii* strains were capable of attacking urea, their activity was markedly slower. At 8 hours every strain gave a positive Nessler test, but 9 strains did not raise the pH in this time. Two days were required for all *morganii* strains to give a pH reaction of 8.0 to 8.2, thus definitely indicating a much slower rate of urease production.

The 31 cultures tentatively classified as Proteus, the 4 strains of *Proteus hydrophilus,* and the single strains of *Proteus icthyosmius** and *Proteus bombycis** neither gave a reaction for ammonia nor changed the pH reaction over a period of 5 days.

To determine the efficiency of urease production in a chemically defined medium, nicotinic acid and pantothenic acid were substituted for the yeast extract since these substances, present in yeast extract, have been recently described as growth factors for Proteus.[5, 6] Representative Proteus strains, inoculated so that no initial turbidity

* American Type Culture Collection.

[5] Fildes, P., *British J. Exp. Path.*, 1938, **19**, 239.

[6] Pelczar, M. J., and Porter, J. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 151.

TABLE I.
Urea Decomposition by Proteus. Control Medium pH 6.8 No Reaction in Any of Cultures at 120 hr. Test Medium pH 6.8.

Cultures	No. of strains	NH_3 (No indicator in medium) Hr 4	8	24	48	96 and 120
Pr. vulgaris	28	Y-YO	YO-O	O		
Pr. mirabilis	81	Y-YO	YO-O	O		
X strains	24	Y-YO	YO-O	O		
X strains	1	—	—	—	—	—
Pr. pseudovaleriei	2	YO	O			
Pr. americanus	1	YO	O			
Pr. ammoniæ	1	Y	O			
Pr. morganii	16	Y	Y	YO	O	
Pr. hydrophilus	4	—	—	—	—	—
Pr. icthyosmius	1	—	—	—	—	—
Pr. bombycis	1	—	—	—	—	—
Unknown strains	31	—	—	—	—	—
		pH (Indicator in medium)				
Pr. vulgaris	28	6.9-7.2	7.2-8.0	9.2-9.4	9.6-9.8	
Pr. mirabilis	81	6.9-7.2	7.2-8.0	9.2-9.4	9.6-9.8	
X strains	24	6.9-7.2	7.2-8.0	9.2-9.4	9.6-9.8	
X strains	1	—	—	—	—	—
Pr. pseudovaleriei	2	6.9	7.4	9.4	9.6	
Pr. americanus	1	7.1	7.8	9.4	9.6	
Pr. ammoniæ	1	6.9	7.4	9.4	9.6	
Pr. morganii	16	6.8	6.8-7.0	7.1-7.3	8.0-8.2	8.2-8.8
Pr. hydrophilus	4	—	—	—	—	—
Pr. ichthyosmius	1	—	—	—	—	—
Pr. bombycis	1	—	—	—	—	—
Unknown strains	31	—	—	—	—	—

Y, weak reaction; YO, moderate reaction; O, strong reaction; —, no reaction.

was present, gave weak to moderate positive reactions in 48 to 72 hours. Urea decomposition was possible in such a synthetic medium but it was apparent that the medium was not nearly as effective as the yeast extract medium.

Discussion. Yeast extract serves to provide the essential growth factors for Proteus. All cultures gave uniform growth in the control medium without producing ammonia or changing the pH in 5 days.

The most recent addition to the Proteus group has been *Proteus morganii* on the basis of Rauss' investigations.[7] The production of the enzyme urease by this organism in our estimation is further justification for its classification under Proteus. Its rate of activity, however, distinctly differs from that of the other species and appears to be constant for all strains of *morganii*.

St. John-Brooks and Rhodes do not consider *hydrophilus* and

[7] Rauss, K. F., *J. Path. and Bact.*, 1936, **42**, 183.

icthyosmius members of the genus Proteus because of their mannitol fermenting property and we would also exclude these strains from the genus because of their inability to attack urea. A similar conclusion can be drawn with regard to *bombycis* since it also failed to attack urea. The 2 strains of *Proteus pseudovaleriei** which decomposed urea did not conform to the description of Bergey and of St. John-Brooks and Rhodes. One strain appeared to be *Proteus vulgaris* and the other *Proteus mirabilis*. On the other hand, we do not agree with St. John-Brooks and Rhodes that the names *vulgaris* and *mirabilis* should be changed.

13055

Selective Action of Sulfanilyl-Guanidine on Different Salmonella Types and Its Practical Importance.*

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Marshall and coworkers[1] have produced a new sulfa-compound, sulfanilyl-guanidine, and studied its bacteriostatic effect. The solubility of this substance in water at 37.5°C is 220 mg %, its absorption from the intestines comparatively poor. These properties make the drug particularly fit for use in intestinal infections.

In order to study the action of sulfanilyl-guanidine† on different organisms of the Salmonella group, we tested 74 types listed in the latest edition of the Kauffmann-White schema[2] and 13 types which were described since. Fifteen strains of *E. coli* isolated from human feces, urine and blood, 4 strains of Shigella (2 Flexner, 1 Sonne, 1 Shiga type) and a strain of Aerobacter were also tested.

For preliminary information on differences in susceptibility the agar strip method was used. Strips 1 cm wide were cut out of agar plates and filled in with a suspension of 1% sulfanilyl-guanidine in agar. The cultures were streaked out across the inlaid strips.

* Aided by a grant from the Ella Sachs Plotz Foundation.

1 Marshall, E. K., Jr., Bratton, A. C., White, H. K., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

† We received the sulfanilyl-guanidine through the courtesy of the research department of E. R. Squibb & Sons.

2 *J. Hyg.*, 1934, **34**, 33; Report of the Salmonella Subcommittee of the Nomenclature Committee of the International Association of Microbiologists, June, 1939.