of escape from the lung. The perfusion fluid and later the blood could have drained *via* the bronchial arteries and veins into the distal ends of the intercostal arteries and the azygos and the hemiazygos veins respectively and from there into the capillaries of body tissues. If additional ligatures were applied to these vessels, the donor lung should be able to maintain pressure for a longer period of time, and hemorrhage of the recipient animal through the donor lung into the donor animal should be minimized.

There was no indication in this study that immunological reactions played a significant role since the lambs recovered soon after they had been disconnected from the donor lungs and the regulation of respiration was returned to their own respiratory centers. Kimoto *et al*(3), who used dog lungs for dialysis of human blood, give a list of diseases which preclude the utilization of dogs afflicted with them, and they also list several drugs that can be used for disinfection of seemingly healthy dog lungs. With these precautions taken, they feel that the donor lung specimen "could be used without any harm to a human." Nevertheless, further tests for the development of immunological safety are in order. It seems at present that a carefully developed biological donor lung might offer two distinct advantages for temporary assistance of a newborn with a malfunctioning lung: It is a compact structure that is easy to operate, and it is relatively inexpensive.

1. Niden, A. H., Mittman, C., Burrows, B., J. Appl. Physiol., 1962, v17, 885.

2. Rosenberg, E., ibid., 1963, v18, 269.

3. Kimoto, S., Inou, T., Ishii, J.-i., Trans. Am. Soc. Artif. Intern. Organs, 1959, v5, 15.

4. Barclay, A. E., Franklin, K. J., Prichard, M. M. L., The Foetal Circulation, Blackwell Scientific Pub., Oxford, 1944.

5. Miller, M. E., Christensen, G. C., Evans, H. E., Anatomy of the Dog, W. B. Saunders Co., Philadelphia, 1964.

6. Benninghoff, A., Lehrbuch der Anatomie des Menschen, Urban & Schwarzenberg, Munich & Berlin, 3rd ed., 1948.

7. Benninghoff, A., Goerttler, K., ibid., 7th ed., 1964.

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Intestinal Transport of Calcium and Phosphate in Experimental Magnesium Deficiency.* (32003)

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Alcock and MacIntyre(1) reported that in magnesium deficient rats calcium absorption was increased in the absence of magnesium, while magnesium absorption was increased if calcium was absent from the diet. They advanced the hypothesis that magnesium and calcium are absorbed by a common transport mechanism. An *"in vitro"* relationship between the absorption of these two ions was found by Schachter and Rosen(2) who showed that concentrative calcium transport across the intestinal wall *in vitro* was depressed by the presence of magnesium in the medium. In conflict with this, Clark(3) reported that under normal dietary conditions, an increase of magnesium intake promoted calcium absorption rather than inhibiting it.

In the rat nutritional magnesium deficiency not only causes decreased concentrations of magnesium in serum and bone but also produces hypercalcemia, hypophosphatemia, hypocalciuria, hyperphosphaturia and renal calcification(4,5). To determine the relationship between intestinal function and the hypercalcemia and hypophosphatemia of the mag-

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nesium deficient rat studies of calcium and phosphate transport by rat small intestine *in vitro* were made using preparations from magnesium deficient and control rats. The effect of vitamin D depletion on the response to magnesium deficiency was also determined.

Methods. Experiments were conducted with male weanling Sprague-Dawley rats of dams which were deprived of vitamin D during lactation. For each experiment the animals were randomly divided into groups and were studied following a period of 3 weeks on their respective diets. Four groups of rats were studied, a control and a magnesium deficient group from both vitamin D treated and vitamin D deficient animals. All rats were fed a vitamin D free diet that differed only in the magnesium content (controls—0.397 mg/g of diet; magnesium deficient 0.022 mg/g of diet). This diet is described in detail elsewhere(6). In addition the vitamin D treated animals received 100 or 1000 i.u. of vitamin D_2 in a single dose P.O. given weekly. Following a period of 3 weeks on their respective diets the rats were put in metabolic cages which prevented contamination with feces or food and urine was collected for 24-hours periods under toluene. At the end of the collection period they were anesthetized with pentobarbital and bled from the abdominal aorta. The small intestine was removed and intestinal loops were prepared for measurement of transport of calcium and phosphate as described below. The left tibia was removed, dried at 105°C for 48 hours and defatted with ether. The fat free dry bone was analyzed for calcium, magnesium and phosphorus.

The following methods were used: serum and bone Mg, Schachter(7), serum calcium, Harrison and Harrison(8), bone calcium by the Clark-Collip modification of the Kramer-Tisdall method(9), inorganic phosphate in serum, bone and urine. Fiske and Subarrow (10). Statistical analysis of the data was performed as described by Natrella(11). The calcium and phosphate transport studies were designed on a $2 \times 2 \times 2$ factorial plan and the analysis of variance of the means was performed by the Yates Procedure(12).

Determinations of concentrative calcium transfer by everted duodenum *in vitro* and of intestinal permeability to calcium by everted jejunal and ileal loops were made by techniques previously described(13). Two general types of experiments were performed. In type A concentrative transport of calcium by everted duodenum was measured. The initial concentration of calcium and of calcium-45 in the solution within the loops and outside bathing the mucosal surface was the same, *i.e.*, 0.2 mM calcium/l and 2.5 μc calcium-45/l. The intestinal loops were shaken in a Dubnoff metabolic shaker at 37°C in an atmosphere of 95% O_2 and 5% CO_2 for 90 minutes. Buffer solutions with and without magnesium were used to determine the effect on concentrative calcium transport of magnesium in the medium as well as the effect of magnesium depletion of the animal. The index of concentrative transport was the ratio of the concentration of calcium-45 in the serosal fluid to that in the mucosal fluid at the end of incubation. (Cs/Cm). In type B experiments the everted intestinal segments used were jejunum and ileum. Calcium and calcium-45 were added only to the buffer solution on the mucosal side of the intestinal wall. The magnesium concentration in the medium was constant, 1.3 mM/l. In these experiments the active transport of sodium and the associated transport of water were blocked by addition of N-ethylmaleimide to the incubation medium in a concentration of 0.5 mM/l. Under these conditions the volume of fluid in the serosal phase was unchanged during 60 minutes incubation. Concentrative transport of calcium is also inhibited by N-ethylmaleimide so that the transfer of calcium from mucosal to serosal fluid presumably is by diffusion along the concentration gradient and the rate of this transfer measures the permeability to calcium of the intestinal mucosa(13). The data are recorded as μ moles of calcium per loop per hour entering the serosal fluid.

At the end of the period of incubation the loops were removed from the solution and calcium-45 was determined in both mucosal and serosal solutions by plating 100 μl aliquots onto stainless steel planchets, drying with an infrared heater and measuring the radio-activity in a continuous gas flow counter with a

TABLE I. Body Weights and Serum, Bone and Intestinal Analyses of Magnesium Deficient and Control Rats With and Without Vitamin D.

	Vitamin D deficient		Vitamin D treated	
	Mg deficient (20)	Control (17)	Mg deficient (13)	Control (13)
Body wt (g)	71 ± 3.9 *	94 ± 4.4	79 ± 3.1 *	114 ± 5.8
Serum (mg/100 ml)				
Mg	.31 ± .05 *	1.55 ± .19	.49 ± .07 *	1.64 ± .09
Ca	5.5 ± .33	5.5 ± .51	11.5 ± .57 *	10.5 ± .32
Bone (mg/g dry wt)				
Mg	1.37 ± .41 *	2.56 ± .28	1.21 ± .17 *	2.46 ± .29
Intestine (mg/g dry wt)			(6)	(6)
Mg			.96 ± .18 †	1.10 ± .19

The vitamin D treated animals received 1000 i.u. of D_2 weekly in a single dose P.O. The basal diet provided a Ca/P ratio of 1:1; and the magnesium content was 0.397 mg/g of diet for control and 0.022 mg/g of diet for magnesium deficient groups.

Data are means ± 95% confidence intervals. Numbers in parentheses indicate No. of observations.

* Statistically significant differences between adjacent means at a level of $P < .005$ are depicted by an asterisk.

† The magnesium content of the small intestine was determined by the atomic absorption method and the differences between means are at $.09 < P < .1$ level.

micro mil window (Nuclear-Chicago D47).

Everted proximal and distal jejunal intestinal loops were used for determination of concentrative phosphate transport. The loops were filled with 0.6 ml of modified K-H buffer and the everted loops were immersed in 5 ml of the same solution in a 20 ml beaker. They were incubated for 90 minutes with shaking (110 oscillations/min) in a Dubnoff metabolic shaker in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. The Krebs-Henseleit bicarbonate buffer(14) was modified by increase of potassium concentration to 18 mEq/l. Buffers with and without magnesium salt were also employed. At the end of the incubation the volume of fluid recovered was measured and the protein was precipitated with trichloracetic acid in a final concentration of 8%. Inorganic phosphate was determined in the filtrate by the method of Fiske and Subarrow(10). The data are expressed as the concentration ratio between the concentrations of phosphate in serosal and mucosal solutions (Cs/Cm) at the end of the incubation.

Results. Table I summarizes body weights, serum concentrations of magnesium and calcium, and bone and small intestine magnesium concentrations of the 4 groups of rats. It should be noted that the magnesium content of the intestine was not significantly reduced in the hypomagnesemic rats. Fig. 1 shows the results of experiments measuring concentrative transport of calcium *in vitro* of intestinal preparations of the 4 groups of animals studied. The concentration ratios between the serosal and mucosal fluid (Cs/Cm) achieved by everted duodenal loops were the same for all vitamin D deficient groups. Vitamin D treatment, 1000 i.u. per week, enhanced the concentrative transport of calcium in the loops from both control and magnesium deficient animals ($P<0.005$), and the response of the magnesium deficient animals was not greater than that of the controls. The concentrative transport of calcium was, however, influenced by the concentrations of Mg^{++} in the solution bathing the intestine (Fig. 1). Transport of calcium by loops from vitamin D treated controls incubated in a magnesium free buffer was greater than that by loops incubated in a magnesium containing buffer. The inhibitory effect of magnesium in the medium was not significant in the experiments in which loops from magnesium deficient vitamin D treated rats were employed nor in the experiments with intestinal loops from vitamin D deficient rats.

The effect of magnesium deficiency on the diffusibility of calcium across the intestinal

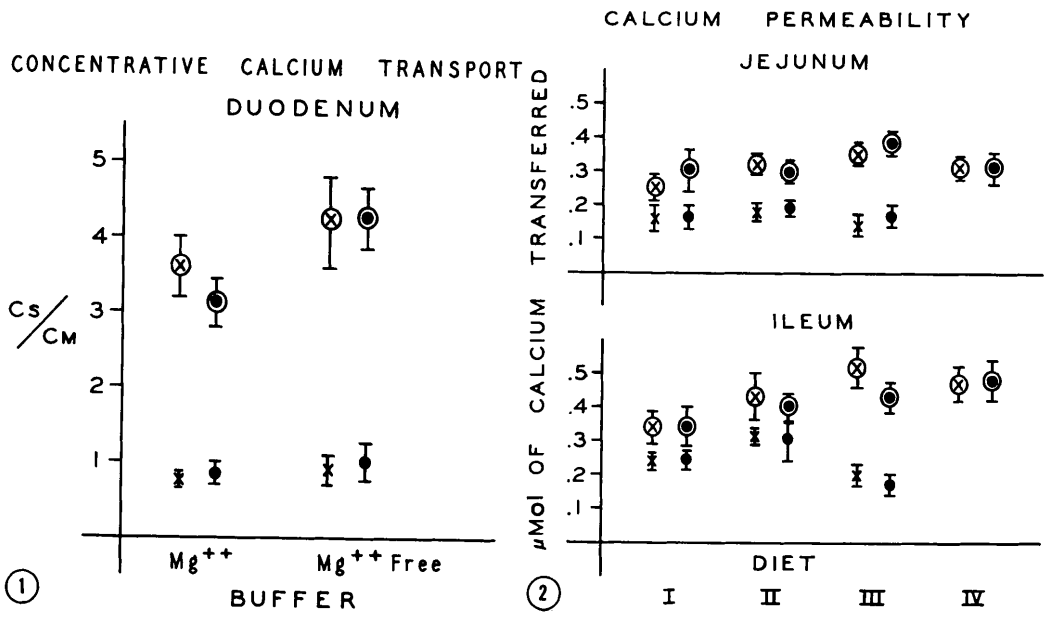

FIG. 1. Comparison of concentrative transport of calcium by duodenal loops from 4 groups of rats incubated in a magnesium free and magnesium containing K-H buffer. Vitamin D treated rats received 1000 i.u. vitamin D_2 weekly P.O. The inhibitory effect of Mg in the medium was significant. ($P<0.05$) in the loops from vitamin D treated control rats. Code: X magnesium deficient, vitamin D deficient; ● control-vitamin D deficient; x circled Magnesium deficient-vitamin D treated; ● circled control-vitamin D treated; I mean ± 95% confidence intervals.

FIG. 2. Permeability *in vitro* to calcium of jejunal and ileal loops from rats of the 4 groups are shown. In these studies loops from rats on 4 diets were studied. Diets I, III and IV provided a Ca:P ratio of 1:1, 5:1 and 10:1 respectively, and magnesium content was the same in all. (Legend Table I). Diet II provided a Ca:P ratio of 1:1 and magnesium content was 0.106 mg/g of diet for the magnesium deficient group and 0.512 mg/g of diet for the controls. Vitamin D treated animals received 100 i.u. vitamin D_2 weekly P.O. Code as in Fig. 1.

wall was studied in experiments of type B. Figure 2 shows the results of these experiments. The data for loops from the jejunum and ileum are shown separately but no significant differences were seen due to the location of the intestine from which loops were made, nor were there differences between loops from magnesium deficient and control rats. The only consistent and significant effect noted was that of vitamin D in increasing the permeability to calcium of the intestinal preparation.

The magnesium deficient rats in the experiments described above grew less rapidly than the control animals (Table I). Since malnutrition and growth disturbance may diminish intestinal calcium transport another group of experiments was done to evaluate the specific effects of magnesium deficiency. The diet of the magnesium deficient rats was altered so that it contained 0.106 mg of Mg per g of diet for the magnesium deficient groups and 0.512 mg/g of diet for the control group. Animals on these diets grew well, the mean body weight at the end of 3 weeks on the diet was 96 g for the magnesium deficient and 100 g for the control vitamin D deficient groups. The mean body weight for the vitamin D treated groups was 126 g for both magnesium depleted and control animals. Nevertheless, in these groups of animals serum and bone magnesium concentrations were as low as those in the group that received the diet with the lower magnesium content. The results of calcium transport experiments of type A are depicted in Fig. 3. The concentrative calcium transport in a magnesium containing buffer was the same for the loops from magnesium deficient and the control animals. The diffusibility of calcium across the in-

TABLE II. Comparison of Serum P, Urine P Excretion and Bone Composition of Magnesium Deficient and Control, Vitamin D Deficient and Vitamin D Treated Rats.

	Vitamin D deficient			Vitamin D treated		
	Control (9)		Mg deficient (10)	Control (12)		Mg deficient (9)
Serum P (mg/100 ml)	9.1 ± .59	†	7.7 ± .60	9.2 ± .73	†	7.2 ± 1.06
Bone						
P (mg/100 g B.W.)	8.9 ± 1.36	‡	11.5 ± 1.31	9.5 ± .91	†	12.4 ± 1.49
Ca/P ratio	2.03		1.93	1.96		2.04
Mg (mg/g of P)	5.9	*	2.6	5.3	*	2.4
Urine P (mg 24 hr/100 g B.W.)	12.2 ± 1.47		14.8 ± 2.71	12.2 ±2.84	‡	16.2 ± 2 28

The 4 groups of animals were studied after 3 wk on the experimental regime. The vitamin D treated group received 1000 i.u. of vitamin D_2 P.O. as a single dose weekly.
Mean ± 95% confidence intervals.
Numbers in parentheses indicate No. of animals studied.
* Differences significant at $P < .005$ level.
† " " " $P < .01$ " .
‡ " " " $P < .05$ " .
The above signs depict statistically significant differences between adjacent means.

testinal wall was also not altered (Fig. 2).

Magnesium deficiency reduced the concentration of serum phosphorus in vitamin D deficient and vitamin D treated rats (Table II). The vitamin D treated magnesium deficient rats also showed an increase in urinary excretion of phosphorus in comparison with vitamin D treated controls ($P<0.005$) which is generally consistent with reports by previous investigators(5). The magnesium and vitamin D deficient animals did not show a significantly increased urinary phosphorus excretion (Table II).

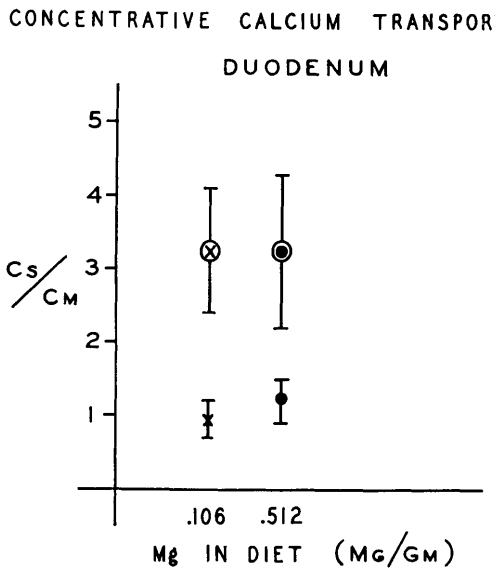

FIG. 3. Comparison of concentrative transport of calcium by duodenal loops from the 4 groups of rats. All loops were incubated in Mg containing K-H buffer. Rats in this series were fed Diet II. Vitamin D treated groups received 100 i.u. vitamin D_2 weekly P.O. Code as in Fig. 1.

The bone content of inorganic phosphorus was higher in the magnesium deficient animals than in controls. This effect was seen irrespective of vitamin D treatment (Table II). These differences in bone phosphorus content were associated with similar increase in the bone calcium content, so that the Ca:P ratio remained constant.

Fig. 4 shows the concentration ratios of inorganic phosphate in serosal and mucosal solutions of everted intestinal loops prepared from the proximal and distal jejunum which show the most efficient concentrative transport of inorganic phosphate(15). In agreement with previous reports vitamin D treatment of the rat enhances concentrative transport of phosphate. Concentrative transport of phosphate of loops incubated in Mg^{++} containing buffer was less than that of loops incubated in Mg^{++} free buffer. Everted intestinal loop preparations from magnesium deficient animals transported phosphate to a greater extent than those obtained from control rats and this effect was independent of the presence or absence of Mg^{++} in the buffer solutions. Means from the 3 described effects were significantly different. The vitamin D effect was highly significant ($P<0.005$) and the inhibitory effect of

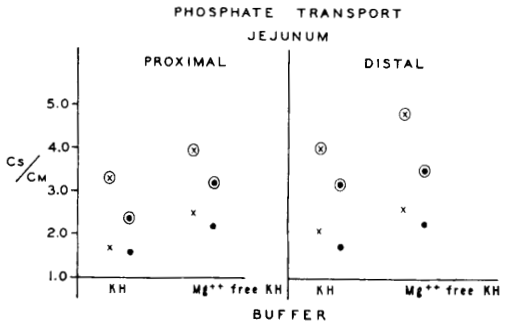

FIG. 4. Effect of magnesium deficiency and concentration of magnesium in medium on concentrative transport of inorganic phosphate by rat small intestine *in vitro*. Concentration ratios of inorganic phosphate in serosal and mucosal solutions (Cs/Cm) of everted loops are shown. The Krebs-Henseleit bicarbonate buffer was used with Mg^{++} (KH) or without Magnesium salt (Mg^{++} Free KH) modified to give a potassium concentration of 18 mEq/l. Statistical analysis of these data showed that differences between means were significant for the 3 described effects, the enhancing effect of vitamin D, the inhibitory effect of Mg^{++} in the buffer and the enhancing effect of magnesium deficiency of the rats. Code: Data are means, each point represents a mean of 6 to 14 loops. X = magnesium and vitamin D deficient; ● = control, vitamin D deficient; x circled = magnesium deficient, vitamin D treated; ● circled = control, vitamin D treated.

Mg^{++} in the buffer as well as the enhancing effect of magnesium deficiency of the animals were significant at a level $P<0.05$.

Discussion. These experiments indicate that concentrative transport of calcium by small intestine *in vitro* is not necessarily correlated with *in vivo* studies of intestinal absorption of calcium. Our results suggest that the behavior of rat intestine in terms of calcium transport *in vitro* is altered by the presence or absence of magnesium in the medium but not by magnesium depletion of the rat from which the intestine was obtained. In a recent similar study, however, Kessner and Epstein found the reverse to be true(16). They showed that the capacity to transport calcium against a concentration gradient by everted sacs of rat intestine of magnesium deficient animals was augmented, but they found no effect related to the presence or absence of magnesium in the medium.

The discrepancy between our findings and those of Kessner and Epstein(16) might depend on differences in experimental conditions. In our studies a bicarbonate buffer containing phosphate, potassium and glucose was used. Schachter and Rosen(2) who also found an inhibitory effect of magnesium *in vitro* on concentrative calcium transport used a phosphate buffer containing potassium and glucose. Kessner and Epstein(16) on the other hand employed a potassium free tris buffer in which fructose was present instead of glucose. In Kessner and Epstein's experiments(16) the control rats were fed a higher magnesium diet than in our studies and the mean serum magnesium concentration was 2.9 mg/100 ml in comparison with 1.64 mg/100 ml for the control rats in the present experiments. It is not known whether this higher intake of magnesium could alter the behavior of the intestine *in vitro*. *In vivo* a low magnesium diet may produce the same effect as the magnesium free medium *in vitro* and thus increase the calcium absorption as reported by Alcock and MacIntyre(1).

The present study indicates that Mg^{++} does play an important role in the system for concentrative transport of inorganic phosphate. The inhibitory effect of Mg^{++} *in vitro* on phosphate transport was evident in loops from magnesium deficient as well as in loops from control rats. The phosphate transport of loops from magnesium deficient rats was greater than that of loops from magnesium fed rats. Since the concentrative transport of phosphate is activated by calcium(15) the inhibitory effect of Mg^{++} could be due to a decreased concentration of Ca^{++} in an intracellular compartment as the result of diminished Ca^{++} transport across the intestinal wall in the presence of Mg^{++}(2). Alternatively this phenomenon may represent another example of Mg^{++} — Ca^{++} antagonism in the activation of an enzyme or carrier system. This would also be consistent with the increased concentrative transport of phosphate by intestinal loops from magnesium deficient rats.

The question whether the effect of magnesium deficiency on phosphate transport is specific for the intestinal mucosal cell or whether it occcurs in other tissues is of interest. The relationship of this phenomenon to the transport of phosphate across the renal tubular cell warrants further exploration. It has been suggested that magnesium deficiency

in rats produces a hyperparathyroid state(17). Hyperparathyroidism alone cannot explain the phosphate diuresis nor the hypophosphatemia observed in magnesium depleted animals since these effects have also been observed in parathyroidectomized rats fed a low magnesium diet(17). The effect of magnesium deficiency on phosphate transport is likewise present in the vitamin D deficient rat. Although the 24-hour urinary excretion of phosphate is not significantly greater in vitamin D deficient, magnesium deficient rats than in vitamin D deficient controls the 24 hour phosphate clearance is greater owing to the lower concentration of serum phosphate. These findings suggest that magnesium deficiency directly affects the phosphate transport systems controlling inorganic phosphate concentrations in extracellular fluid.

Summary. The concentrative transport of calcium by everted intestinal loops *in vitro* and the permeability of the small intestinal wall to this ion were determined in intestinal preparations from vitamin D deficient and vitamin D treated magnesium deficient and control rats. These experiments indicate that concentrative transport of calcium by rat small intestine *in vitro* is altered by the presence or absence of magnesium in the medium but not by magnesium depletion of the animals. The permeability to calcium of the intestinal preparations was not influenced by magnesium deficiency. The effects of magnesium depletion of the rat and of the presence of magnesium in the medium on concentrative transport of inorganic phosphate by the small intestine *in vitro* were also studied. Concentrative transport of phosphate by intestinal loops incubated in Mg^{++} containing buffer was less than that by those incubated in Mg^{++} free buffer. Intestinal loop preparations from magnesium deficient animals transferred phosphate to a greater extent than those obtained from control rats; this difference was observed both in the presence and absence of Mg^{++} in the buffer solution. Magnesium deficiency in the rat also reduced the concentration of serum phosphorus and increased the excretion of phosphate in the urine. These findings are consistent with the theory that magnesium influences the phosphate transport systems which control phosphate concentrations in extracellular fluids.

1. Alcock, N., MacIntyre, I., Clin. Sci., 1962, v22, 185.
2. Schachter, D., Rosen, S. M., Am. J. Physiol., 1959, v196, 357.
3. Clark, I., Nature, 1965, v207, 482.
4. MacIntyre, I., J. Chron. Dis., 1963, v16, 201.
5. Welt, L. G., Yale J. Biol. & Med., 1964, v36, 325.
6. Lifshitz, F., Harrison, H. C., Bull, C. E., Harrison, H. E., Metabolism, 1967, v16, 345.
7. Schachter, D., J. Lab. Clin. Med., 1959, v54, 763.
8. Harrison, H. E., Harrison, H. C., J. Lab. Clin. Med., 1955, v46, 662.
9. Clark, E. P., Collip, J. B., J. Biol. Chem., 1925, v68, 461.
10. Fiske, C. H., Subarrow, Y., ibid., 1929, v81, 629.
11. Natrella, H. C., National Bureau of Standards, Washington, D. C., Handbook 91, 1963.
12. Yates, F., Technical communications No. 35, Imperial Bureau of Soil Science, Harpenden, England, 1937.
13. Harrison, H. E., Harrison, H. C., Am. J. Physiol., 1960, v199, 265.
14. Krebs, H. A., Henseleit, K., Hoppe-Seyler Z., 1932, v210, 33.
15. Harrison, H. E., Harrison, H. C., Am. J. Physiol., 1961, v201, 1007.
16. Kessner, D. M., Epstein, F. H., Proc. Soc. Exp. Biol. & Med., 1966, v122, 721.
17. Heaton, F. W., Clin. Sci., 1965, v28, 543.

Received October 5, 1966. P.S.E.B.M., 1967, v125.