these patients has shown evidence of relapse during this interval.

The possibility that the bedsoniae isolated in this study were the agents of trachoma or inclusion conjunctivitis appears to be ruled out on several counts. Exclusion of the agent of LGV was less certain. Several reports of mycoplasma involvement in Reiter's syndrome have not established the role of these organisms in the disease(7). As suggested in a previous report(3), Reiter's syndrome may represent a symptom-complex. As such, several etiologic agents might produce similar clinical manifestations. Alternatively, the provocation of the first infection may set up fertile ground for repeated infection by other agents. Thus, mycoplasmas or bedsoniae might be the causative agents, individually or conjointly. Once the agent or agents have been isolated, these conjectures should be amenable to experimental investigation. Although we have not attempted mycoplasma isolations, the possibility of their involvement in the present cases cannot be ignored.

In the future, attempts to isolate bedsonia and mycoplasma organisms from patients with various types of arthritis, including Reiter's syndrome, will be carried out. Currently, attempts are being made to produce arthritis by injection of the isolates into animals. In preliminary studies, intraperitoneal injection of yolk-sac suspensions of the isolates in guinea pigs has produced arthritis in some instances. Of greater significance, arthritis has been regularly produced by intra-articular injection of the isolate into subhuman primates. These findings will be reported later.

Summary. Studies in 16 patients with Reiter's syndrome, consisting of arthritis, urethritis and/or conjunctivitis, suggest that bedsoniae (psittacosis-lymphogranuloma venereum-trachoma group of microorganisms) may play a role in arthritis in man. Attempts to isolate bedsoniae from synovial material or urethral and conjunctival scrapings from 8 patients were successful in 5 instances. Five of the 16 patients had significant complement-fixation titers against a psittacosis group antigen.

1. Mendlowski, B., Segre, D., Am. J. Vet. Res., 1960, v21, 68.

2. Siboulet, A., Galistin, P., Brit. J. Ven. Dis., 1962, v38, 209.

3. Bauer, W., Engleman, E. P., Trans. Assn. Am. Phys., 1942, v307, 57.

4. Jones, B. R., Brit. J. Ven. Dis., 1964, v40, 3.

5. Meyer, K. F., Eddie, B., in Diagnostic Procedures for Viral and Rickettsial Diseases, 2nd ed., Am. Public Health Assn., New York, 1965, p399.

6. Schachter, J., Doctoral Dissertation, Univ. of California, Berkeley, 1965.

7. Channock, R. M., New Engl. J. Med., 1965, v273, 1199.

Received February 8, 1966. P.S.E.B.M., 1966, v122.

Acute Effect of Metal Ions on Uptake of Alpha-Aminoisobutyric Acid (AIB) by Rat Parathyroids *in vivo*.* (31114)

WILLIAM Y. W. AU, JUDITH R. ENGERMAN, AND LAWRENCE G. RAISZ†
(Introduced by Harold C. Hodge)
Departments of Pharmacology and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

In a previous study(1), we demonstrated that the rat parathyroid responds to chronic hypocalcemia by an increase in size and in ability to concentrate amino acids as measured by the distribution ratio of the non-metabolized amino acid analogue, alpha-amino isobutyric acid (AIB). In short-term experiments *in vitro* the uptake of AIB and the uptake and incorporation into proteins of several natural amino acids by the parathyroid were found to be inversely related to the calcium concentration of the medium(2). In

* This study was supported by Grants AM 08205 and AM 07871 from USPHS.

† Burroughs Wellcome Scholar in Clinical Pharmacology.

TABLE I. *Experiment 212.* Effect of Administration of Various Salts on Serum Calcium (Ca), Phosphorus (P) and Magnesium (Mg) Concentrations, Parathyroid Dry Weight (Dry Wt) and AIB Distribution Ratios (T/S) of Parathyroid, Thyroid, Diaphragm and Kidney Cortex.

		Serum			Parathyroid				
Treatment	n	(Ca) mg/100 ml	(P) mg/100 ml	(Mg) mM/l	Dry wt, μg	T/S	Thyroid T/S	Diaphragm T/S	Kidney cortex T/S
Sodium chloride	5	8.0 ± .2	10.1 ± .2	1.0 ± .1	86 ± 7	8.0 ± .7	.8 ± .3	3.7 ± .8	36 ± 3
Calcium gluconate	5	13.6 ± .9†	9.2 ± .3	—	88 ± 6	5.8 ± .5*	1.3 ± .1	4.2 ± .4	25 ± 2*
Sodium phosphate	5	6.3 ± .2†	15.6 ± .4†	—	70 ± 9	12.6 ± .9†	.8 ± .1	4.0 ± .2	43 ± 4
Sodium oxalate	5	6.2 ± .5†	11.6 ± .6*	—	62 ± 8	11.3 ±1.2*	1.1 ± .3	4.2 ± .7	18 ± 2†
Magnesium sulfate	5	7.9 ± .3	9.6 ± .2	4.5 ± .1†	91 ± 5	5.8 ± .5*	1.3 ± .2	4.9 ± .6	48 ± 6
Strontium chloride	5	7.5 ± .5	10.3 ± .3	—	76 ± 5	4.5 ± .5†	1.0 ± .2	3.6 ± .6	38 ± 5

Values are mean ± standard error. Each rat received 0.25 mM/100 g body wt of a 0.25 M solution subcutaneously at 2 and 1 hr before sacrifice.

Significant difference from sodium chloride treated (control) rats: * $p < .05$; † $p < .01$.

the present study, we have investigated *in vivo* the effects of acute changes in serum calcium, magnesium, or strontium on the distribution ratio of AIB in rat parathyroids. The acute effects on parathyroid function of parathyroid hormone, Vitamin D, or thyrocalcitonin administration were also examined.

Methods. Male, albino rats of the Rochester Wistar strain weighing between 150 and 250 g were maintained on standard Purina Chow diet. Rats fasted for 18 hours were used in the first 2 experiments (Experiment 203 and 212, Table I and II); non-fasted rats in the others. The rats were injected with 20 μc AIB-1-C^{14} (specific activity, 3.5 mc/mM) subcutaneously 24 hours before sacrifice. On the experimental day, the rats were divided into treatment groups of 5 to 7 rats. Each rat received 2 subcutaneous injections (at 2 hours and at one hour before sacrifice)

TABLE II. The Effect of Administration of Various Substances on Serum Calcium (Ca) and Phosphorus (P) Concentrations, Serum Magnesium (Mg) Concentration, and on AIB Distribution Ratio (T/S) of Parathyroid.

			Serum			
Exp No.	Treatment	n	(Ca) mg/100 ml	(P) mg/100 ml	(Mg) mM/l	Parathyroid T/S
203	Sodium chloride	5	8.4 ± .2	9.9 ± .5		7.6 ± .8
	Calcium gluconate	5	9.4 ± .3*	9.2 ± .5		8.7 ± .7
	EDTA	5	6.7 ± .3†	17.0 ±1.0†		7.1 ± .4
	Parathyroid extract 2 hr	5	8.9 ± .1	9.4 ± .6		7.1 ± .7
	Thyrocalcitonin	5	6.4 ± .2†	8.0 ± .2†		11.7 ±1.3*
231	Sodium chloride	6	10.1 ± .2	9.0 ± .4		6.7 ± .8
	Parathyroid extract 24 hr	5	11.2 ± .2†	8.1 ± .4		3.7 ± .6†
	Vitamin D	5	10.1 ± .1	9.2 ± .3		7.4 ±1.6
240	Cottonseed oil	7	9.2 ± .2	10.0 ± .5		4.3 ± .3
	Dihydrotachysterol	7	10.2 ± .2†	11.6 ±1.0		2.6 ± .4†
237	Sodium chloride	5	9.8 ± .2	11.9 ± .4	.56 ± .1	3.9 ± .2
	Calcium gluconate	5	12.3 ±1.0*	9.4 ± .3†	.52 ± .1	3.3 ± .4
	Magnesium sulfate	4	9.0 ± .1*	10.6 ± .6	2.0 ± .2*	4.2 ± .5
245	Sodium chloride	6	9.5 ± .1	9.6 ± .2	.59 ± .1	7.9 ± .4
	Calcium gluconate	6	12.7 ± .2†	9.9 ± .4	.46 ± .1	4.9 ± .5†
	Magnesium sulfate	6	8.9 ± .3*	9.1 ± .4	2.8 ± .2†	5.9 ± .6†

Values are mean ± standard error. See *Methods* for strength, dosages and frequency of administration of treatment solution.

Significant difference from control (sodium chloride or cottonseed oil): * $p < .05$; † $p < .01$.

of one of the following solutions given at the dosages indicated (expressed as mM per 100 g body weight): 1) calcium gluconate, 0.25 mM or 0.125 mM; 2) magnesium sulfate, 0.25 mM or 0.125 mM; 3) strontium chloride, 0.25 mM; 4) sodium ethylenediaminetetraacetic acid (EDTA) 0.25 mM; 5) sodium phosphate (buffered, pH 7.4), 0.25 mM; 6) sodium oxalate, 0.25 mM (at 2 hours before sacrifice only); 7) sodium chloride 0.15 mM or 0.25 mM. In addition, the following preparations known to affect serum calcium concentration were given to other groups: 1) parathyroid extract (PTE),‡ 50 units/100 g body weight subcutaneously 2 hours before sacrifice; 2) PTE, 50 units/100 g body weight subcutaneously 24 hours and 12 hours before sacrifice; 3) thyrocalcitonin (TC),§ 8 units/100 g body weight subcutaneously 2 hours before sacrifice; 4) vit. D_2 in cottonseed oil, 4,000 IU/100 g body weight orally 24 hours before sacrifice; 5) dihydrotachysterol (DHT) in cottonseed oil, 50,000 IU/rat orally 24 hours before sacrifice. Control animals were given solutions of 0.15 M NaCl or 1.6% glycerol and 2% phenol in 0.15 M NaCl subcutaneously or cottonseed oil orally.

As described previously(1), the rats were killed by decapitation and the parathyroids, thyroids and small pieces of diaphragm and kidney cortex removed. The tissues were extracted for AIB with 5% trichloroacetic acid (TCA) overnight. The TCA-extract was dried and AIB-1-C^{14} counted in a liquid scintillation counter. Aliquots of TCA-extracts of serum were counted in a similar manner so that the AIB tissue/serum ratios could be obtained without correction for quenching. Fat-free dry weight of the tissue was obtained on a Cahn electro-balance after the tissue was extracted with acetone and petroleum ether-diethyl ether and dried at 37°C for 24 hours. Tissue water was calculated as 3 times the fat-free dry weight. Methods for estimation of serum calcium and phosphorus concentrations as well as for calculations of tissue/serum concentration ratio of AIB (T/S ratio) have been described(1). Serum magnesium concentration was estimated by the phototitrimetric method of Beale and Bostrom(3).

Results. All the data for one experiment in which several inorganic compounds were administered are shown in Table I. An inverse relation between parathyroid AIB uptake (T/S ratio) and the serum calcium concentration was seen; *e.g.*, after calcium gluconate administration T/S decreased while calcium concentration increased by 5.6 mg/100 ml or 1.4 mM/l above control values, whereas after sodium phosphate or oxalate administration T/S increased while serum calcium concentration decreased by 1.7 mg/100 ml or 0.4 mM/l. Administration of magnesium sulfate or strontium chloride decreased parathyroid T/S ratio for AIB with no change in serum calcium concentration. Following a magnesium sulfate dose, serum magnesium concentration increased 3.5 mM/l over control values, an increase both absolutely and relatively greater than the increase in serum calcium concentration after calcium gluconate injection. Serum strontium concentration was not measured. Uptake of AIB (T/S ratio) by thyroid, diaphragm and kidney cortex generally was not affected by changes in serum metal ion concentrations. The concentrative uptake of AIB by the kidney cortex was significantly lowered by sodium oxalate injections. The effect of calcium on kidney AIB uptake seen in Table I was not observed in other experiments.

A decrease in parathyroid AIB uptake could be obtained whether serum calcium concentration was increased by administration of calcium, parathyroid extract or dihydrotachysterol. However, these effects were not always consistent (Table II). In one experiment (Exp. 203) calcium gluconate injection produced only a small increase in serum calcium concentration and no decrease in parathyroid AIB uptake was observed, while in another experiment (Exp. 237) the rise in serum calcium concentration was large, but the parathyroid AIB uptake was low in the sodium chloride-injected animals and did not decrease further with calcium injection. No effect on parathyroid AIB uptake was observed 2 hours after parathyroid extract injection (Exp. 203)

‡ Parathyroid Injection, USP (Lilly).

§ Partially purified hog thyrocalcitonin, 4 units/mg; kindly provided by Dr. Philip Hirsch.

when there was no significant increase in serum calcium concentration. Similarly, there was no effect on parathyroid AIB uptake 24 hours after administration of vit. D, 4,000 IU, when serum calcium concentration was not significantly increased (Exp. 231). However, PTE (Exp. 231) or a massive dose of dihydrotachysterol (Exp. 240) given 24 hours before sacrifice caused significant increases in calcium concentration and decreases in parathyroid AIB uptake. Magnesium injection was again seen to decrease parathyroid AIB uptake when control parathyroid uptake was high and the serum magnesium concentration increased to 2.8 mM/l with a slight decrease in serum calcium concentration (Exp. 245). However, in Exp. 237, in which control parathyroid AIB uptake was low, no further decrease in parathyroid AIB uptake was observed after magnesium sulfate injections in spite of an increase in serum magnesium concentration to 1.4 mM/l above control values. Magnesium injection tended to decrease serum calcium concentration in all experiments.

Thyrocalcitonin significantly decreased serum calcium concentration and increased parathyroid AIB uptake. EDTA produced as great a decrease in serum calcium concentration but did not increase parathyroid AIB uptake. In other experiments EDTA produced variable effects on parathyroid AIB uptake. With very large dosages of EDTA which lowered serum calcium concentration to below 1 mM/l the parathyroid T/S ratio occasionally decreased anomalously to values of 3 or less compared with control values between 6 and 8. EDTA was also observed to decrease T/S ratio for AIB of kidney cortex. The pretreatment serum calcium concentrations as reflected by the sodium chloride-injected rats in 2 experiments (Exp. 203 and 212) were lower than those seen in the rest of the experiments (Exp. 231, 237, 240 and 245). This difference in serum calcium concentration can be attributed to prolonged fasting.

Discussion. This study demonstrates that acute decreases in serum calcium concentration are usually accompanied by increases in parathyroid AIB uptake while acute increases in serum calcium concentration are usually accompanied by a decrease in parathyroid AIB uptake. The distribution ratio for AIB between the cell and serum presumably represents a dynamic equilibrium achieved by rapid concentrative uptake into the cell and slow diffusion out of the cell along the concentration gradient established by active inward transport. Increasing calcium concentration in the medium *in vitro* inhibits active transport of amino acids into the cell(2), but not simple diffusion across a cell membrane (Raisz, unpublished observations). Thus, while an acute increase in serum calcium concentration might rapidly suppress AIB transport into the cell, a new equilibrium state will be attained only as slowly as AIB diffuses out of the cell and reaches a lower concentration. On the other hand, an acute decrease in calcium concentration could stimulate active transport of AIB more rapidly. Thus, the present technique should consistently demonstrate increased amino acid uptake, but is inherently less sensitive for detecting acute suppression of amino acid uptake. To circumvent this we made a number of attempts to maintain elevated serum calcium concentration by continuous intravenous administration of calcium salts in intact rats. However, variability of serum calcium concentration in these experiments was great, presumably because factors opposing elevated serum calcium concentration such as secretion of thyrocalcitonin, the ability of the bones to take up calcium, and the excretion of calcium into the intestine and urine varied so greatly among different animals. While it was possible to increase serum calcium concentration and decrease parathyroid AIB uptake by this method(2), the simpler method of subcutaneous injection produced better maintenance of serum calcium concentrations and less variability between animals at the time intervals studied.

The degree to which effects on AIB transport reflect the control of parathyroid function *in vivo* is unknown. Studies on isolated parathyroids *in vitro* indicate that calcium has a direct effect on the transport of a number of natural amino acids, and that this may constitute a mechanism for the con-

trol of a parathyroid hormone synthesis(2). It is probable that secretion of parathyroid hormone is subject to a separate, but parallel, negative feedback control by calcium. Further studies on the uptake and incorporation of natural amino acids are needed to determine whether this relation obtains *in vivo*.

The changes in serum calcium concentration induced by thyrocalcitonin, parathyroid extract, or dihydrotachysterol caused appropriate inverse changes in parathyroid T/S ratio in these experiments. The observation that parathyroid extract or vit. D does not affect parathyroid AIB uptake if serum calcium concentration is not changed supports previous studies that these substances have no direct effect upon parathyroid amino acid transport(1,4). The present data also provide further support for the concept that the short duration of action of thyrocalcitonin is in part due to a rapid parathyroid response (5).

The apparent inhibition of parathyroid AIB transport by magnesium injection *in vivo* is in conflict with previous *in vitro* studies in which high magnesium concentration in the medium did not inhibit AIB transport(4). We have no definite explanation for this difference, although the known pharmacologic hypotensive effect of magnesium(6) may be a factor. However, the decrease in serum calcium concentration after magnesium sulfate injection could be due to decreased PTH secretion. In any case, the increase in serum magnesium concentration which produced a decreased parathyroid AIB uptake was much larger than the increase in serum calcium concentration which produced this effect, indicating that the parathyroid gland is substantially less sensitive to magnesium than to calcium. The observation that large doses of strontium, without elevating serum calcium concentration, also inhibit parathyroid AIB uptake confirms previous data obtained *in vitro*(4).

None of the several anions employed could be shown to have any specific effect on parathyroid function, and there was no consistent correlation between serum phosphorus concentration and T/S ratio for AIB.

EDTA has frequently been employed as a means of lowering serum ionized calcium concentration and stimulating parathyroid glands. In the present study, administration of EDTA resulted in variable effects on parathyroid AIB uptake. Although a stimulation was sometimes encountered with a small decrease in serum calcium concentration(2) further decreases in serum calcium concentration with increasing dosages of EDTA resulted generally in reducing parathyroid AIB distribution ratio. This could be due to a direct toxicity of EDTA since comparable hypocalcemic levels induced by phosphate or oxalate administration increased parathyroid AIB uptake. EDTA toxicity could be due to chelation of other metallic ions essential to cell membrane function, or to direct binding of EDTA on the membrane and interference with amino acid transport. Whatever the mechanism of its effect on the parathyroid glands, the use of high dosages of EDTA as a means of inducing hypocalcemia may not represent an appropriate model for physiologic stimulation of the parathyroids.

Summary. Acute increases in serum calcium concentration produced by administration of calcium salts, parathyroid extract or high dosages of dihydrotachysterol generally resulted in a decrease in the uptake of AIB by the rat parathyroids *in vivo*. Acute decreases in serum calcium concentration produced by administration of sodium oxalate, sodium phosphate, or thyrocalcitonin resulted in increases in the uptake of AIB by the parathyroids. Large increases in serum magnesium or strontium concentration also suppressed parathyroid AIB uptake. Administration of EDTA to decrease serum calcium concentration resulted in variable effect on parathyroid AIB uptake. This study provides further direct evidence for a negative feedback control of parathyroid function by serum calcium concentration.

1. Au, W. Y. W., Raisz, L. G., Am. J. Physiol., 1965, v209, 637.

2. Raisz, L. G., Au, W. Y. W., Stern, P. H., in The Parathyroid Gland: Ultrastructure, Secretion, and Function, P. J. Gaillard, R. V. Talmage, A. M. Budy, eds., Univ. of Chicago, 1965, p37.

3. Beale, R. W., Bostrom, J. O., J. Clin. Path., 1963, v16, 252.

4. Raisz, L. G., O'Brien, J. E., Am. J. Physiol., 1963, v205, 816.
5. Tashjian, A. H., Endocrinology, 1965, v77, 381.
6. Engback, L., Pharmacol. Rev., 1952, v4, 396.

Received February 17, 1966. P.S.E.B.M., 1966, v122.

Studies of the Effect of Dimethylsulfoxide on Permeability of Dermal Connective Tissue.* (31115)

JOHN FABIANEK† AND ANTHONY HERP
Department of Biochemistry, New York Medical College, New York City

Dimethylsulfoxide (DMSO) was found to be an effective histological preservative of mammalian cells when used at low concentrations(1-5). Several other chemical, physiological and therapeutic properties of this compound have also been reported(6,7). The ability of DMSO to translocate drugs through animal tissues has been ascribed to its high solvating power and membrane-permeability enhancing effect(7). The present study was undertaken to investigate the effect of this compound on the permeability of connective tissue in which the hyaluronic acid of the ground substance plays a major role(8).

The experiments were performed on albino male rats of CFN strain 9 to 10 months old. For measuring permeability our previously described method(8,9) based on the rate of dermal diffusion of dye alone or in combination with test substance was used. In this method 0.05 ml of a 0.4% solution of Evans blue in saline (pH 7.3) with or without the test substance was injected intradermally. Three injections on each animal were made for the control (generally dye alone) and 3 for the test compound. The contours of the blue spots thus produced were traced onto semi-transparent paper at 30, 60, 120 and 180 minute intervals after injection. The spots traced on paper were cut out, weighed and their areas calculated as mm^2.

Since 3 spots were obtained on each animal for the control and 3 others for test substance, the results reported in Table I are thrice the number of animals used for the test. Thus each animal served as its own control. The area of each spot was entered as an individual result in the calculation of the average value and standard deviation.

Four concentrations of DMSO were tested: 0.2, 2, 5 and 50%. Since it was previously observed that an association of a factor enhancing tissue permeability with an inhibitor potentiated the effect of the inhibitor(8,9), DMSO at a concentration of 0.2% was tested for its permeability effect in combination with diethyldithiocarbamate of sodium (DEDTC) at 2% concentration. DEDTC was previously found markedly to inhibit connective tissue permeability(9,10). Hence, it was of interest to test the effect of DMSO on permeability in the presence of this strong inhibitor.

The results show (Table I) that at concentrations of 0.2, 2 and 5% DMSO did not enhance the permeability as defined by this method. In fact, a slight inhibition of skin "permeability" (about 6 to 7%) was observed at a concentration of 0.2% DMSO. It behaved like other substances which affect permeability at low concentrations but which were inactive at higher concentrations(8,9). However, at a concentration of 50%, DMSO enhanced markedly the permeability when tested by the diffusion method. When DMSO was used with DEDTC, the inhibitory effect was slightly enhanced as compared to the effect obtained with DMSO alone (Table I).

A striking parallelism was earlier observed between the *in vitro* oxido-reductive depolymerization of hyaluronic acid (tested by rate of reduction in viscosity) and the *in vivo*

* This work was supported by the U.S.P.H.S. Nat. Inst. for Arthritis and Metab. Dis., Grant A-4619. The authors wish to thank Mrs. Anne O'Brien-Botticelli for valuable technical assistance.

† Present address: Laboratory of Experimental Metabolic Diseases, Veterans Admin. Hospital, Long Beach, Calif.