

of a teneral adult of *Periplaneta* (Fig. 4). The epicuticle was recognizable as composed of two layers, only the outer of which stained red, in the intersegmental membranes of *Thermobia*, *Periplaneta* (Fig. 3), and adult *Apis* (Fig. 7), in prospective adult sclerites from old *Apis* pupae, and in both larval and puparial cuticles of *Sarcophaga* (Fig. 2). The outer epicuticle stained red, the inner epicuticle pink, in early developmental stages of the adult cuticle in the pupa of *Apis* (Fig. 6). The epicuticle was unstained, and the procuticle nearly or completely negative also, on the dorsum of *Thermobia*, partly or fully sclerotized sclerites of adult *Periplaneta* and *Apis*, and both sclerites and intersegmental membranes of *Sinea* and *Tribolium*.

Accepting this demonstration of a carbohydrate (polysaccharide?) in the epicuticle of numerous insect species, the question of how to interpret the case of those species that give negative results arises. An answer for the honeybee is available. When the adult cuticle is being formed late in pupal life, the outer epicuticle stains red and the inner epicuticle stains pink over all of the antenna (Fig. 6); later in pupal life the outer epicuticle of the sclerites stains only pink, the inner epicuticle seemingly not at all; and still later, at the time of emergence of the adult from the pupa, the outer epicuticle of the intersegmental membrane stains red (Fig. 7), that over the sensory pore plates stains pink, and that over the general sclerites and setae does not stain at all. Less complete data outlined in the preceding paragraph suggest that the same explanation will hold for *Periplaneta* and *Tribolium*. Also, it seems relevant to point out an observed correlation: when the epicuticle stains intensely, the procuticle stains moderately, whereas when the epicuticle is negative, the chitin-containing procuticle is also negative (*Apis*, *Sinea*, and *Tribolium*) or nearly so (*Thermobia*). Admitting that negative evidence is not proof, the suggestion is still obvious that the insect epicuticle consistently contains a carbohydrate component but that in some species or regions the carbohydrate becomes masked by sclerotization processes.

To the biologist, demonstration of a carbohydrate in the epicuticle is of minor importance in comparison to the concomitant demonstration that epicuticle may vary in composition not only from species to species but also from area to area on one species. Thus, commonly, two types of epicuticle can be shown on one species, and in the honeybee at least three types can be demonstrated on the antennae.<sup>3</sup> Since the work of Wigglesworth and Beament has shown that the epicuticle is the major portion of the effective barrier between the insect and its environment (at least for the passage of water) (3), the demonstration of local gross variations is important and, unfortunately, complicates precise analyses of penetration through the cuticle.

<sup>3</sup> Visible differentiation can also be made with Mallory's connective tissue stain, which at different developmental stages and on various areas gives red, blue, purplish, or no staining to the epicuticle of honeybee antennae.

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## The Fluoride Content of Placental Tissue as Related to the Fluoride Content of Drinking Water<sup>1</sup>

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Current programs to fluoridate water supplies as a dental caries control measure have stimulated interest in the extent to which the fluoride might be retained by the various tissues of the body. Considerable evidence is available to show that much of the fluoride is excreted in the urine, but there is little information on the possible accumulation or storage of the fluoride not excreted.

A previous study of normal human blood fluoride concentrations of residents of Rochester and Newburgh, N. Y. (1), has disclosed blood fluoride as a function of the fluoride concentration in drinking water. Higher levels of blood fluoride were noted for residents of Newburgh, where the fluoridated water supply contains 1.0–1.2 ppm fluoride, than for residents of Rochester, where the supply contains approximately 0.06 ppm.

In this investigation samples of placentae were obtained from the afterbirth of normal patients residing in Rochester and Newburgh, and the fluoride contents determined by the method of Smith and Gardner (2) as described for blood. Table 1 shows the distribution of the fluoride content of the placental samples as found for the two cities.

Of the Rochester samples 58% contained less than 50 µg/100 g of tissue, whereas only 17% of the Newburgh samples were in this range. Only one Rochester sample contained more than 200 µg/100 g, but 6 of the Newburgh samples contained more than this concentration. The Rochester samples had a mean concentration of 0.74 ppm fluoride and the Newburgh samples 2.09 ppm, almost three times as much. In the study of blood fluoride concentrations (1) the Rochester samples had a mean value of 0.014 ppm fluoride and the Newburgh samples 0.040 ppm—also almost three times as much. Thus the increased level of

<sup>1</sup> This article is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.

TABLE 1

DISTRIBUTION OF FLUORIDE CONTENT OF THE NORMAL  
HUMAN PLACENTAL SAMPLES FROM ROCHESTER  
AND NEWBURGH, N. Y.

$\mu\text{g}$ fluoride/ 100 g	Rochester		Newburgh	
	No. samples	Per- centage	No. samples	Per- centage
0-49	7	58	2	17
50-99	2	17	3	25
100-199	2	17	1	8
200-299	1	8	4	33
300-599	0	0	2	17
Totals	12	100	12	100

fluoride in the water resulted in a higher concentration of fluoride in the placental tissue, which may be related to the blood fluoride concentration. Also, it should be noted that both the Rochester and Newburgh placental samples had higher concentrations of fluoride than the respective blood samples. Two explanations are suggested for this observation. First, if fluoride is an essential trace element the placenta may act as a concentrating organ for fluoride, to ensure that the fetus will have adequate fluoride for the developing tissues. Second, since excessive fluoride is toxic, this organ may be acting as a barrier to prevent more than trace amounts of fluoride from reaching the fetus. How much of this accumulating fluoride passes from the placenta to the fetus is yet to be determined. At any rate, the placental concentrations are not of the order of magnitude to cause deleterious effects in the mother.

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## The Significance of Intravas pH in Relation to Sperm Motility<sup>1</sup>

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Important contributions in the field of reproductive physiology have concerned the relation of mammalian sperm activity to the hydrogen ion concentrations of the dilution media (1-4). These investigations were studies *in vitro*, generally on seminal sperm, in which the criteria of activity included motility, glycolysis, and oxidative respiration. Emmens' extensive work on rabbit spermatozoa indicated that the pH optimum for motility is in the pH range 7.2 to 7.9, whereas the results of Lardy and Phillips demonstrated a pH of

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6.8 as optimum both for motility and respiration. Insofar as they go these data are of considerable practical importance, and probably the apparent discrepancy in results can be resolved on a basis of the chemical nature of the diluents and the relative dependability of the methods used in rating sperm motility. However, in these investigations two fundamental biological problems have been neglected, problems of more significance than the determination of the absolute pH values for optimum activity: (1) the mechanism by which pH changes affect sperm motility, and (2) the biological significance of pH in controlling the motility of sperm *in vivo*, particularly within the male reproductive ducts. This note concerns the second of these problems.

The spectacular achievement of motility by mammalian spermatozoa immediately following ejaculation represents a sudden change in metabolic pattern. When first obtained from the epididymis or from the vas deferens, sperm are immotile but rapidly become active under the new environmental conditions. The initiation of activity of vas sperm *in vitro* has prompted several suggestions as to the nature of the intravas inhibition of motility. Oxygen deficiency, high acidity caused by acid metabolites, and lack of space have been suggested as being mainly responsible for the immotility of sperm while still within the ducts, each of which conditions is modified at the time of ejaculation.

The data presented here indicate that a low pH is not normally found in the vas; an acid condition brought about by lactic acid or carbon dioxide accumulation does not prevail. On the other hand, we have evidence (to be presented elsewhere) that a very low intravas oxygen tension combined with a deficiency of carbohydrate substrate may indeed be the significant limiting factor for sperm motility and that this, rather than the hydrogen ion concentration, inactivates the vas sperm. This brings into line the motility regulation of mammalian sperm with that of sea-urchin sperm, in which it is now clear that oxygen lack is the important inactivating physical factor (5). Although the present suggestion is not new in regard to the relative insignificance of hydrogen ion concentration in inhibiting the motility of mammalian sperm in the vas deferens, no direct measurements of intravas pH have yet been recorded.

Mature, breeding buck rabbits were investigated under Nembutal anesthesia; pH determinations were made with the Beckman (Model G) pH meter, using a standard microglass electrode of approximately 5- $\mu\text{l}$  capacity. The capillary containing the material to be measured was carried in a vessel of hydrochloric acid-quinhydrone solution connected to the instrument through an HCl-platinum wire junction. A calomel electrode completed the circuit. The electrodes were standardized against buffers at pH 7.00 and 4.00 before, and usually after, each determination. All measurements were made at room temperatures. Following the abdominal incision and exposure of the vas deferens, this duct was clamped off and removed. The