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

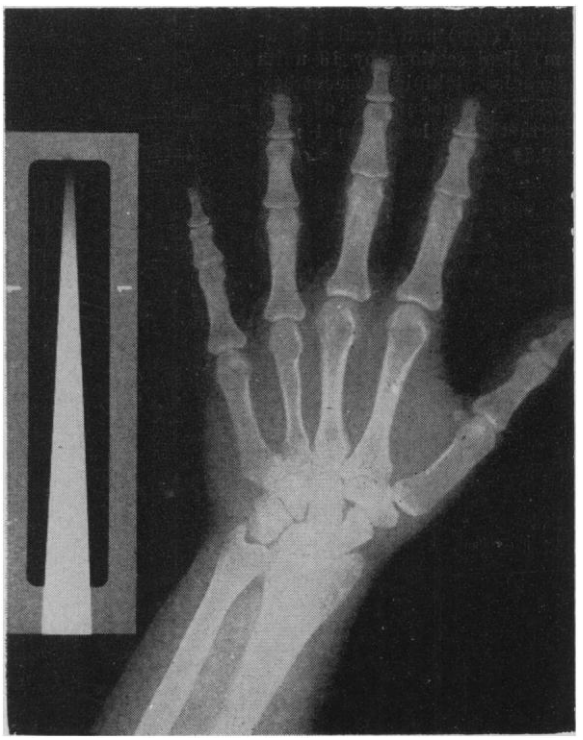

Fig. 1. A typical roentgenogram for bone-density evaluation.

first step is to produce a calibration curve by measuring the light transmitted through various points along the wedge image. The calibration curve so obtained is different for each film, owing to differences in exposure and developing. The function transformer is then set according to the calibration curve. When one measures the light transmitted through the bone image on the film, the result is computed automatically in terms of the calibration curve previously determined. This feature makes this method unique and reproducible.

The soft tissue surrounding a bone is a source of error (*2*), and we find that the soft-tissue mass must be determined by special computations to give a correct figure for bone density. Owing to this source of error, most of the applications of this technique have been limited to the phalanges, radius and ulna, and os calcis where soft-tissue corrections can be made quite accurately. We are currently investigating techniques that may eliminate soft tissue as a source of error.

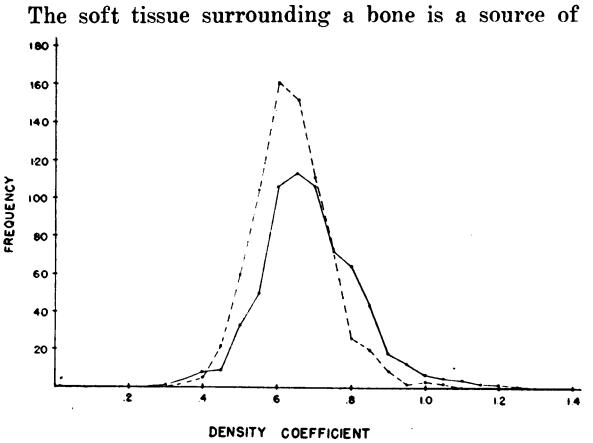

Fig. 2. Range of bone-density coefficients for the os calcis in approximately 1200 individuals. Frequency represents the number of times the stated value appears. The solid line represents males; the dashed line, females.

The bone-density results are computed and reported as an average of "density coefficient" for the particular bone evaluated. This density cofficient can be multiplied by a constant factor to find the density of the bone in grams of bone ash per cubic centimeter of bone.

Bone-density determinations from films from approximately 1200 "normal" persons of all ages and both sexes reveal a 2-to-1 range of variation in general (Fig. 2).

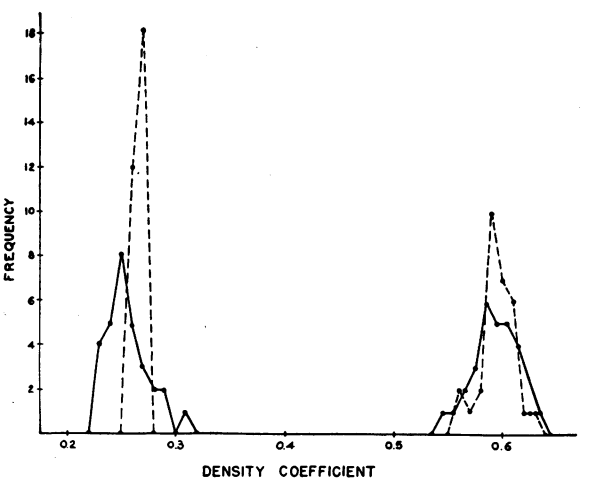

Fig. 3. Reproducibility of the technique. Frequency represents the number of times the stated value appears. The curve on the left is for the soft tissue near the os calcis; the curve on the right is for the os calcis. The solid line represents 30 films of one subject taken at 1-hr intervals; the dashed line represents 30 evaluations of one film in the same series.

It appears possible to divide "normal" human adults into approximately 10 classes of bone mineralization by this technique. At present, we are attempting to determine average bone-density coefficients for different sex and age groups.

The technique is highly reproducible, as is shown by Fig. 3. This graph shows the results of evaluating 30 films of one subject and 30 evaluations of one film from the same series. The reproducibility in the case of different films is on the order of 5 percent, which appears to be much more accurate than other known methods.

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