eliminate the artifact option suggested by Hülsmann *et al.* (*20*), and rather strongly indicate that loosely coupled mitochondria can be an important source of nonshivering thermogenesis in skeletal muscle.

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References and Notes

1. L. Jansky and J. S. Hart, *Can. J. Biochem. Physiol.* **41**, 953 (1963).
2. L. Jansky, *Biol. Rev. Cambridge Philos. Soc.* **48**, 85 (1973).
3. J. Himms-Hagen *et al.*, in *Regulation of Depressed Metabolism and Thermogenesis*, L. Jansky and X. J. Musaccia, Eds. (Thomas, Springfield, Ill., 1976), pp. 243-260; W. A. Behrens and J. Himms-Hagen, *J. Bioenerg. Biomembr.* **9**, 41 (1977); J. Himms-Hagen, J. Cerf, M. Desantels, G. Zaror-Behrens, *Experientia Suppl.* **32**, 119 (1978).
4. A. S. Blix *et al.*, *Am. J. Physiol.*, in press.
5. The abbreviations used in this report are ATP, adenosine triphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; and FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.
6. Cytochrome c oxidase activity was measured with a Clark electrode (Yellow Springs Instruments) after treatment of minced tissue in a modified Chappell-Perry medium (1 m*M* ATP, 50 m*M* potassium-containing Hepes buffer, *p*H 7.4, 0.1*M* KCl, 5 m*M* $MgCl_2$, 1m*M* EDTA, 5 m*M* EGTA (*5*) with low concentrations of Lubrol added according to A. Aulie and H. J. Grav (*Comp. Biochem. Physiol.*, in press).
7. The aerobic nature of the metabolism in this muscle was further reflected in a peculiar fiber composition: Standard adenosinetriphosphatase staining (*p*H 9.4) of tissue slices from 10-day-old pups exposed only one type of fibers. Prior incubation of the slices at *p*H 4.1, however, revealed that two subtypes were present. Both fiber types were rich in triglyceride but completely devoid of glycogen (*8*). Electron microscopic examination likewise suggested an apparent increase in the number of both mitochondria and triglyceride droplets in the course of the 10-day neonatal period.
8. V. Dubowitz and M. H. Brooke, *Muscle Biopsy: A Modern Approach* (Saunders, London, 1973).
9. G. Bullock, E. E. Carter, A. M. White, *FEBS Lett.* **8**, 109 (1970).
10. B. Chance and B. Hagihara, *Proc. Int. Congr. Biochem.* **5**, 3 (1961).
11. The homogenization medium consisted of 0.21*M* mannitol, 0.07*M* sucrose, 10 m*M* Hepes buffer, *p*H 7.4, and 10 m*M* EDTA.
12. The homogenate was placed in a cooled Sorvall SS-1 or RC-2B centrifuge equipped with an SS-34 rotor running with half-filled 50-ml tubes. The pellet obtained at a field of 1500*g* (average) × minute was discarded and the supernatant was filtered through gauze. The mitochondrial fraction was then obtained at a field of $9200g_{av}$ min. This fraction was washed twice, with the same centrifugal field being used. The final pellet was resuspended in homogenization medium (*11*) and stored at 0°C at a concentration of 30 to 50 mg of mitochondrial protein per milliliter. Protein was determined by a biuret procedure.
13. The P/O ratios were determined by the glucose-hexokinase trap method as follows. Adenosine triphosphate (2 m*M*) was added to the incubation medium and the reaction started by adding 25 units of hexokinase and 5 m*M* KH_2PO_4 with $NaH_2\,^{32}PO_4$ as a tracer. Phosphate uptake was determined by the isotope distribution method [O. Lindberg and L. Ernster, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1956), vol. 10, p. 1], as modified by Grav *et al.* (*14*). The inorganic phosphate (P_i) taken up was related to the equivalent oxygen consumption as measured from polarographic traces.
14. H. J. Grav, J. I. Pedersen, E. N. Christiansen, *Eur. J. Biochem.* **12**, 11 (1970).
15. L. Ernster and R. Luft, *Exp. Cell Res.* **32**, 26 (1963).
16. B. Chance and G. R. Williams, *J. Biol. Chem.* **217**, 409 (1955).
17. R. J. Guillory and E. Racker, *Biochim. Biophys. Acta* **153**, 490 (1968).
18. J. Rafael, D. Klaas, H. J. Hohorst, *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1711 (1968).
19. D. G. Nicholls, *FEBS Lett.* **61**, 103 (1976).
20. W. C. Hülsmann, J. W. DeJong, A. Van Tol, *Biochim. Biophys. Acta* **162**, 292 (1968); W. C. Hulsmann, A. E. F. Meijer, J. Bethlem, G. K. Van Wijngaarden, in *Muscle Diseases*, J. N. Walton, N. Canal, G. Scarlato, Eds. (Exerpta Medica, Amsterdam, 1970), pp. 319-322.
21. We thank M. C. Keyes and D. Wooliver for assistance in the field, H. Behrisch for laboratory space at the University of Alaska in Fairbanks, R. Grammeltvedt for histochemical tests, and M. A. Smith of the University of Alaska for help with EM examinations. The L-oleyl carnitine and L-caproyl carnitine were gifts from J. Bremer, University of Oslo. Supported in part by grants GM-10402 and HL-16020, National Institute of Health, U.S. Public Health Service, and the National Marine Fisheries Service.
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Differential Mortality by Sex in Fetal and Neonatal Deaths

Abstract. *Vital statistics data for the United States from 1922 to 1936 and from 1950 to 1972 were used to analyze fetal and early neonatal mortality. This analysis corroborates the previously established pattern of the sex ratio of fetal deaths—highest from months 3 to 5, lower from months 6 to 7 or 8, and increasing at term. It also indicates a postponement of late fetal deaths into the early infant period. Whereas earlier research reports have described the pattern of the sex ratio of fetal deaths, this report repeats this analysis for a recent national data base. This line of analysis is extended by using the patterns observed in the data to produce an empirical estimate of the primary sex ratio. For 1950 to 1972, this ratio (male to female) is conservatively estimated to be 120:100.*

A sex ratio at conception (primary sex ratio) in excess of the sex ratio at birth (secondary sex ratio) is a necessary condition for differential mortality by sex in utero. Although discussions of the primary sex ratio focus on the existence and extent of sex differential mortality in utero (*1, 2*), there is no clear-cut consensus on the primary sex ratio. In fact, estimates of the primary sex ratio (males:females) range from 110:100 to 170:100. The variability in these estimates derives in part from the different data under consideration (*3*) and in part from the subjective judgments that result from each reseacher's examination of the data on hand. Despite the discrepancies among these studies, one overriding similarity emerges in the pattern of the sex ratio of fetal deaths by month of gestation; the sex ratio of fetal deaths is reported to be highest between months 3 and 5, lower between months 6 and 7 or 8, and increases at term (*4*).

Cavalli-Sforza and Bodmer (*2*) have contended that vital statistics provide the best data source for analyzing sex-differential mortality in utero, as vital statistics encompass enough cases to assure the significance of the relatively small differences in sex ratio. Thus, I used annual data for the sex ratio of fetal deaths by month of gestation for the United States from 1922 to 1936 and from 1950 to 1972 (*5*) in conjunction with data on early infant mortality (*6*).

The sex ratios were calculated as the ratio of males to females; after a preliminary graphic analysis of the general trend, least-squares and polynomial regression techniques were used to fit second-degree equations to the fetal death data. This analysis was performed on the arithmetic means of the sex ratio of fetal deaths by months (*7*) (Fig. 1).

Analysis of fetal death data for 1922 to 1936 reveals a nonlinear pattern that reflects the patterns reported in earlier studies (*3*). This pattern can be described by the second-order equation (*8*).

$$SR = 7.5637 - 1.7470M + 0.1155M^2 + e$$
$$\qquad\qquad (0.3370) \quad (0.0256)$$

$$R^2 = .8965$$

where SR = sex ratio of fetal deaths, M = month of gestation, and e = the least-squares residual; the values in parentheses are the standard errors.

The 1950 to 1972 fetal death data are limited to data from months 5 to 10; these data can be described by the second-order equation (*9*).

$$SR = 2.1958 - 0.2295M + 0.0119M^2 + e$$
$$\qquad\qquad (0.1176) \quad (0.0076)$$

$$R^2 = .9116$$

The nonlinear pattern of the 1950 to 1972 data differs from the pattern of the earlier data. The difference may be attributed to several factors. (i) The pattern observed from months 5 to 7 in the 1922 to 1936 data is present at a lower level in the 1950 to 1972 data. (ii) Available data from spontaneous and induced abortions in the first trimester of gestation support the notion that sex ratios of early fetal loss are higher than subsequent fetal death sex ratios (*10*). (iii) The largest difference occurs among fetal

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Table 1. Male stillbirth mortality loss proportions.

	y_1 = 0.5652 (130 : 100)				y_1 = 0.5455 (120 : 100)			
	f = 0.2	f = 0.3	f = 0.4	f = 0.5	f = 0.2	f = 0.3	f = 0.4	f = 0.5
				1950 (y_2 = *0.5131*)				
m	0.3515	0.4325	0.5136	0.5947	0.2976	0.3854	0.4732	0.5610
$m - f$	0.1515	0.1325	0.1136	0.0947	0.0976	0.0854	0.0732	0.0610
				1964 (y_2 = *0.5115*)				
m	0.3556	0.4361	0.5167	0.5972	0.3021	0.3893	0.4766	0.5638
$m - f$	0.1556	0.1361	0.1167	0.0972	0.1021	0.0893	0.0766	0.0638

deaths between months 8 and term. The data from 1950 to 1972 indicate that the sex ratio of fetal deaths for month 8 to term remains approximately the same as that of months 6 and 7, whereas data from 1922 to 1936 reflect an increase in the sex ratio of fetal deaths from month 7 to term. Teitelbaum (*11*) observed a similar change in his analysis of perinatal mortality data from five Western European countries, from 1901 to 1963 (*12*). He concluded that decreases in the sex ratio of late fetal deaths (deaths occurring from month 7 on) are associated with increases in the sex ratio of early infant deaths (deaths occurring within 7 days of birth). Data from the United States are less clear. In fact, the sex ratio of late fetal deaths declines over time, whereas the sex ratio of early infant deaths shows no clear trend. Close examination of these data reveals that the sex ratio of perinatal deaths declined from 136:100 in 1922 to 127:100 in 1972. Although the sex ratio of early infant deaths does not increase over time, the decline in the sex ratio of late fetal deaths, from 134:100 in 1922 to 109:100 in 1972, results in an increase in the absolute difference between the sex ratio of late fetal deaths and the sex ratio of early infant deaths (2 in 1922 versus 31 in 1972). Thus, there is a trend toward an increasing excess in the sex ratio of the early infant deaths relative to the sex ratio of the late fetal deaths; Teitelbaum's argument that there is a postponement of late fetal deaths into the early infant period is supported.

Cavalli-Sforza and Bodmer (*2*) presented a model that allows us to consider the implications of these mortality differentials relative to the primary sex ratio. This model provides for the computation of the amount of sex-differential mortality in utero necessary to go from a primary sex proportion to a given secondary sex proportion (*13*). More specifically, the product of the proportion of males surviving to term $(1 - m)$ and the proportion of males at conception (y_1) yields a survival index for males; similarly, the product of the proportion of females surviving to term $(1 - f)$ and the proportion of females at conception (x_1) yields a survival index for females. The survival index for males is divided by the sum of these two indices to produce an empirical estimate of the proportion of males at birth (y_2):

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

Thus, if we know the male secondary sex proportion (y_2), and if we can make certain assumptions about the proportional female zygote loss (f) we can deduce the associated levels of male zygote loss (m). From this we can deduce the excess male loss $(m - f)$ that would result from specific male primary sex proportions (*14*). We compare these calculations with the observed values of $(m - f)$ to decide on a minimum estimate of the male primary sex proportion.

The 1950 to 1972 data indicate that the observed male proportions of live births ranged from 0.5115 in 1964 (low) to 0.5131 in 1950 (high) (*15*). The female zygote mortality proportions of 0.2, 0.3, 0.4, and 0.5 are based on reported estimates of fetal loss (*16*). These two sets of values are used in conjunction with primary sex ratio estimates of 130 (y_1 = 0.5652) and 120 (y_1 = 0.5455) to produce the range of possible values of male fetal mortality proportions (Table 1). By way of example, in 1964 the male secondary sex proportion (y_2) was 0.5115. If for that year, we assume that 40 percent of the female zygotes did not survive (f = 0.4), under an assumption of a primary sex ratio of 120 (y_1 = 0.5445), 47.66 percent of the male zygotes did not survive (m = 0.4766). The difference between the mortality proportions $(m - f)$ reflects the proportion of male fetal mortality in excess of female fetal mortality required to meet each set of assumptions. This value is 0.0766 or 7.66 percent excess of male fetal mortality relative to female mortality for the assumptions in our example.

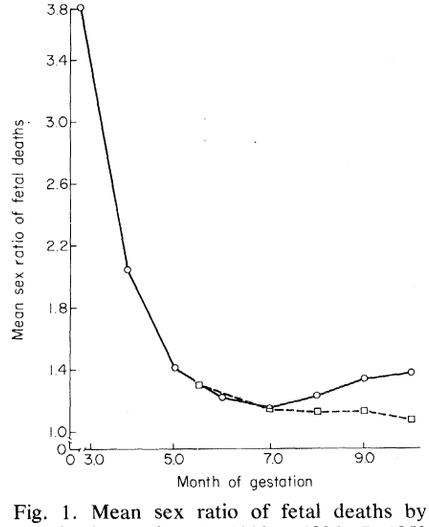

Fig. 1. Mean sex ratio of fetal deaths by month of gestation. ○, 1922 to 1936. □, 1950 to 1972.

The data in this analysis support the existence of a primary sex ratio of at least 120:100. More specifically, we expect excesses of male fetal mortality over female fetal mortality on the order of 6 to 10 percent; 1950 $(m - f)$ = 0.0610 to 0.0976 and 1964 $(m - f)$ = 0.0638 to 0.1021. Although we do not have specific observed values of the proportion loss of male and female zygotes, we can compute the excess of male loss relative to female loss. The observed proportions of male fetal deaths by month of gestation are on the order of 0.52 to 0.59, with the corresponding female proportions ranging from 0.48 to 0.41. Thus, the observed excesses of 4 to 18 percent support the 6 to 10 percent estimates of excess male fetal mortality predicted by this analysis.

This analysis uses data for mortality sex differentials in utero, the observed proportion of males at birth, and estimates of zygote loss, to produce empirical estimates of the primary sex ratio. The resulting estimate of the primary sex ratio differs from earlier estimates insofar as it is based on an empirical analysis of observed data. The data for the sex ratio of fetal deaths indicate a disproportionately high level of male mortality in utero; the pattern of this mortality differential is systematic and tends to be relatively constant over the 38 years of available data. A primary sex ratio of at least 120:100 is possible. This is a conservative estimate with confirmation of a primary sex ratio greater than 120:100 contingent upon more complete knowledge of the sex ratios of stillbirths in the first two trimesters of gestation.

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References and Notes

1. A. Ciocco, *Hum. Biol.* **10**, 235 (1938); E. Takahashi, *ibid.* **23**, 41 (1951); B. K. Sladen and F. B. Bang, *Biology of Populations* (Elsevier, New York, 1969); A. Scheinfeld, *Heredity in Humans* (Lippincott, New York, 1972).
2. L. L. Cavalli-Sforza and W. F. Bodmer, *The Genetics of Human Populations* (Freeman, San Francisco, 1971).
3. These estimates are based on data from Paris, 1896 to 1902 (from 4 months to term); Washington, D.C., 1874 to 1902 (from 4 months to term); United States, 1922 to 1928 (from 3 months to term); Vienna, 1893 to 1910 (from 3 months to term); Budapest, 1903 to 1907 (from 4 months to term); and Paris, 1901 to 1909 (from 4 months to term).
4. J. B. Nichols, *Am. Anthropol. Assoc.* **1**, 247 (1905–1907); S. J. Holmes and V. P. Mentzer, *Hum. Biol.* **3**, 560 (1931); S. Winston, *ibid.* **4**, 272 (1932); C. Tietze, *ibid.* **20**, 156 (1948); T. McKeown and C. R. Lowe, *ibid.* **23**, 41 (1951).
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, $R^2 = .8017$.
10. V. Tricomi, D. M. Serr, G. Solish, *Am. J. Obstet. Gynecol.* **79**, 504 (1960); D. Serr and B. Ismajovich, *ibid.* **83**, 63 (1963). These studies report ratios of 135 to 166 males per 100 females.
11. M. S. Teitelbaum, *Demography* **8**, 541 (1971).
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).
16. M. G. Kerr, *J. Biosoc. Sci.* **3**, 223 (1971).

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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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