without corresponding decrease in absorption of Ca^{45}. This apparent difference in effect of sodium alginate on absorption of calcium and strontium probably does not reflect solely the relative ion-binding of alginate with Sr and Ca, since the observed difference in binding is quite small (*3*).

Ratios of guluronic acid to mannuronic acid, based on Manucol SS/LD at 1.0 (five determinations), were determined at: Manucol SA/LD, 0.36 (four determinations); Manucol SA/LM, 0.43 (four determinations); and calcium alginate, 0.45 (two determinations). We conclude that a sodium alginate with a high guluronic : mannuronic ratio more effectively reduces uptake of radioactive Sr from the diet.

Our results confirm that sodium alginate, unlike most other therapeutic agents used to reduce uptake of radioactive strontium, does not interfere with calcium absorption; they also show that even greater protection from such strontium is afforded by supplementing the diet with alginate containing a higher proportion of guluronic acid.

GEORGE E. HARRISON
ERIC R. HUMPHREYS
ALICE SUTTON, HILDA SHEPHERD
Medical Research Council,
Radiobiological Research Unit,
Harwell, Didcot, Berkshire, England

Reference and Notes

1. F. G. Fischer and H. Dörfel, *Z. Physiol. Chem.* **302**, 186 (1955); A. Haug and B. Larsen, *Acta Chem. Scand.* **16**, 1908 (1962).
2. D. Waldron-Edward, T. M. Paul, S. C. Skoryna, *Can. Med. Assoc. J.* **91**, 1006 (1964); *Nature* **205**, 1117 (1965).
3. A. Haug, *Acta Chem. Scand.* **15**, 1794 (1961).
4. We thank Roy Brooks and Jill Pollard for technical assistance, and Alginate Industries, Ltd., Bedford St., London, W.C.2, for supplying the alginates.

25 February 1966

Intestinal Alkaline Phosphatase: Regulation by a Strain-Specific Factor in Mouse Milk

Abstract. *Two inbred strains of mice show a threefold difference in duodenal phosphatase activity at 11 days of age. When half-litters of the two strains are interchanged between the two mothers on the day of birth, enzyme activity in young of the low-activity strain is unaffected at 11 days by the source of milk, but is significantly reduced in high-activity young nursed by a low-activity mother.*

Some enzymes that have been studied in higher organisms appear to be constitutive in the sense that their formation is not "electively provoked by a substrate," the level of activity characteristic of a tissue or organ rather being genetically determined (*1*). Nevertheless, an increasing number of enzymes are known to be regulated by mechanisms that are not directly genetic. Tryptophane pyrrolase of rat liver is affected by substrate and by glucocorticoids (*2*), the effect of substrate being apparently to stabilize the enzyme (*3*). Adrenocortical hormones raise the levels of several enzymes, including alkaline phosphatase (*4*) and invertase (*5*) in the small intestine of the mouse and rat and gluconeogenic enzymes in the rat liver (*6*). Creatine represses development of arginine-glycine transaminidase in the liver of the chick embryo (*7*), and dietary constituents regulate threonine dehydrase activity in rat liver (*8*).

Alkaline phosphatase activity of the duodenum of the mouse has been extensively investigated (*9*). Preliminary studies with eight inbred strains revealed marked differences in intestinal phosphatase activity. The highest-activity strain (SWR/J; Jackson Laboratory) has activity almost three times that of the lowest-activity strain (LAS) (Swiss; maintained in this laboratory since 1949), and this difference has been consistently maintained through six generations during our investigation. Although a genetic basis is thus indicated, differences in amount or quality of milk produced by the two strains may influence phosphatase activity during nursing stages. We have found that high-activity-strain (HAS) milk does contribute to phenotypic expression of the HAS genotype. This is a heretofore unrecognized mode of enzyme regulation.

Because duodenal phosphatase begins to be affected by endogenous corticoids at about 13 days (*9*), we studied 11-day-old mice. When two litters were born on the same day, one to HAS (SWR/J) and one to LAS (local Swiss) parents, half of each litter was switched to the other mother on the day of birth; they were readily accepted by the foster mothers and gained weight as rapidly as their foster siblings. At 11 days the young of both litters were decapitated. An 8-mm piece of duodenum, just distal to the entrance of the common bile duct, was excised and homogenized in iced distilled water in a Ten Broeck grinder. The homogenate was assayed for protein content (*10*) and phosphatase activity against phenylphosphate (PhP) and beta-glycerophosphate (bGP) under optimal conditions for each substrate (*11*).

Mixtures of homogenates of HAS and LAS duodenum yielded average phosphatase values, indicating that the strain differences are not due to dissociable activators or inhibitors; this conclusion had been shown to hold for differences in phosphatase levels between infant and juvenile stages (*12*). It had also been shown that litter size (and consequent availability of milk) does not influence phosphatase activity (*13*); this fact we verified for both strains by reducing numbers in litters from ten to three at birth: there was no effect on enzyme level at 11 days, even though members of small litters weighed about 50 percent more than members of large litters.

When the infants of one strain were nursed by mothers of the other, however, striking differences appeared. Duodenal phosphatase was unaffected in LAS mice fostered by HAS mothers (Table 1). But in the opposite situation two changes occurred: (i) phosphatase activity was significantly lower in HAS young when they were raised by LAS mothers than when raised by their own mothers; (ii) activity on PhP was more severely affected than activity on bGP, so that the PhP : bGP ratio dropped. This ratio (which expresses the amount of P cleaved from PhP to that cleaved from bGP under optimal conditions for each substrate) is proportional to level of activity during the course of

Table 1. Duodenal alkaline phosphatase activity (micrograms of phosphate per milligram of protein per 30 minutes) in 11-day-old mice of both strains nursed by mothers of both high-activity (SWR/J) and low-activity (local Swiss) strains. Five litters of ten mice each were used in the exchanges of offspring between mothers. PhP, phenylphosphate; bGP, beta-glycerophosphate; LAS, low-activity strain; HAS, high-activity strain.

Mother	Phosphatase against:		PhP:bGP
	PhP	bGP	
	LAS offspring *		
LAS	546	700	0.78
HAS	561	698	0.80
	HAS offspring †		
HAS	1792	1723	1.04
LAS	1087	1197	0.91

*Difference between the two mothers not significant. † $P < .001$ for all three columns.

development (*11*); it is higher at 11 days in HAS duodenal phosphatase than in LAS (Table 1).

This reduction in duodenal phosphatase activity in HAS mice indicates that some strain-specific difference distinguishes the milks of the two strains. Since changing litter size (and milk availability) does not alter phosphatase activity within either strain, it appears that the milk of one strain contains a constituent that is absent from (or present in ineffective concentration in) the milk of the other. Attempts to identify the factor by injecting likely substances (serine phosphate, glycerophosphate, glucose, hypertonic saline) into the stomachs of young mice have so far proved unsuccessful.

In normally developing young mice, the relatively low-ratio, low-activity phosphatase present at 11 days is replaced in the third week by two isozymes of higher ratio and activity (*14*). The replacement may be elicited precociously by actinomycin D, puromycin, cycloheximide, and ethionine, and thus appears to depend on conversion of low- to high-ratio forms rather than to *de novo* synthesis of the latter (*15*). It has been proposed that the inhibitors of protein synthesis act by removing a regulatory protein that restrains the conversion reaction, perhaps by limiting the rate at which the phosphatase-containing microvillar surface is allowed to mature (*16*). Since the normal tendency in development is toward higher ratio and higher activity (*11*), it seems reasonable to assume that the postulated restraining mechanism is weaker during the first 11 days in HAS duodenum than in LAS; thus the alkaline phosphatase activity is permitted to rise to higher levels in HAS infants than in LAS. If this is so, the role of the milk factor may be to oppose the action of the regulatory protein in the HAS, which strain loses activity when the young are taken off the milk of their natural mothers. Only the HAS is susceptible to the influence of the milk factor; since both strains are inbred, this distinction seems to be basically genotypic.

Another significant observation is that the reduced enzyme activity in HAS mice nursed by LAS mothers remains almost double the activity in the LAS. The obvious inference is that the difference between the two strains reflects at least two regulatory factors: in part the higher level of activity in the HAS must result from inherent genetic differences with respect to the determination of alkaline phophatase; but in part it reflects the action of a strain-specific milk factor that functions as an environmental determinant of variation.

P. R. V. NAYUDU
FLORENCE MOOG
Department of Zoology, Washington University, St. Louis, Missouri 63130

References and Notes

1. F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).
2. W. E. Knox, *Brit. J. Cancer Res.* **32**, 462 (1951); P. Feigelson, M. Feigelson, O. Greengard, *Recent Progr. Hormone Res.* **18**, 491 (1962); O. Greengard, M. A. Smith, G. Acs, *J. Biol. Chem.* **238**, 1548 (1963).
3. R. T. Schimke, E. W. Sweeney, C. M. Berlin, *Biochem. Biophys. Res. Commun.* **15**, 215 (1964).
4. F. Moog, *J. Exp. Zool.* **124**, 329 (1953); R. Halliday, *J. Endocrinol.* **18**, 56 (1959).
5. R. Doell, G. Rosen, N. Kretchner, *Proc. Nat. Acad. Sci. U.S.* **54**, 1268 (1965).
6. G. Weber, M. C. Henry, S. R. Wagle, D. S. Wagle, *Advan. Enzyme Regulat.* **2**, 335 (1964).
7. J. B. Walker, *Proc. Soc. Exp. Biol. Med.* **112**, 245 (1963).
8. H. C. Pitot and C. Peraino, *J. Biol. Chem.* **239**, 1783 (1964).
9. F. Moog, *Federation Proc.* **21**, 51 (1962).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
11. F. Moog, *Develop. Biol.* **3**, 153 (1961).
12. ———, in *Cell. Organism and Milieu*, D. Rudnik, Ed. (Ronald Press, New York, 1959).
13. ———, *J. Exp. Zool.* **118**, 187 (1951).
14. ———, H. R. Vire, R. D. Grey, *Biochim. Biophys. Acta* **113**, 336 (1966).
15. F. Moog, *Science* **144**, 414 (1964); *Advan. Enzyme Regulat.* **3**, 221 (1965).
16. ———, *J. Exp. Zool.*, in press; ——— and R. D. Grey, in *Symposium on Development of Metabolism in Relation to Nutrition*, O. Koldovsky, Ed. (Academic Press, New York, in press).
17. Supported by PHS grant GM 03937.

11 February 1966

Metabolism of Alveolar Cells: Histochemical Evidence and Relation to Pulmonary Surfactant

Abstract. *Large alveolar cells of normal dog lung are rich in enzymes concerned with oxidative and synthetic pathways. In three experimental situations where ability of the lung to produce surfactant was impaired, the enzyme-rich cells were lacking or absent. Findings support the concept that these cells are sites of active metabolism, possibly including production of surfactant.*

There is evidence that the lung is not a passive organ merely for exchanging oxygen and carbon dioxide, but that it also performs active metabolic functions (*1*). One function that has received special attention is the synthesis of fatty acids and phospholipids that are important ingredients of the surface-active lining, essential to alveolar stability (*2*).

To learn more about metabolic pathways in lung tissue and to identify the cellular elements most active biochemically, we examined fresh, quick-frozen lung sections for oxidative enzyme activity. To test the hypothesis that alveolar cells may have a special role in the production of pulmonary surfactant, we compared these enzyme reactions in lungs known to synthesize phospholipids and to contain surfactant (normal dog) with those in lungs whose ability to lower surface tension is either questionable (chicken) or lacking (frog, and in dogs after pulmonary artery ligation). Alveolar cells in lungs of normal dogs showed evidence of considerable metabolic activity, consistent with a biosynthetic function. The large enzyme-rich cells were lacking or absent in the other lungs, which suggests a correlation with capacity of the lung to form pulmonary surfactant.

Under anesthesia and through an open chest incision, a segment of lung was infiltrated with liquid gelatin introduced into the airway. Gelatin was allowed to solidify by pouring cold saline on the surface of the lung (*3*). A sample of lung was then removed; it was immediately quenched in isopentane cooled to about −150°C by

Table 1. Oxidative enzymes in alveolar cells of normal dog. Abbreviations: DH, dehydrogenase; NAD, nicotinamide-adenine dinucleotide; and NADP, nicotinamide-adenine dinucleotide phosphate.

Enzyme *	Coenzyme	Intensity of reaction †
NAD and NADP diaphorases		+ + + +
Isocitrate DH	NAD	+ + +
Succinate DH		+
Malate DH	NAD	+ + + +
α-Glycero-phosphate DH	NAD	+ +
Lactate DH	NAD	+ + +
Glucose-6-phosphate DH	NADP	+ +
6-Phospho-gluconate DH	NADP	+ +
Cytochrome oxidase		+ +

* Technique for diaphorase reactions was that used by Novikoff *et al.* (*4*); for dehydrogenases, as described in Pearse (*5*); and for cytochrome oxidase, according to Burstone (*6*). † Graded without count of granules.