residual enzyme-coenzyme activity (in the absence of added coenzyme). Evidence is presented for a decrease in coenzyme content of dystrophic muscle and for a correlation of these enzyme changes with age of mice and severity of myopathy.

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Effect of Lactose on the Utilization of Phosphorus from Colloidal Phosphate by Chicks.* (25376)

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The effect of lactose on absorption of calcium and phosphorus has been of considerable interest, and a great deal of work has been done on the over-all effects of lactose feeding. Lactose, when included at a level of 25% in the basal diet, increased the amount of calcium and phosphorus absorbed due to formation of lactic acid by fermentation in the intestine(1). Simultaneous feeding of lactose and bone meal to dairy calves resulted in increased calcium retention from bone meal (2). These authors found that feeding a diet containing 25% lactose caused marked lowering of pH of contents of the digestive tract below the duodenum. Inclusion of lactose at a level of 40% in the diets of young chicks of a rachitogenic ration produced a favorable effect on calcium absorption, as well as having a positive influence on maintaining a more acid condition in the entire intestinal tract (3). Colloidal phosphate (soft phosphate with colloidal clay), obtained as by-product in the mining of rock phosphate, has been offered as a phosphorus supplement, to be used in animal rations. Recent workers(4,5, 6) reported that this supplement, when used as sole source of phosphorus in the diet, gave lower growth rate and (percent) bone ash in chicks than was produced by tricalcium phos-

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phate. Since colloidal phosphate is a cheap source of phosphorus, it was our object to study the effect of various levels of lactose on availability of phosphorus from colloidal phosphate.

Methods. Three hundred and sixty day-old Vantress x Nichols 108 chicks were randomly divided into 18 groups of 20 chicks (equal number of males and females in each group) and 2 groups were fed on each diet. Composition of basal diet was as follows: ground corn 59.5%, soybean oil meal 23%, dehydrated alfalfa 5.0%, dried whey 3.0%, sodium chloride 0.5%, $MnSO_4$ (70%) 120 mg/lb and vitamin mixture 1.0%. The following vitamins were supplied/lb of diet: vit. A 4540 I.U., vit. D_3 600 I.C.U., riboflavin 2 mg, calcium pantothenate 5 mg, niacin 12.5 mg, vit. B_{12} 4.0 μg, choline chloride 400 mg and aureomycin hydrochloride 10 mg. The vitamins were premixed with cerelose to give a level of 1 lb/100 lb of diet. The basal diet fed to Group 1 contained 0.34% phosphorus from dibasic sodium phosphate and those of the other 4 groups were supplemented with 0.2% phosphorus from colloidal phosphate. Lactose was added at levels of 0, 5, 10 and 15% of basal diet. Each diet was maintained at the same calorie-protein ratio by adjusting levels of soybean oil meal and ground corn. The calcium:phosphorus ratio was maintained at 2:1 in all diets. The birds were housed in electrically-heated batteries with raised wire floors, and feed and water were supplied *ad lib.* All groups were observed daily and group weights were recorded at weekly intervals. Birds were weighed individually at end of the 4-week experimental period and feed consumption was recorded. Five birds were selected at random from each replication and the left tibia was removed for bone ash determination according to the A.O.A.C. method (7). Intestinal pH was measured with Beckman pH meter, on the same birds used for bone ash determination. Birds were anesthetized and killed with chloroform vapor. The intestine was removed and pH measured at duodenal loop, 4 inches posterior to duodenum, caecum, and 4 inches anterior to caecum, by procedure similar to that decided by March *et al.*(8). In a second experiment, lactose was added as supplement to a purified diet which consisted of the following ingredients: Purified soybean protein 25%, soybean oil 2.5%, vit. mixture 1.41%, and mineral mixture 2.37%. Cerelose was added to make the diet 100%, after addition of calcium carbonate and phosphorus supplements. The Ca:P ratio was maintained at 2:1. The following vitamins were added/kg of diet: thiamin HCl 10 mg, riboflavin 10 mg, *D*-calcium pantothenate 20 mg, pyridoxine HCl 6 mg, inositol 500 mg, biotin 0.2 mg, niacin 75 mg, folic acid 4 mg, vit. A 10,000 I.U., vit. D_3 2000 I.C.U., α-tocopheryl acetate 20 I.U., choline chloride 1.5 g, menadione sodium bisulfite 0.5 g, vit. B_{12} 20 μg, methionine 7500 mg, glycine 3500 mg, aureomycin HCl 10 mg and BHT (25%) 500 mg. The mineral supplement consisted of the following ingredients/kg of diet: $MnSO_4 \cdot H_2O$ 1.1 g, $MgSO_4 \cdot 7H_2O$ 5.7 g, KI 0.04 g, NaCl 9.5 g, $FeC_6H_5O_7 \cdot 5H_2O$ 1.6 g, $CuSO_4 \cdot 5H_2O$ 0.016 g, $ZnCl_2$ 0.014 g, $CoCl_2$ 0.005 g and KCl 5.72 g. Four groups of chicks were fed 0.4% phosphorus as dibasic sodium phosphate, and 4 groups were fed 0.4% phosphorus as a combination of dibasic sodium phosphate and colloidal phosphate, each supplying 0.2% phosphorus to the diet. The diets were supplemented with lactose at levels of 0, 5, 10 and 15% of the basal diet. Other experimental procedures were the same as described for previous experiment.

Results. Addition of lactose to a practical diet containing dibasic sodium phosphate as source of phosphorus had no appreciable effect on either percent bone ash or body weight at 4 weeks of age (Table I). Slight decreases in weight were observed when lactose was added at levels of 5 and 10%, but at the 15% level the weight was equal to that of Group 2 which received dibasic sodium phosphate, with no added lactose.

Groups of chicks fed colloidal phosphate as sole source of phosphorus showed improvement in growth rate with addition of 10 or 15% lactose. Addition of 5% lactose had very little effect on growth rate, but increased availability by 8.3 percentage points when measured in terms of bone ash. Increasing lactose level in the diet containing colloidal

TABLE I. Effect of Addition of Lactose to a Practical-Type Diet on Chick Growth and Bone Formation at End of 4 Weeks, in 9 Groups.

Lactose level (%)	Added phosphorus (%)	Source	4 wk wt (g)	Bone ash (%)	Feed conversion	Availability (%)*
0	0		290.8	33.58	2.49	
0	.2	Na_2HPO_4	343.1	41.83	1.97	
5	"		335.6	42.65	2.05	
10	"		314.8	42.10	2.05	
15	"		343.3	43.30	1.90	
0	"	Colloidal phosphate	283.2	38.55	2.37	82.6
5	"		285.0	38.77	2.10	90.9
10	"		300.5	39.52	2.05	93.8
15	"		303.2	40.15	2.14	94.9

* Availability relative to that of Na_2HPO_4 was determined by comparing bone ash values of groups with corresponding lactose levels when different sources of phosphorus were used.

phosphate to 10% resulted in increased growth as well as bone ash. Groups of birds fed 10% lactose showed an 8.5% increase in growth rate over that of the group fed a diet containing no added lactose. Phosphorus availability, based on comparison of bone ash with that of corresponding groups fed dibasic sodium phosphate, indicated that addition of 10% lactose to the diet increased 11.3 percentage points in availability of phosphorus in colloidal phosphate. Increase of lactose level to 15% had very little effect on rate of growth and percent bone ash, as compared to group fed 10% lactose. Phosphorus availability in this case was 12.4 percentage points greater than that observed in the group fed colloidal phosphate alone.

The pH measurements at different parts of intestine are summarized in Table II. With increases in lactose level of the diet, a regular decrease was observed in pH of duodenum from 6.8 to 6.5. The pH of caecal contents dropped less consistently from 6.3 to 5.4. These data are in general agreement with the findings of Beach *et al.*(9), that with increase of lactose level of the diet an increase in intestinal acidity takes place.

TABLE II. Effect of Lactose on Intestinal pH of Chicks Fed a Practical-Type Diet.

	pH*			
Added lactose (%)	Duodenum	4" posterior to duodenum	Caecum	4" anterior to caecum
0	6.8	6.5	6.3	7.0
5	6.7	6.3	5.8	6.8
10	6.6	6.4	5.4	6.4
15	6.5	6.3	5.7	6.1

* Avg of 10 values/treatment.

Increased availability of colloidal phosphate (Groups 7, 8 and 9) with increased lactose content of diet may have been due to increased acidity of the intestinal tract. This could create favorable conditions for bringing the colloidal phosphate into a soluble state. Lactose is hydrolyzed and absorbed with difficulty. For this reason, it remains for a considerable length of time in the intestinal tract, and may produce a favorable environment for growth of aciduric types of intestinal flora. An increase in acidity following growth of these aciduric bacteria may cause increased release of phosphorus and calcium from colloidal phosphate, resulting in increased availability of phosphorus to the chicks.

Efficiency of feed conversion improved considerably from 2.37 for birds fed colloidal phosphate alone to 2.10, 2.05 and 2.14 with groups fed colloidal phosphate supplemented with 5, 10 and 15% lactose, respectively (Table I).

Addition of lactose to a purified chick diet depressed growth rate in Groups 2, 3 and 4 fed dibasic sodium phosphate (Table III). Growth of birds receiving phosphorus as equal parts of colloidal phosphate and dibasic sodium phosphate was stimulated by supplementation with lactose (Groups 6, 7, and 8) as compared to Group 5, fed phosphorus from colloidal phosphate and dibasic sodium phosphate with no added lactose. This indicated a favorable effect of lactose on utilization of phosphorus from colloidal phosphate. Relative availability increased from 93 to 101% with increase in lactose level up to 10% of the diet.

TABLE III. Effect of Addition of Lactose to a Purified Diet on Chick Growth and Bone Formation at the End of 4 Weeks.

	Groups and supplements	Added phosphorus (%)	Lactose (%)	4 wk wt (g)	Bone ash (%)	Availability (%)*
1.	Na_2HPO_4	.4	0	408.5	36.08	
2.	"	"	5	378.1	35.61	
3.	"	"	10	383.3	35.48	
4.	"	"	15	377.1	34.86	
5.	½" + ½ colloidal phosphate	"	0	278.0	33.56	93
6.	*Idem*	"	5	362.5	35.96	101
7.	"	"	10	341.9	35.84	101
8.	"	"	15	351.5	33.16	95

* Availability relative to that of Na_2HPO_4 was determined by comparing bone ash values of groups with corresponding lactose levels when different sources of phosphorus were used.

Summary. The effect of lactose, at levels of 5, 10 and 15% of purified and practical diets, on utilization by chicks of phosphorus from colloidal phosphate was studied. Data presented here demonstrated that utilization of colloidal phosphate by chicks was improved when diets were supplemented with lactose at levels to 10%. The pH of intestinal tract was lowered by lactose supplementation, although this effect was not uniform as lactose level increased.

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Estimation of Cardiac Output by Rapidly Repeated Dye-Dilution Technic. (25377)

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A method for estimating cardiac output every 5-8 seconds has been devised. The procedure, based on the indicator dilution principle, has been used in acute experiments where rapidly repeated measurements of cardiac output were required.

Method. A small (Intramedic, PE-100) polyethylene catheter was introduced into the left atrium of anesthetized dogs *via* a pulmonary vein. The small intercostal incision in the fourth left interspace was left open and mild artificial respiration was continued throughout the experiment. The catheter was connected to a specially designed injection valve which controlled the injection of the dye-indicator solution into the left atrium. Time between injections and duration of injection could be pre-determined and phased with other events, such as respiration, by means of electronic controls. A schematic diagram of the injection valve is shown in Fig. 1. The dye-indicator was a tricarbocyanine dye (Cardio-Green®)* prepared as a 0.1% solution. Approximately 0.5 mg (for 10-12 kg

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