phological studies of South American forage plants in collaboration with Dr. Agnes Chase at the United States National Museum; AGESILAU ANTONIO BITANCOURT, subdirector of the São Paulo Biological Institute, who will make a study of the virus diseases of citrus and of fungus parasites on other economic plants, with Dr. H. S. Fawcett at the Citrus Experiment Station of the University of California, Riverside, and with scholars in the U. S. Department of Agriculture, and EDGARD DO AMARAL GRANER, technical assistant in the Institute of Agronomy and lecturer in the "Luiz de Queiroz" School of Agriculture, Piracicaba, Brazil. His project is to study the citogenetics of corn and tobacco chiefly with Professor T. H. Goodspeed, at the University of California.

SPECIAL ARTICLES

THE ORIGIN OF THE RETE APPARATUS IN THE OPOSSUM

THE system of male genital ducts in vertebrates is mainly derived from mesonephric structures, preserved and readapted to another function. The mesonephric duct and a number of anterior tubules become the ductus deferens and ductus epididymidis, and the epididymal tubules, respectively; while connection with the gonad is established by means of the rete elements, which occupy an intermediate position at the hilum. These elements typically anastomose freely in the hilar region but connect as discrete ductules with the prospective epididymal tubules at the glomerular capsules.

While there is agreement as to the development of ductus deferens and epididymis, the problem of the origin of the rete apparatus has received many answers. Historically, it has been derived, according to different studies, from (1) cord-like outgrowths of the glomerular capsules which penetrate the hilum of the gonad to unite with the medullary cords; (2) conversely, by extensions of the medullary cords through the mesorchium to unite with the capsules; (3) as an independent, local development at the hilum, with connections established secondarily in both directions. Considering the diversity of forms studied, the differences of opinion are not surprising. The extensive literature is best approached through the medium of recent general discussions.[1, 2, 3]

In *mammals* the problem is complicated by the rudimentary nature and transitory development of certain of the structures involved. Consequently, if we accept for the purpose of this discussion the prevailing view that the rete apparatus has an independent origin locally, we still encounter widely different opinions as to the nature and precise manner of origin of the earliest visible primordia. One view of doubtful value holds merely, (*a*) that the rete connections arise as local condensations from the primary mesenchyme; a second (*b*) derives them from an ill-organized cellular blastema, probably originating from the adjacent coelomic epithelium;[4, 5, 6, 7, 8] while a third opinion (*c*) asserts a highly specific origin from discrete epithelial cords (sometimes tubular ingrowths) arising from the coelomic epithelium of the anterior genital ridge.[4, 9, 10, 11] In the last category, the resemblance which these structures bear to abortive nephrostomes or nephrostomial canals is frequently noted and discussed; however, only one author without hesitation describes them as such.[10] If this interpretation is correct there is a considerable accumulation of evidence from a number of mammals placing this group in line with a condition clearly established in certain lower vertebrates.[12, 13] This view would also identify the rete system with other parts of the male duct system, as a transformed mesonephric structure.

Much the same problem exists regarding the origin of the Mullerian duct, in particular the ostial ingrowth. In selachians this duct is still held to arise by a gradual backward splitting of the Wolffian duct, but in higher classes it develops by invagination of a localized area of ciliated coelomic epithelium at the anterior pole of the mesonephros. The ostium thus formed gives rise to the remainder of the duct by backward growth. The invaginating epithelium has been frequently, if rather loosely, identified with one or more mesonephric (or pronephric) nephrostomes.[2, 3, 4]

In view of the interest attaching to these structures, from the standpoint of comparative morphology as well as embryology, their development in the North American opossum (*Didelphys virginiana*), a primitive type of marsupial, deserves description. The process is similar to that described by Fraser[11] for certain Australian marsupials (especially *Trichosurus*),

[1] B. H. Willier, "Sex and Internal Secretions," 2nd Ed. Baltimore: Williams and Wilkins, 1939.
[2] F. W. R. Brambell, "The Development of Sex in Vertebrates," London: Sidgwick and Jackson, Ltd., 1930.
[3] E. S. Goodrich, "Studies on the Structure and Development of Vertebrates," London: Macmillan, 1930.
[4] S. E. Wichmann, *Anat. Hefte*, 45: 629, 1912.
[5] W. Felix, Keibel and Mall, "Manual of Human Embryology," II, Phila.: Lippincott, 1912.
[6] H. M. de Burlet and H. S. de Ruiter, *Anat. Hefte*, 59: 321, 1921.
[7] C. S. Simkins, *Acta Zool.*, 4: 241, 1923.
[8] K. W. Wilson, *Contr. Emb. Carnegie Inst. Wash.*, 17: 69, 1926.
[9] B. M. Allen, *Am. Jour. Anat.*, 3: 89, 1904.
[10] F. W. R. Brambell, *Proc. Roy. Soc.*, Series B, 102: 206, 1928.
[11] E. A. Fraser, *Jour. Anat.*, 53: 97, 1919.
[12] A. Brachet, "Traité d'Embryologie des Vertébrés," Paris, 1921.
[13] B. M. Allen, *Am. Jour. Anat.*, 5: 79, 1905.

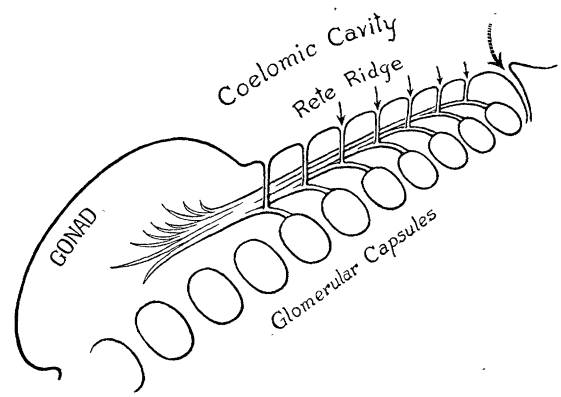
and to the brief outline given for *Didelphys aurita* by the same author; however, the clear-cut relationship of the rete cords to the coelomic epithelium, and the characteristics which relate them to nephrostomial canals, are particularly distinct in *Didelphys virginiana.*[14] The account that follows is a brief recapitulation, emphasizing features that are especially clear in the material. A full account will appear elsewhere at a later date.

At birth the genital ridge (as in Trichosurus) is already distinctly marked off into an anterior region, the *rete ridge,* slender and virtually lacking in germ cells, and a posterior *gonad region.* The latter is still sexually undifferentiated save in exceptional cases. Both gonad and rete ridge are closely applied to the ventro-medial surface of the mesonephros, in intimate relation with a series of glomerular capsules. Within the rete ridge appear numerous irregular epithelial cords, frequently in contact with one another and with the capsules. At intervals these cords are seen to reach the surface of the ridge, where they are continuous with the covering coelomic epithelium. Frequently they appear tubular even at this early stage.

Development is slow for a time, but by 10 days, when the gonads are well differentiated, the cords within the ridge form a compact bundle of rete canals, lined with a densely staining epithelium. Toward the hilum of the gonad they merge gradually into a single large duct; toward the mesonephros they diverge as discrete tubules which unite individually with glomerular capsules. The primitive connections with the coelomic epithelium are now wide, funnel-like canals (6–8 typically) lined with the same massive epithelium, which also spreads over the surface of the ridge, with an abrupt transition to mesothelium of ordinary coelomic type. Longitudinally, this epithelium forms an irregular band along the crest of the ridge, and is continuous at the anterior end of the ridge with the ostium of the Mullerian duct. It is during this period that the Mullerian duct is developing. The plan and relationships of the system, at the height of its development, are shown in Fig. 1.

At twelve to fourteen days a sex difference is usually found, although there is great variability. In most males the funnel-like ducts joining the rete canals with the coelom, are retrogressing. In some specimens they have virtually disappeared, but may be seen in others as late as twenty days. In females they remain open indefinitely and at seven weeks still retain their original relationships, opening on the surface of the mesovarium in continuity with the fimbriated margin of the ostium.

Thus in males the rete canals, in adaptation to their eventual function, lose their primary communications with the coelom comparatively early. In the female they remain open indefinitely, and the rete itself persists as a well-developed structure in the adult ovary. It has been shown that male hormones evoke this sex difference in females by effecting early closure of the



PLAN OF THE RETE APPARATUS IN DIDELPHYS VIRGINIANA

FIG. 1. Plan of the rete system in *Didelphys virginiana;* a composite of conditions encountered between 10 and 20 days, pouch life. The large arrow indicates the ostium of the Mullerian duct, small arrows coelomic funnels of the rete canals.

funnels, with development of a rete and epididymis comparable with the normal male parts.[15]

SUMMARY AND CONCLUSIONS

The rete apparatus in the opossum appears first as a series of cellular cords, or slender canals, arising from an irregular band of thickened coelomic epithelium covering the rete region of the genital ridge. At the cephalic end of the ridge this band is directly continuous with and indistinguishable from the area that gives rise to the ostium tubae. A number of these cords become canalized and appear as wide, funnel-like openings leading from the body cavity into the rete canals, which are now also tubular. During the period when the funnels are becoming patent the ostial invagination is also developing. In males the funnels retrogress early, converting the rete canals into closed passages, while the ostium tubae also atrophies. In females, in which the ostium is a functional structure, the rete invaginations also remain open indefinitely.

From the manner of origin and early relationships of these canals, from their appearance at the height of differentiation, and from their behavior during later development, it is strongly indicated that, together with the ostial invagination, they represent a series of persistent nephrostomial canals. Thus the rete canals, like other parts of the male genital duct system, are derived from preexisting mesonephric structures, while

[14] R. K. Burns, Jr., *Anat. Rec.,* 79 (Supplement), 1941.

[15] R. K. Burns, Jr., *Jour. Morph.,* 69: 79, 1939.

the individual rete invaginations and the ostial funnel are morphologically members of a homologous series.

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ARGINASE

ARGINASE as compared with the starting material—a watery extract from ground beef liver—was purified 50 times, using the common protein separation methods. The ferment solution obtained showed absence of catalase, amylase and proteolytic enzymes. Spectroscopic examination revealed the absence of hemoglobin, myoglobin and cytochrome C.

10γ of this protein solution hydrolyzed 30 to 35 per cent. of 17.4 mg arginine within 10 minutes under the condition of the experiment.

While the addition of Mn^{++}-ions[1] to a crude liver extract is frequently without any effect upon the activity of the enzyme, it was found that in the further stages of purification Mn^{++}-ions are essential for obtaining maximum activity of the enzyme. The activation of arginase by metallic ions, for instance, Mn^{++}, is a time reaction requiring about 15 minutes under the condition of the experiment for obtaining optimum activation. The optimum pH for the purified enzyme (with or without Mn^{++}-ions) is about 9.5. Other ions, such as Fe^{++}, Ni^{++} and Co^{++} also activate the enzyme but to a less extent than Mn^{++}. Here again, however, the optimum pH for these ions was found to be about 9.5.

Arginase (no addition)	.35
" + Mn^{++}	.71
" + Mn^{++}—vitamin C	.71
" + Co^{++}	.53
" + Co^{++}—vitamin C	.56
" + Ni^{++}	.50
" + Fe^{++}	.48
" + Fe^{++}—cysteine	.59

10γ arginase; 17.4 mg arginine; heavy metal salts 0.5γ; vitamin C or cysteine 1γ; total volume 1.5 cc adjusted to pH 9.5; incubated for 10 minutes at 37.5° C. Numbers are n/20 KOH. Method of determination was the Linderstroem-Lang method[2] for titration of ornithine, modified to semi-micro.

In the purified state arginase is quite stable, tolerating dialysis for 48 hours at 4° C. without loss of activity. The ferment solution is also stable at this temperature for weeks and its activity not altered by evaporating from the frozen state to dryness and subsequent redissolving.

The isoelectric point of the enzyme was found by electrophoresis to be at pH 5.7. The investigation of the ash content of the purified enzyme gave in one case an Mn-content of 0.08 per cent.

[1] G. Klein and W. Ziese, *Klin. Woch.*, 14: 205, 1935.
[2] K. Linderstroem-Lang, L. Weil and H. Holter, *Z. physiol. Chem.*, 233: 174, 1935.

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THE INDUCTION OF FERTILITY IN GENETICALLY SELF-STERILE PLANTS

IN hybridization experiments designed for the production of new colors in the various classes of commercially important Petunias, a strain of Golden Rose was discovered that has been found to be completely self-sterile under natural conditions. The self-sterility in this strain of Petunia behaves in inheritance as a simple Mendelian recessive character. By means of cuttings the strain has been maintained in the Botanical Laboratory of Bucknell University for the past five years. The plants are unusually floriferous and everblooming, due, in part at least, to their inability to produce seeds unless they receive pollen from plants which are not homozygous recessive for the self-sterility gene. Although many thousands of flowers were produced during this period and although repeated attempts were made to self-fertilize the plants, not a single seed capsule was ever produced until recently when self-fertility was induced by the techniques described below.

Dr. H. Clyde Eyster, of the Botany Department of the University of South Dakota, made microscopic observations of the pollen tubes in the stigmas and styles of the self-pollinated self-sterile plants and found that the pollen grains germinate well and develop into tubes which extend into the neck of the style but rarely if ever grow as far as one half the distance from the stigma to the ovary. In styles that had been cut three days after they had been self-pollinated, Dr. Eyster found that most of the pollen tubes grow only about one tenth of the distance from the stigma to the ovary, while an occasional tube grows somewhat less than one half of the distance down the style. Before any of the tubes reach the ovary, the style is cut off from the top of the ovary by the formation of an abscission layer. From these observations it appears that the self-sterility in the Golden Rose Petunia studied is caused by the very slow rate of growth of the pollen tubes and the formation of an abscission layer which severs the style from the ovary before any of the tubes enter the latter.

The pollen of the self-sterile plants was placed on the stigmas of normal self-fertile plants and allowed to develop for forty-eight hours. At the end of this time the styles were cut, preserved and sent to Dr. Eyster for microscopic examination. Approximately 75 per cent. of the pollen grains were found to have developed pollen tubes of varying lengths, including many which extended all the way down the style and,

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