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5. These data provide the most comprehensive record of stillbirths in the United States. Despite the improvement in data collection over the years, the reporting of fetal deaths remains one of the weakest links in the vital statistics system. Within any one year, the coverage is probably best at term and weakest in the earliest months of gestation. There is, however, no reason to assume that there is a disproportionate failure to report male fetal deaths relative to female fetal deaths, or vice versa. In addition to these qualifications, a subset of fetal deaths for which the month of gestation was not reported were excluded from this analysis.
6. Fetal deaths are defined by the World Health Organization as "death prior to the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy." [World Health Organization: Third World Health Assembly, *Official Records of the World Health Organization, No. 28* (Geneva, December 1950), pp. 16–17.] United States Vital Statistics uses this definition. Data for 1922 to 1936 are from the U.S. Bureau of the Census [*Births, Stillbirths, and Infant Mortality* (Government Printing Office, Washington, D.C., annual)]. Although coverage of birth registrations was not completed until 1933, my analysis uses ratios rather than actual counts; I know of no reason to expect these ratios to change with the inclusion of more states. The data are based on fetal deaths by sex and period of gestation for time intervals less than 3 months and consecutive 1-month intervals to 10 months and more, and infant deaths by sex for the first 6 days of life, under 1 month, 1 month, and 2 months. Data for 1950 to 1972 are from the U.S. Department of Health, Education, and Welfare [*Vital Statistics of the U.S.*, vol. 2, part A, *Mortality* (Government Printing Office, Washington, D.C., annual)]. The data include fetal deaths by sex and period of gestation for months 5 through 10 and more and infant deaths by sex for the first 6 days of life, under 1 month, 1 month, 2 months, and 3 months.
7. Examination of these data by year revealed little annual variability within the years 1922 to 1926 and within the years 1950 to 1972. Thus, for the sake of simplicity the monthly means of each of these groups of years were used in this analysis; this procedure should also yield more robust equations.
8. The linear equation for these data yields a poorer fit, $R^2 = .4760$.
9. The linear equation for these data yields a poorer fit, $R^2 = .8017$.
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12. Perinatal mortality comprises deaths occurring from month 7 of gestation through the first 7 days of life.
13. The sex ratio is defined as the ratio of males to females, whereas the sex proportion reflects the proportion of all individuals who are males.
14. The calculation formula for m is:

$$m = \frac{y_2(1 - x_1 f) - y_1}{y_1 y_2 - y_1}; \quad x_1 = 1 - y_1$$

15. These data are used because the registration system was more complete in the period from 1950 to 1972 than it was for the earlier data (1922 to 1936).
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17. I thank R. Schoen for his help and encouragement at all stages of this research and K. Land, L. Waite, and R. Fagen for their helpful comments. Computer time and facilities were provided by the Social Science Quantitative Laboratory, University of Illinois, Urbana.

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Tight Junctions in a Fluid-Transporting Epithelium of an Insect

Abstract. *Occluding junctions have been found between the lateral cell borders at the base of the rectum of* Periplaneta americana. *They appear as punctate membrane appositions in thin sections, and after incubation in physiological solutions containing lanthanum before fixation the inward penetration of tracer is impeded in this same basal area. Moreover, freeze-fracture studies of this region reveal simple linear ridges on fracture face P and grooves on fracture face E, which are similar to the less complex vertebrate tight junctions. The luminal clefts, which permit free inward diffusion of tracers, present no tight junctions, but do have septate junctions. These results support the contention that, contrary to earlier speculation, arthropods do possess tight junctions; these, rather than septate junctions, appear to form the morphological basis of at least some of the permeability barriers observed in invertebrates.*

The existence of true tight junctions or zonulae occludentes in a number of tissues of vertebrates has been well established (*1*), and they have been shown to provide the structural basis for a variety of physiologically important permeability barriers (*2, 3*). Moreover, it has been considered that tight junctions are a diagnostic feature of chordates, since the invertebrate groups originally appeared not to possess them (*4*). Recently it has become apparent that tight junctions, recognizable both in thin sections and by their freeze-fracturing characteristics, do exist in invertebrates, at least in insects (*5, 6*), where they form the morphological basis of the blood-brain barrier, observed to be present in the central nervous system (CNS) of insects by electrophysiological criteria (*7*) and by the exclusion of exogenous tracers (*5, 6*).

Discovery of tight junctions in an arthropod tissue other than the nervous system would further strengthen the contention that these junctions not only exist in invertebrates but may play important physiological roles. Evidence is presented here for the existence of such junctions in the insect rectum.

The fluid-transporting epithelium composing the rectum of insects is primarily concerned with reabsorption of water and ions, the feces being concentrated and water loss prevented. Since insects have a small body weight relative to their surface area, this is an important consideration in survival.

The mode of action of the rectal cells in dictyopteran insects such as the cockroach has been thought to involve uptake of water by absorption of ions over the luminal plasma membrane; the ions are then pumped over the lateral cell membrane into the intercellular spaces. The high concentration of ions there produces an osmotic inflow of water, which then makes its way to the hemolymph via the intercellular spaces around the tracheae, ion reabsorption occurring en route (*8, 9*). The exit route via the sinuses around the tracheae may be somewhat modified by the possible presence of basal cells (*10*), but this does not affect the model system as described or the interpretation of our results. This report describes new observations made after freeze-fracturing the rectum of *Periplaneta americana* and incubating it, before fixation, in physiological saline containing the exogenous tracer lanthanum. The results show that true tight junctions exist in this system; earlier, without en bloc staining, gap junctions were incorrectly described as "tight" (*8*).

In previous investigations the ultrastructure of the rectum in the cockroach has been studied in detail in thin sections (*8, 10*), but not by freeze-fracture or for tracer uptake. The tissue consists of a luminal cuticle under which lie columnar epithelial cells, the lateral borders of which are associated at the luminal surface by septate junctions and desmosomes. These lateral borders are thrown into complex interdigitations there and also deeper into the tissue, where scalariform junctions occur lying in intimate association with mitochondria; this is thought to be the site of the ion transport from the cytoplasm to the intercellular spaces or sinuses (*8*). Toward the base of the cells, close to the basement membrane, and by the muscles and circulating hemolymph, septate junctions again occur. But here our investigations reveal the presence not only of gap junctions but also of tight junctions, seen in thin sections as punctate membrane appositions below the sinuses and near, or in

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the midst of, the basal septate junctions (Fig. 1).

After incubation of the outer wall of the intact rectum of *P. americana* in lanthanum, the tissue was fixed and examined. The tracer was found to have made its way through the basement membrane and part of the basal intercellular junctions, but was then stopped (Fig. 1); the site of this occlusion corresponds to the position of the tight junctions. In a different set of incubations, the lumen of the intact rectum was injected, through the anus, with saline containing lanthanum. Under these conditions, the tracer makes its way through the cuticle and into the luminal intercellular clefts, where it is found not only in the septate junctions, but also beyond them.

Freeze-fracturing of rectal tissues demonstrates the presence of a simple unbranched, intramembranous P face ridge and E face groove system (Fig. 2) localized in the basal part of the rectal cells; this is typical of the insect tight junctions observed elsewhere (*5, 6*) and comparable to the simpler of the vertebrate tight junctions seen after freeze-cleaving (*2*). These beadlike ridges, about 10 nm in diameter, lie in simple linear arrangements (Fig. 2, inset), over the P face. The ridges sometimes overlap and often lie in parallel arrays (Fig. 2, inset), up to eight having been found in any one area. These P face ridges sometimes display short discontinuities; this may mean that the ridges at that point have cleaved onto the E face, or it may reflect the complexity of the interdigitating intercellular cleft, which, as with the cells forming the insect blood-brain barrier in the CNS (*5, 6*), does not apparently require such a complex network of tight junctional strands as do the vertebrate permeability barriers (*5*). The complementary E face grooves lie on the outer half of the membrane (Fig. 2). The ridges and grooves are often found near arrangements of intramembranous particles which are characteristic of septate junctions and gap junctions in replicas, and in thin sections these junctions are also often found together.

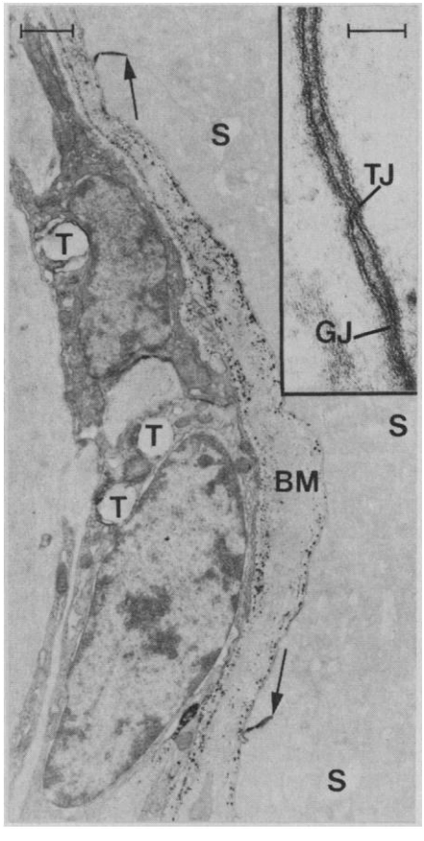

Fig. 1. Thin section through the basal region of rectal cells from the cockroach, after incubation for 30 minutes in 10 m*M* lanthanum in Ringer solution followed by fixation in phosphate-buffered glutaraldehyde and processing. The tracer can be seen in the basement membrane (*BM*) and, although it enters the basal intercellular clefts, its further penetration is stopped (arrows) in the regions where tight junctions (*TJ*) (as in inset) are situated, often near basal septate junctions and gap junctions (*GJ*). Other abbreviations: *T*, tracheoles; *S*, intercellular sinuses. Scale bar, 1 μm; inset scale bar, 100 nm.

Since tracers appear to have free access from the lumen to the intercellular clefts, it may be that water enters the rectal system through the septate junctions near the luminal surfaces rather than, as previously thought (*8*), across the plasmalemma. This theory of water uptake via the extracellular clefts is supported by evidence such as that from gallbladder (*11*), where it has been suggested that water flow occurs via the intercellular space, not by intracellular osmotic "pulling," as previously thought to occur. This theory of water transport avoids the difficulty inherent in the traditional idea, which is that there is no obvious driving force for water flow across the luminal plasma membrane; the ion levels in the cells are much too low to produce such an effect (*12*).

It seems likely that ionic uptake would nevertheless occur across the luminal plasma membrane because these areas are enriched with numerous closely packed intramembranous P face particles; the level of ion transport across membranes increasingly appears to be related to particle density (*13*).

Water flowing out of the rectum would have to attain the hemolymph via the intercellular and tracheal sinuses, since the intercellular passage in the clefts at the base of the cells is blocked by the tight junctions (Fig. 1). At the same time, these basal occlusions would prevent water in the hemolymph from rushing back through the intercellular junctions and subsequently into the rectal sinuses, drawn by the osmotic gradient created by the high ionic concentration in the clefts of the scalariform junctions. The presence of basal tight junctions would therefore ensure unidirectional flow of the conserved water from the lumen of the rectum into the intercellular sinuses and out into the hemolymph. Originally it was thought to be the septate junctions at base and lumen which sealed off the rectal surfaces (*8*), but the contention presented here, that it is the basal tight junctions which are the occluding structures, is supported by the lanthanum studies. These junctions in *Periplaneta* appear analogous to the multilayered cellular sheath of low permeability present in *Blaberus* and *Blattella* (*14*).

The coexistence of septate junctions and tight junctions in the insect rectum is not unprecedented in arthropods; it also occurs in the perineurium, which forms the blood-brain barrier in the CNS of most larval and adult insects (*5*) [with the exception of the moth *Manduca sexta* (*6*), where tight but no septate junctions

Fig. 2. Freeze-fracture replicas from the base of the cockroach rectum. The cytoplasmic or inner membrane half (fracture face P) contains a simple system of ridges (arrows in inset), while the extracellular or outer membrane half (fracture face E) contains complementary grooves (thick arrows). This linear system of intramembranous ridges and grooves forms the rectal tight junctions. Scale bars, 100 nm.

are found] as well as in insect testis (*15*) where a blood-germ cell barrier occurs. Other investigators who have not found insect tight junctions, possibly because they are difficult to detect by virtue of their relative simplicity, have suggested that septate junctions are the basis of all occluding phenomena in invertebrates (*10, 16*). However, I have found that tracers are able to move completely through septate junctions, and comparable events also occur in such insect tissues as Malpighian tubules, where inulin (molecular weight, 5200) readily traverses the septate junctions between adjacent cells (*17*). The tight junctions observed appear to prevent the inward movement of lanthanum as they do in the insect CNS (*5, 6*) and so may be presumed to be the structures which impede the inward diffusion of molecules, forming a blood-basal rectal cleft barrier.

It would be premature, however, to generalize from these observations that the role of the septate junctions in controlling transepithelial permeability has been completely eliminated in all situations. Studies of more primitive systems such as coelenterates and planarians (*18*) strongly implicate septate junctions as a barrier to the paracellular flow of water and small molecules across epithelia in their tissues.

In summary, this report presents another example of tight junctions in arthropods, which strengthens the case for their existence in invertebrates; it also weakens the argument that septate junctions are the invertebrate equivalent of the vertebrate tight junctions in forming the structural basis of permeability barriers. Moreover, the restriction of occluding tight junctions to the basal region of a fluid-transporting epithelium involved in water and ion resorption, wherein the luminal clefts are open to inward diffusion of tracers, suggests the possibility that the junctions are actively involved in the regulation of unidirectional fluid flow in a way that would permit intercellular transport of the water flowing into and through the system.

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Brain Norepinephrine and Dopamine in Schizophrenia

Farley *et al.* (*1*) reported that increased levels of norepinephrine (NE) were found in certain limbic regions of schizophrenic brain. However, we offer a word of caution about using small numbers of cases from a heterogeneous population. We have measured dopamine (DA) and NE in the striatum and three limbic regions in more than 50 patients who died with a hospital diagnosis of schizophrenia (Table 1). We found a significant increase in DA concentration in the nucleus accumbens, confirming our finding reported in an earlier, smaller series (*2*). In addition, the DA concentration in the anterior perforated substance was significantly increased (Table 1). Although we, like Farley *et al.*, found that the NE concentration was increased in the nucleus accumbens, this result was not statistically significant. Similarly, the apparent increase in NE in anterior perforated substance was not statistically significant when analyzed by a nonparametric test (since the NE values show a skew distribution the Student's *t*-test is not applicable). When the NE values obtained by Farley *et al.* in the nucleus accumbens are compared with our own, it seems likely that differences in defining this anatomical region may exist between our laboratories. Although the NE values they report for the ventral septum and hypothalamus are in accord

Table 1. Norepinephrine and dopamine in limbic and basal ganglia regions of postmortem brain from psychotic patients and controls. The statistical significance was determined with a two-tailed *t*-test; S.E.M., standard error of the mean; *N*, number of brains.

Brain region	Norepinephrine (μg per gram of protein)		Dopamine (μg per gram of protein)	
	Mean ± S.E.M.	*N*	Mean ± S.E.M.	*N*
Nucleus accumbens				
Controls	1.3 ± 0.13	40	12.2 ± 0.95	46
Psychotic	1.8 ± 0.18*	47	16.3 ± 1.03†	51
Anterior perforated substance				
Controls	0.7 ± 0.11	25	1.9 ± 0.3	32
Psychotic	1.4 ± 0.24‡	35	3.7 ± 0.58§	37
Ventral septum				
Controls	4.2 ± 0.71	35	1.4 ± 0.14	35
Psychotic	4.2 ± 0.10	32	1.6 ± 0.15	32
Caudate				
Controls	0.7 ± 0.10	47	17.3 ± 1.27	51
Psychotic	0.5 ± 0.06	44	19.7 ± 1.35	50
Putamen				
Controls			22.0 ± 2.3	29
Psychotic			22.9 ± 2.2	37

*P = .129, Mann-Whitney U test. †$P < .005$ when compared with control, Student's *t*-test or Mann-Whitney U test. ‡P = .067, Mann-Whitney U test. §$P < .02$, when compared with control, Student's *t*-test or Mann-Whitney U test.

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