It has been suggested that the hypocalcemia that occurs in magnesium deficiency is the result of diminished end-organ responsiveness to parathyroid hormone (*1*). However, normal end-organ responsiveness to parathyroid hormone has been observed in patients with primary hypomagnesemia (*8, 17*) as well as in magnesium-depleted dogs (*4*). If diminished end-organ responsiveness to parathyroid hormone were the cause of hypocalcemia in magnesium deficiency, elevated rather than low levels of serum IPTH would be an expected finding. In our patient, the levels of serum IPTH were consistently low in the presence of hypomagnesemia and hypocalcemia. Following the administration of intramuscular magnesium the serum IPTH increased and the parathyroid glands appeared to respond appropriately to the level of ionized calcium in blood. The results of this study, therefore, provide evidence for the occurrence of parathyroid failure in the magnesium-deficient state in man. The possibility of magnesium depletion should be considered in patients with unexplained hypocalcemia.

CONSTANTINE S. ANAST
JAMES M. MOHS
SHELDON L. KAPLAN
THOMAS W. BURNS
Department of Pediatrics and *Medicine, University of Missouri*, and *Veterans Administration Hospital, Columbia, Missouri 65201*

References and Notes

1. H. Estep, W. A. Shaw, C. Watlington, R. Hobe, W. Holland, S. G. Tucker, *J. Clin. Endocrinol. Metab.* **29**, 842 (1969).
2. F. P. Muldowney, T. J. McKenna, L. H. Kyle, R. Freaney, M. Swan, *N. Engl. J. Med.* **281**, 61 (1970).
3. L. M. Sherwood, *ibid.* **282**, 752 (1970).
4. S. M. Suk, A. Csima, D. Fraser, *J. Clin. Invest.* **50**, 2668 (1971).
5. C. S. Anast, G. D. Hawker, R. P. Utiger, J. M. Mohs, T. W. Burns, *Endocrine Society Program of the Fifty-Third Meeting*, pp. A-65 (1971).
6. J. Salet, C. Polonowski, F. DeGouyon, G. Peiu, B. Melekian, J. P. Fournet, *Arch. Franc. Pediat.* **23**, 749 (1966).
7. M. Friedman, G. Hatcher, L. Watson, *Lancet* **1967-I**, 703 (1967).
8. L. Paunier, I. C. Radde, S. W. Kooh, P. E. Conen, D. Frasier, *Pediatrics* **41**, 385 (1968).
9. I. MacIntyre, S. Hanna, C. C. Booth, A. E. Read, *Clin. Sci.* **20**, 297 (1961); M. Walser, *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.* **59**, 201 (1967); J. W. T. Dickerson and E. M. Widdowson, *Biochem. J.* **74**, 247 (1960); B. J. Hunt, *Clin. Chem.* **15**, 979 (1969).
10. H. Conaway and C. Anast, in preparation.
11. C. H. Fiske and Y. Subbarow, *J. Biol. Chem.* **66**, 375 (1925).
12. D. M. Greenberg and M. A. Mackey, *ibid.* **98**, 765 (1932); H. E. Harrison and H. C. Harrison, *Metabolism* **13**, 952 (1964).
13. R. M. Buckle, A. D. Care, C. W. Cooper, H. J. Gitelman, *J. Endocrinol.* **42**, 529 (1968).
14. P. Pletka, D. S. Bernstein, C. L. Hampers, J. P. Merrill, L. M. Sherwood, *Lancet* **1971-II**, 462 (1971).
15. J. H. Targovnik, J. S. Rodman, L. M. Sherwood, *Endocrinology* **88**, 1477 (1971).
16. J. W. Hamilton, F. W. Spierto, R. R. MacGregor, D. V. Cohn, *J. Biol. Chem.* **246**, 3224 (1971).
17. J. H. Stromme, R. Nesbakken, T. Norman, F. Skjorten, D. Skybert, B. Johannessen, *Acta Paediat. Scand.* **58**, 433 (1969).
18. Supported by PHS research grants AM 01351, HD 02756, and FR 00287. C.S.A. is the recipient of Career Research Development Award No. HD 39654 of the National Institute of Child Health and Human Development. J.M.M. was supported by PHS training grant AM 5316. We thank Mrs. Marcia Schweiss, Mrs. Noyce Noteboom, and Mrs. Sammy Langeluttig for their excellent technical assistance.

3 April 1972; revised 18 May 1972

Intestinal Uptake of Macromolecules: Effect of Oral Immunization

Abstract. *Animals were orally immunized with horseradish peroxidase and bovine serum albumin, and absorption of these antigens was studied. In comparison with controls, a consistent and significant decrease in peroxidase uptake was noted in both germ-free and conventional rats immunized with peroxidase; a similar decrease in serum albumin uptake was also noted in animals immunized with serum albumin. There was no difference in the uptake of an unrelated macromolecule. These observations suggest that local immunization interferes specifically with the intestinal uptake of macromolecular antigens.*

Although the intestinal absorption of intact macromolecules (*1, 2*) and the ability of such molecules to induce both a local and systemic immune response has been demonstrated under certain natural and experimental conditions (*2–4*), the effect of immunization on intestinal uptake of macromolecules has not been fully investigated (*5*). In previous studies from this laboratory, the absorption of horseradish peroxidase (HRP) by the small intestine of the rat was investigated using everted gut sacs in vitro (*6*), as well as using jejunostomy infusion (*7*) and instillation into ligated ileal loops in vivo (*8*). These studies indicated that an exogenous macromolecule was taken up by pinocytosis into the membranous subcellular system of the rat small intestinal absorptive cells and that the macromolecules were subsequently transported into the extracellular space of the lamina propria and from there into the lymph (*7, 8*). The present study was designed to investigate the effect of active oral immunization on the absorption of HRP and bovine serum albumin (BSA) by rat small intestinal segments in vitro.

Ten 40-day-old female germ-free rats (CD^R strain, Charles River Breeding Laboratories) were orally immunized according to the method of Crabbé *et al.* (*4*) by exposure to HRP (1 mg/ml) in the drinking water for a 10-day period; the rats were studied 2 weeks after the start of immunization. Two sets of 12 adult white female conventional Sprague-Dawley rats weighing approximately 175 g were fed either HRP (1 mg/ml) or BSA (1 mg/ml) in drinking water for 2 weeks and studied 1 week later. At the time of study, animals fasted 24 hours were subjected to a laparotomy under ether anesthesia, and the small intestine was removed. Five-centimeter everted gut sacs (*9*) were prepared and incubated for 60 minutes at 37°C in oscillating flasks containing oxygenated Krebs-Ringer bicarbonate solution and 10 μM HRP (Sigma, 250 purpurogallin units per milligram of protein). After incubation, the serosal contents were drained from the sacs and the concentration of HRP was determined enzymatically. A 0.1-ml sample of test solution was mixed with 2.9 ml of a reaction mixture containing 0.003 percent H_2O_2 in phosphate buffer (0.1M, *p*H 6.0) and 0.025 ml of an aqueous solution of *o*-dianisidine, dihydrochloride (10 mg/ml). With a Gilford recording spectrophotometer, the rate of increase in optical density at 460 nm was determined. At dilutions of HRP standard solutions below 10 μM, the relation between enzyme activity and enzyme protein concentration was not linear. However, when serial dilutions of standard solutions were assayed in phosphate buffer containing 1 percent BSA, a linear relation was noted (*10*). The concentration of enzyme protein in sac fluid was determined from a standard curve relating enzyme activity to enzyme protein. The viability of gut sacs during the test period was monitored by measuring active transport of L-[^{14}C]histidine. In order to permit comparisons of uptake by sections of intestine with different surface areas, uptake was expressed as picomoles of HRP per milligram of mucosal protein per hour.

To assure that any observed decreases in HRP absorption, as measured enzymatically, reflected actual changes in HRP uptake and not simply altered enzyme activity, ^{125}I-labeled HRP (*11*) uptake was also determined. Serosal fluid from sacs incubated in [^{125}I]HRP (10 μM) were dialyzed extensively to remove any free ^{125}I and counted in a Beckman gamma counter. Absorption of HRP (pmole/mg per hour) was determined from the specific activity. The intestinal absorption of BSA (Nutritional Biochemicals) labeled with NA^{125}I (*11*) was examined in a similar manner in control, BSA-immunized, and HRP-immunized conventional rats.

Antigen-binding activity of serum, mucosal extracts, and intestinal secretions was tested by a modification of the ammonium sulfate method of Minden and Farr (*12*). Secretions were obtained by rinsing the entire small intestine with iced saline. The mucosal surface of the gut was cleaned with gauze and removed by scraping with a glass slide. The mucosal scrapings were homogenized in a tissue grinder with a small amount of saline. Secretions and mucosal extracts were clarified by centrifugation at 105,000*g*, followed by dialysis and lyophilization. Antigen-binding activity of serum and mucosal extracts was also tested by radioimmunodiffusion (*13*), except that x-ray film was exposed for 5 to 6 days to the gel plates. Rabbit antiserum to rat immunoglobulins G_1 and A (IgG_1 and IgA) was prepared as described (*13, 14*). Mucosal extracts were mixed with an equal volume of normal rat serum as "carrier."

The uptake of HRP, measured enzymatically, by both jejunal and ileal gut sacs obtained from conventional rats orally immunized with HRP was significantly less than the uptake of gut sacs obtained from control animals ($P < .005$ and .001, respectively). Similarly, the uptake of [^{125}I]HRP by gut sacs from specifically immunized animals was significantly less than that of controls ($P < .01$ for both jejunal and ileal sacs). There was no significant difference in the uptake of [^{125}I]BSA by gut sacs obtained from HRP-immunized versus control animals (Fig. 1).

Table 1 summarizes the results obtained with conventional rats exposed to BSA in their drinking water prior to testing the in vitro uptake of homologous and unrelated protein. There was a marked decrease in uptake of [^{125}I]BSA by jejunal gut sacs and a moderate decrease in uptake by ileal sacs from the same animals; there was no decrease in uptake of HRP (measured enzymatically).

Table 1. Antigen uptake in rats orally immunized with BSA. Numbers in parentheses indicate the proportion of observations showing a significant decrease in uptake.

Antigen	Absorption (% of normal)	
	Jejunum	Ileum
[^{125}I]BSA	51 (30/30)	70 (22/30)
HRP	99 (7/7)	97 (6/6)

In experiments performed with germ-free rats orally immunized by exposure to HRP, there was a significant decrease in uptake of HRP, measured enzymatically and by the isotope technique, by gut segments tested in vitro. No decrease in uptake of [^{125}I]BSA was observed. The specific decrease in uptake of HRP in these animals was nearly identical with that observed in conventional immunized rats.

Specific binding activity for ^{125}I-labeled HRP or BSA was found in nearly all secretions and mucosal extracts obtained from immunized conventional and germ-free rats. No antigen-binding activity was noted in the serum from these same animals. Gel diffusion studies disclosed the presence of IgG_1 and IgA in secretions and mucosal extracts. In preliminary radioimmunodiffusion studies with ^{125}I-HRP, specific binding to IgG_1 but not to IgA was observed. There was no binding to the IgG_1 precipitin arcs formed between serum of orally immunized rats and monospecific rabbit antiserum to rat IgG_1.

Based on the observations that patients with a deficiency of serum (and secretory) IgA have an unusually high incidence of circulating antibodies to proteins of cow's milk (*15*), it has been suggested that intestinal absorption of intact proteins occurs more readily in patients lacking secretory antibodies to such antigens. The present study provides experimental evidence that oral immunization may specifically interfere with the uptake of protein antigens, HRP and BSA, in germ-free and conventional animals.

Bockman has demonstrated enhanced uptake of ferritin from ligated loops of small intestine in hamsters parenterally immunized with this antigen and concluded that immunized animals have an altered permeability to

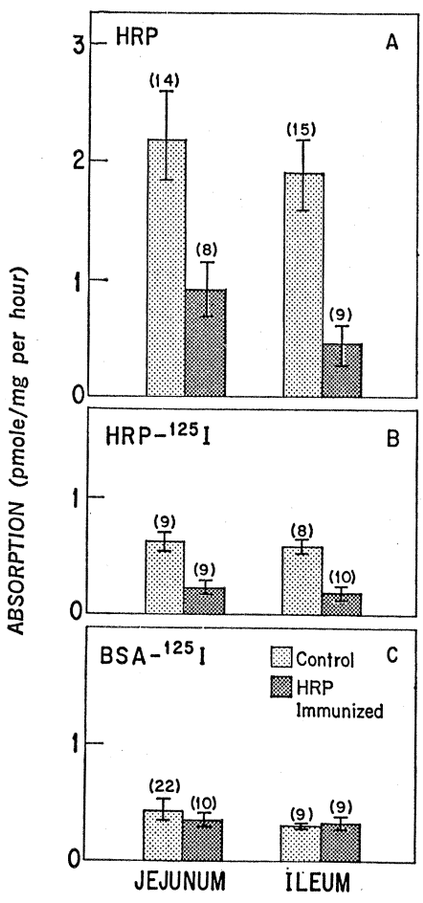

Fig. 1. Absorption of antigens into the serosal fluid of rats orally immunized with HRP and of control rats. Number of observations per group of animals is expressed in parentheses. Uptake of HRP as measured by enzymatic assay in 12 orally immunized conventional rats was significantly less than in control animals (A). The average absorption ± S.E., per hour, in jejunum of immunized animals was 0.92 ± 0.22 pmole/mg and 2.18 ± 0.37 pmole/mg in control animals ($P < .005$); in the ileum, 0.45 ± 0.15 pmole/mg was absorbed, compared with 1.89 ± 0.30 pmole/mg in controls ($P < .001$). Uptake of HRP as measured by radioactivity techniques was also significantly less in immunized animals than in controls (B). Average absorption ± S.E. of [^{125}I]HRP, per hour, in jejunum of immunized animals was 0.23 ± 0.04 pmole/mg and 0.61 ± 0.06 pmole/mg in controls ($P < .01$); in the ileum, 0.19 ± 0.06 pmole/mg was absorbed compared with 0.58 ± 0.05 pmole/mg in controls ($P < .01$). However, no significant difference in absorption of [^{125}I]BSA in jejunum (0.35 ± 0.04 pmole/mg versus controls 0.45 ± 0.07 pmole/mg) or ileum of immunized rats (0.32 ± 0.04 pmole/mg versus controls 0.29 ± 0.02 pmole/mg) was noted (C).

macromolecules which might be related to the specific antibodies produced in response to immunization (*5*). It remains to be determined whether the differences between his observations and ours are related to the species of animal, the route of immunization, or the techniques for studying macromolecular uptake.

W. ALLAN WALKER
KURT J. ISSELBACHER
Gastrointestinal Unit, Massachusetts General Hospital, Boston 02114
KURT J. BLOCH
Arthritis and Clinical Immunology Units, Massachusetts General Hospital

References and Notes

1. D. H. Alpers and K. J. Isselbacher, *J. Biol. Chem.* **242**, 5617 (19[illegible] J. P. Kraehenbuhl and M. A. Campiche, *J. Cell Biol.* **42**, 345 (1969); E. Danforth and R. O. Moore, *Endocrinology* **65**, 118 (1959).
2. I. D. Bernstein and Z. Ovary, *Int. Arch. Allergy* **33**, 521 (1968).
3. P. E. Korenblat, R. M. Rothberg, P. Minden, R. S. Farr, *J. Allergy* **41**, 226 (1968).
4. P. A. Crabbé, D. R. Nash, H. Bazim, H. Eyssen, J. F. Heremans, *Lab. Invest.* **22**, 448 (1970).
5. D. E. Bockman and W. B. Winborn, *Anat. Rec.* **155**, 603 (1966).
6. W. A. Walker, R. Cornell, L. Davenport, K. J. Isselbacher, *J. Cell Biol.*, in press.
7. A. L. Warshaw, W. A. Walker, K. J. Isselbacher, *Lab. Invest.* **25**, 675 (1971).
8. R. Cornell, W. A. Walker, K. J. Isselbacher, *ibid.*, p. 42.
9. T. H. Wilson and G. Wiseman, *J. Physiol.* **123**, 116 (1954).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
11. F. C. Greenwood, W. M. Hunter, J. S. Glover, *Biochem. J.* **89**, 114 (1963).
12. P. Minden and R. S. Farr, *Handbook of Experimental Immunology* (Blackwell, Oxford, 1967), pp. 463–492.
13. K. J. Bloch, H. C. Morse, K. F. Austen, *J. Immunol.* **101**, 650 (1968).
14. D. J. Stechschulte and K. F. Austen, *ibid.* **104**, 1052 (1970).
15. R. H. Buckley and S. C. Dees, *N. Engl. J. Med.* **281**, 465 (1969).
16. Supported by grants from NIH (AM-03014, T1AM-05146, AI-10129); U.S. Army Medical Research and Development Command (contract DADA-17-70-C-0113); and Massachusetts Chapter, Arthritis Foundation. We thank L. M. Davenport for technical assistance.

30 May 1972

Deficiency of Kallikrein Activity in Plasma of Patients with Cystic Fibrosis

Abstract. *Total kallikrein activity and kallikrein activity inhibited by soybean trypsin inhibitor are significantly reduced in the plasma of patients with cystic fibrosis compared to age-matched controls. The level of the STI inhibited kallikrein activity in the plasma of heterozygotes was significantly different from that in either controls or affected children. However, the individual heterozygote could not be reliably identified in each case.*

Cystic fibrosis (CF) is a familial metabolic disorder in which the basic defect is unknown (*1*). Several studies have indicated that saliva and serum of patients with cystic fibrosis contain "factors" which may be unique to this disease (*2–4*). Saliva of patients with cystic fibrosis has been reported to contain a macromolecular factor which inhibits sodium reabsorption (*2*), while serum of these patients contains factors which induce dyskinesis in the rhythmic beat of cilia from rabbit trachea (*3*) and from oysters (*4*).

Earlier studies in our laboratory, undertaken to explain the presence of the macromolecular factor in saliva of patients with cystic fibrosis, indicated that trypsinlike activity was deficient in the saliva of these patients (*5*). The trypsinlike activity present in normal saliva was similar in a number of its properties to those of kallikreins. Since kallikreins are present in human plasma (*6*), we have extended our studies to include the determination of kallikrein activities in plasma of controls and patients with cystic fibrosis. The results of these studies show that plasma of patients with cystic fibrosis is deficient in kallikrein activity as compared to that of either age-matched or adult controls.

We have assayed kallikrein activity as arginine esterase activated by treating plasma with chloroform and ellagic acid. This assay is valid in view of the findings of Colman *et al.* (*6*), who have shown that the level of arginine esterase activity is proportional to the concentration of Hageman factor activated by ellagic acid. Since Hageman factor is a physiologic activator of prekallikrein, we, like Colman *et al.* (*6*), assume that the arginine esterase activity reported below represents kallikrein activity.

Blood was collected in citrated plastic tubes, and contact with glass surfaces was avoided. The plasma was separated by centrifugation and either used immediately or stored at −20°C. The plasma, in siliconized tubes, was treated with an equal volume of cold chloroform and centrifuged at 4°C. The kallikrein in the plasma fraction was activated by treating with ellagic acid (final concentration 0.05 m*M*) for 15 minutes at 25°C. A portion (0.3 ml) of this plasma was transferred to tubes containing 0.015*M* α-*N*-(*p*-toluenesulfonyl)-L-arginine methyl ester (TAME), 0.10*M* phosphate buffer (*p*H 7.6), and 0.15*M* NaCl. The reaction mixture [final volume, 1.0 ml (*6*)] was incubated at 37°C for 15 minutes, and the reaction was terminated by the addition of 1.0 ml of 10 percent trichloroacetic acid. The mixture was centrifuged and the methanol in the supernatant was assayed according to Siegelman *et al.* (*7*). Activity was assayed in the presence and absence of soybean trypsin inhibitor (STI).

Plasma treated with chloroform and ellagic acid contains at least two types of activities. One type of activity is inhibited by low concentrations of STI (5 μg/ml), whereas the other is resistant even to very high concentrations of inhibitor. In order to assay for both

Table 1. Kallikrein activity in plasma. The results are expressed as micromoles of TAME utilized per hour per milliliter of plasma.

Sample	*N*	Total activity		STI-inhibited activity		STI-resistant activity	
		M ± S.D.	Range	M ± S.D.	Range	M ± S.D.	Range
Normal adults	15	43.3 ± 17.7	20.2–73.5	30.2 ± 15.3	12.9–69.1	13.1 ± 13.1	1.1–51.6
Normal children	20	41.2 ± 7.6	30.5–55.1	23.2 ± 6.2	14.1–35.6	17.9 ± 5.4	10.1–31.4
Cystic fibrosis (CF_1)	20	16.8 ± 6.9	2.4–27.6	9.6 ± 4.2	0.5–17.6	7.2 ± 4.0	1.9–17.2
Cystic fibrosis (CF_2)	17	26.6 ± 12.3	9.9–50.4	13.0 ± 6.9	3.4–29.5	13.3 ± 8.7	3.0–32.5
Parents	27	32.5 ± 10.4	14.2–64.0	18.4 ± 8.4	6.1–47.2	14.8 ± 8.8	1.8–30.0
All controls	35	42.1 ± 13.0	20.2–73.5	26.2 ± 11.6	12.9–69.1	15.9 ± 9.8	1.1–51.6
All cystic fibrosis	37	21.3 ± 10.9	2.4–50.4	11.2 ± 5.9	0.5–29.5	10.0 ± 7.2	1.9–32.5