by Gould and Morgan (*7*) for the laboratory rat, and by Schleidt (*8, 9*) for several small, wild rodents. In addition, Kahmann and Ostermann (*10*) have recently shown that hamsters when deprived of their vision can jump to a feeding stand without hesitation. These high-frequency sounds may serve for communication between individual rats; and it is also conceivable, though certainly not yet demonstrated, that rodents use high-frequency sounds for orientation in some manner comparable to the process of echolocation employed by bats.

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The Influence of Hydrocortisone on the Epithelial Phosphatase of Embryonic Intestine *in Vitro**

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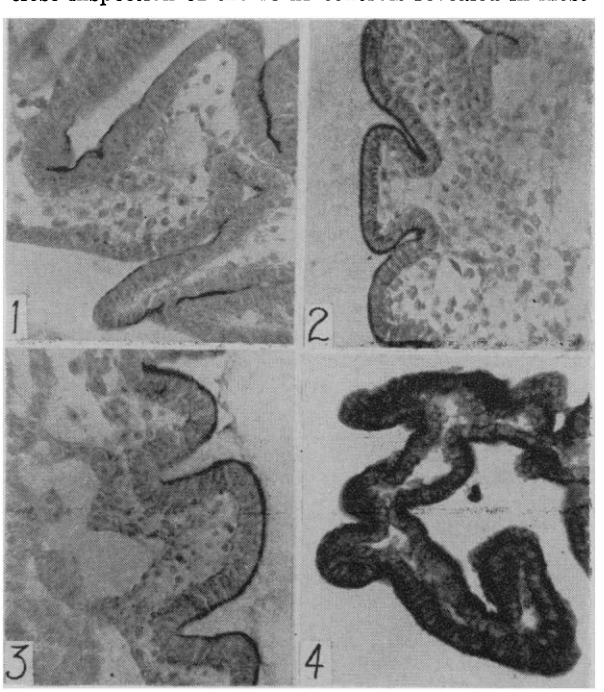
The high concentration of alkaline phosphomonoesterase characteristic of duodenal epithelium is accumulated, in both chick embryos and young mice, in short periods immediately preceding onset or change of digestive function (*1, 2*). In the mouse the accumulative phase is under the control of the pituitary-adrenal axis (*3*), and in the chick embryo *in ovo* duodenal phosphatase can be raised precociously to the hatching level by injection of cortisone (*4*). These studies bring into question the locus of action of the cortisone that was administered: does it affect the intestinal mucosa directly, or does it act indirectly, as for example by interfering with growth (*3, 5*) and thus enlarging the supply of raw material available for specific syntheses?

To determine whether exogenous corticoids can influence intestinal phosphatase concentration without the intermediation of other organs, fragments of the duodenal loop of 16-day chick embryos were raised *in vitro*. Each fragment was supported in a small piece of cellulose sponge (*6*) in a Maximov dish; the medium consisted of a drop of chicken plasma plus chick embryo extract, to which was added a tiny droplet of either saline or solution containing 0.05 gamma of hydrocortisone as free alcohol. The 16-day stage was chosen because it is the time at which the intestine acquires its maximal sensitivity to corticoids (*4*); hydrocortisone has been shown in this laboratory to be just as effective as cortisone in promoting intestinal differentiation. After 24 or 48 hr in culture, the fragments were fixed in iced 85 percent alcohol, embedded in paraffin, and sectioned serially at 7 microns (7 μ). Segments of each ribbon were stained with hematoxylin and eosin, or for phosphatase according to Gomori's current method (*7*).

In the hydrocortisone-treated fragments, the villi present at the time of explanatation were maintained without elongating, during the 48-hr period considered here, whereas in the controls they tended to shorten somewhat. Long, richly branched outgrowths (Figs. 1, 4) consisting of lamina propria covered by columnar epithelium were also produced, and these were more numerous and more elaborate in the experimentals than in the controls. The mucosa in general preserved a quite normal appearance between the muscular layer and the bases of the villi.

The epithelial cells clothing the surfaces of the cultures developed according to the normal pattern, growing progressively longer and narrower and the apical cytoplasm becoming increasingly dense; in both experimentals and controls, these events proceeded slightly faster than *in vivo*. The cut edges of the fragments became covered with an epithelium that sometimes remained squamous but often produced highly differentiated columnar cells. Although the differentiation appeared grossly normal in all series, close inspection of the 48-hr controls revealed in most



Figs. 1 and 2: control (1) and hydrocortisone-treated (2) explants, cultured 24 hr, incubated in phosphate medium 15 sec. Figs. 3 and 4: control (3) and hydrocortisone-treated (4) explants cultured 48 hr, incubated 10 min. (×440)

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cases an excessive number of nuclei crowded together within a given length of surface. In pursuance of this observation, mitotic counts are now being made.

The activity and distribution of phosphatase were studied in sections incubated for 15 sec, 10 min, and 30 min in the buffer-substrate solution. Staining was limited to the nuclei of the lamina propria and to the cytoplasm, nuclei, and free border of the epithelial cells. Of these sites, only the border and cytoplasm showed any clear-cut differences according to the presence or absence of the hormone. These differences are summarized in Table 1, in which the stain intensity has been estimated by visual inspection: 0 means no stain; 1, occasional lightly-stained border patches or pale grey cytoplasm; 2, deeply but irregularly stained border or moderately grey cytoplasm; 3, solid black continuous border or blackened cytoplasm.

The striated border stained heavily after only 15 sec incubation in most of the experimentals (Fig. 2), but in the controls it stained irregularly or not at all (Fig. 1). After 10 min, the border stained deeply in most of the controls, although two of the 24-hr controls remained negative. Since the 15-sec reaction in the experimentals was about equivalent to that found in the borders of intact embryos of 17 or 18 days, it appears that the controls had failed to undergo the increase that normally occurs after 16 days.

Ten minutes of incubation produced some darkening of the cytoplasm of the epithelial cells of the hydrocortisone-treated cultures, some of which stained intensely (Fig. 4). In the *in vivo* controls of the same ages, the cytoplasm did not stain after 10 min and the reaction was also weak or negative in the explant controls (Fig. 3). After 30 min of incubation, the epithelial cytoplasm of the cultured controls became somewhat grey, but that of the experimentals was blackened.

These data show that hydrocortisone is able to promote accumulation of phosphatase by acting directly on intestinal tissue. Quantitatively the difference between the treated explants and controls is probably substantial, for correlated quantitative and histochemical studies indicate that only a large difference can be visualized (*2*). It is not clear, however, that the synthesis of the enzyme is entirely dependent on the hormone, for comparison of the cytoplasmic staining in the 24- and 48-hr controls suggests that some increase occurs in these too (Table 1), although there are not enough cases to prove this point. It has previously been shown that, even in explants from 14-day embryos, the border reaction increases slightly without addition of any hormone (*8*).

Table 1. Numbers of explants showing various degrees of phosphatase staining (see text). C = control; E = hydrocortisone-treated.

Region and stain time	Hours in culture		Stain intensity 0	1	2	3
Striated border (15 sec)	24	C	5	1	3	0
		E	0	1	1	9
	48	C	4	3	2	0
		E	1	0	1	9
Cytoplasm of epithelial cells (10 min)	24	C	7	1	1	0
		E	0	2	4	5
	48	C	4	2	3	0
		E	0	1	4	6

A previous report that adrenocorticoids decrease intestinal phosphatase *in vitro* (*9*) is not in conflict with our results, for the study was made on 13-day or 14-day tissue, which is not yet sensitive to corticoids *in vivo* (*4*), and employed culture conditions that permitted the epithelium to spread in sheets. Probably the maintenance of normal differentiation is essential to normal enzyme production, although our results do show that differentiation and enzyme synthesis may be partly dissociated, for on many of our explants we found areas where a strong border reaction had occurred on cells that were still cuboidal or had elongated only slightly. Further work now in progress in this laboratory is concerned with quantitating the present data as well as extending these studies to other aspects of total functional differentiation.

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Evaluation of Bone Density from Roentgenograms

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For years clinical roentgenologists and research workers have sought a method for precise, reproducible, roentgenographic determination of bone mineralization. An apparatus was described by Brown in 1951 (*1*), with which it is possible to obtain an accurate reading for bone density in the light of exposure and developing factors for the particular film. This consists of a modified densitometer, a function transformer, and recording, integrating, and counting devices.

Roentgenograms are taken with a specially designed, standardized, aluminum alloy wedge exposed in proximity to the bone under consideration as shown in Fig. 1. The standardized wedge and detailed instructions are available from our laboratory.

To determine the bone density from such a film, the