

In 27 of 28 comparisons, the polymer caused decided increases in uptakes of sodium from the seven silt loams, and in all cases the larger uptakes of sodium were from the limed soil.

The findings demonstrate capacity of the additive sodium to repress uptake of calcium and magnesium, while causing increase in the uptake of potassium by the above-ground growth of the several crops; substantial soil retention of the sodium of the polymer throughout the growth of three successive crops; and appreciable passage of the polymer sodium into the fourth of four crops.

In general, decisive effects upon the chemical compositions of the several crops were induced only by the 0.05 and 0.1% inputs. No substantial repressions in plant response were indicated, and the incorporated polymer functioned in like manner in the unlimed and limed soils.

The pot cultures of Experiments I and II were not subjected to leaching and the crops were feeding upon sodium contents that were different from those upon which the several vegetations would have been growing in soils subjected to natural rainfall, or to a simulation of it.

The two experiments demonstrate that the incorporated polymer induced changes in the proportions of the cations that prevailed in solute and exchangeable forms in the soil. Therefore, the

authors believe it essential to obtain a comprehensive inventory and understanding of the chemical and biochemical effects of incorporations of soil conditioners, sodium or potassium. It is believed those objectives could be attained through lysimeter experiments conducted at several points where soil type and reaction and rainfall are the controlling factors, with parallel fallow and cropping. Such an experimental approach should indicate to what extent rain-water leachings and plant uptake govern persistence and fate of an incorporated polymer and correlate the physical effects induced by the polymer.

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PIG NUTRITION

Selection of Diet for Studies of Vitamin B₁₂ Depletion Using Unsuckled Baby Pigs

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THE BABY PIG has been used extensively and very successfully as an experimental animal in nutritional research. However, most of the reported studies involved pigs which were allowed to nurse for 1 or 2 days before being put on an experimental diet. In studying nutritional factors which are accumulated or stored, it would be desirable to start the animal on the nutritional regime immediately after birth. Early attempts by other workers to raise baby pigs taken at birth were unsuccessful (1, 10), and colostrum was considered essential for survival.

Young and Underdahl (20) have recently demonstrated that pigs which

have not nursed can be raised on supplemented cow's milk if the animals are separated from the dam and the herd environment at birth and are maintained in strict isolation. Their work indicated that the essential feature of colostrum involves protection against disease by antibody transfer, and therefore colostrum is not necessary if exposure to disease is prevented by adequate isolation. To accomplish this separation the pigs were caught at birth in sterile bags, or more recently removed from the sow by hysterectomy (19), and placed immediately in an isolated environment.

Sheffy *et al.* (17) have reported the successful rearing of 12 pigs to the age of 4 weeks on a semisynthetic diet. Their work indicated that phospholipides were necessary for survival, and their ration

contained antibiotics. Catron and co-workers (5) successfully raised 14 pigs without colostrum to 8 weeks of age on a supplemented skim milk ration, but were unable to repeat their experiment because of disease interference. The present paper relates experience in raising newborn pigs on purified diets using moderate isolation procedures, and presents evidence of having obtained a definite depletion of vitamin B₁₂ reserves using this technique.

Methods

Preparation of Rations In experiments I and II the cow's milk fed was commercial homogenized "vitamin D" milk supplemented with mineral salts and egg as

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Although the 1- to 2-day-old baby pig has been used extensively as an experimental animal in nutritional investigations, very few reports have appeared concerning the use of pigs that have not been allowed to nurse. This work was conducted to examine the feasibility of maintaining pigs from birth on purified rations, and to select a ration that would be suitable for studies of vitamin B₁₂ depletion. It was found that unsuckled baby pigs isolated from the herd environment could be raised to 8 weeks of age on purified rations, and that some rations were more suitable than others. On a ration consisting of glucose, a purified soybean protein, methionine, hydrogenated vegetable oil, phospholipides, mineral salts, and vitamins, but no added vitamin B₁₂, a marked depletion of the vitamin B₁₂ reserves of the animals was obtained in 8 weeks, as evidenced by the vitamin B₁₂ activity present in selected tissues.

described by Young (20). In experiments II and III the purified casein and soybean protein diets were prepared according to the formulation of Neumann, Krider, and Johnson (13)—i.e., 30.0% protein source, 37.4% glucose, 26.6% lard, 6.0% salts, and adequate amounts of water-and fat-soluble vitamins. Antibiotics and vitamin B₁₂ were omitted from these rations. When soybean proteins were used, 0.6% of DL-methionine was added to the ration, replacing that amount of protein source. These rations were fed as "milk" containing 13% solids for the first 11 to 18 days, then fed as a solid feed with free access to water. Attempts to disperse the lard by passing the milk through a hand-operated homogenizer and a colloid mill were not successful, there still being some separation of the lard.

The constituents of the diet used in experiment IV are listed in Table I, as they differ appreciably from those of the purified rations used previously. In preparing this milk ration the glucose, salts, methionine, and protein source were added to hot (80° C.) water and allowed to settle. Part of the supernatant was then used to form an emulsion with the phospholipides, melted Crisco, and Tween 80, using a hand-operated homogenizer. The mixtures were combined, passed through a colloid mill, and then frozen in appropriate daily amounts. Before use, the milk was heated to 80° C. and cooled, and the vitamins were added. No separation of fat occurred with this milk ration. The solid ration was prepared in 5- to 20-kg. batches using a Hobart mixer, the Crisco being added in melted form, and the ration was stored in a refrigerator.

Treatment of Animals The pigs were obtained either by catching them in sterile bags at birth (20) or by cesarean section. By the latter method the pigs were placed in sterile bags as soon as they were removed from the uterus; the operation was performed under local anesthesia, so that the pigs were active immediately. The pigs were taken to an isolation room

which had been previously cleaned and fumigated by the permanganate-formaldehyde technique (7), and were placed in individual screen-bottomed cages. A heat lamp was suspended above each cage, and the room was kept at 80° F. or above. The methods used in handling the animals were designed to provide isolation from other swine and to keep the immediate surroundings as clean as possible. To isolate them individually and from the caretaker would require apparatus such as described by Young and Underdahl (19) and was considered as probably unnecessary for nutritional work. Within a few hours after birth the pigs were given a 1-mg. tablet of vitamin K and several days' allotment of a vitamin A, D, E mixture, and offered their respective rations in individual feeding pans. The milk was warmed during the first week only. The pigs were fed four times daily until weaned to solid ration, after which time they were fed three times daily. The

animals which received the cow's milk were not weaned.

Certain of the animals received intramuscular injections of vitamin B₁₂, a sterile isotonic solution of the crystalline material being used for this purpose. During the course of the experiments, blood samples were obtained from the anterior vena cava (8). Some or all of the following determinations were performed on these samples: red cell count, white cell count, hemoglobin, hematocrit, total and differential (electrophoretic) plasma proteins, plasma vitamin B₁₂, and whole blood vitamin B₁₂. On autopsy only a gross examination was performed, and tissue samples were obtained for vitamin B₁₂ assay.

Vitamin B₁₂ Assay Methods The assay organism was *Lactobacillus leichmannii* 313 and the medium of Peeler, Yacowitz, and Norris (15) with minor modifications was used. The assay tubes (10 ml.) were steamed 15 minutes, brought to 37° C., inoculated, incubated at 37° C. for 72 hours, and

Figure 1. Effect of vitamin B₁₂ supplementation on growth of baby pigs fed a purified ration

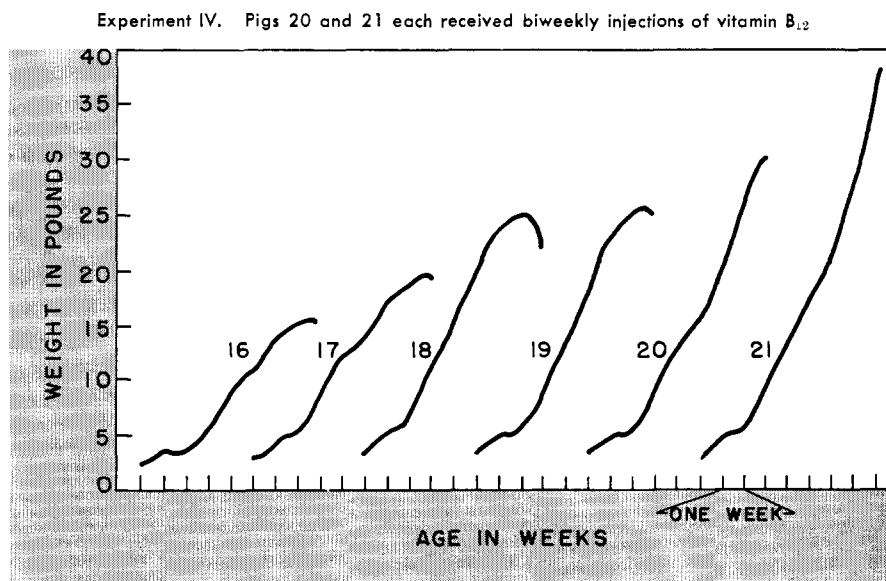


Table I. Composition of Ration Used in Experiment IV

Ingredient	G./Liter Milk	G./Kg. Dry Feed
Drackett Protein 220 ^a	45.0	350.0
DL-Methionine	1.0	10.0
Glucose, technical	56.0	368.0
Crisco	20.0	160.0
Phospholipides ^b	2.0	16.0
Salts premixture ^c	6.0	46.0
	Mg./Liter	Mg./Kg.
Thiamine hydrochloride	0.75	6.0
Riboflavin	1.50	12.0
Nicotinic acid	3.00	24.0
Pyridoxine hydrochloride	0.75	6.0
Calcium pantothenate	1.50	12.0
Biotin	0.01	0.08
Inositol	25.0	200.0
p-Aminobenzoic acid	2.50	20.0
Folic acid	0.05	0.40
Menadione	0.30	2.40
α-Tocopherol	1.00	8.0
Choline chloride	0.26 g.	2.0 g.
Vitamin A	2500 IU	20000 IU
Vitamin D	250 IU	2000 IU
Nonnutritive bulk ^d	...	48.0 g.
Tween 80 ^e	0.50 ml.	...

^a The Drackett Co., Cincinnati, Ohio.

^b Alcolic Granules, American Lecithin Co., Woodside, L. I., N. Y.

^c Composition of salts premixture (grams)

CaHPO ₄	350.0
CaCO ₃	230.0
K ₂ HPO ₄	220.0
NaCl	120.0
MgSO ₄ ·7H ₂ O	42.0
FeSO ₄ ·7H ₂ O	26.0
MnSO ₄ ·H ₂ O	8.4
CuSO ₄	1.8
KI	1.2
ZnCl ₂	0.3
CaF ₂	0.3
	1000.0

^d Alphacel, Nutritional Biochemicals Corp., Cleveland, Ohio.

^e Hill Top Laboratories, Inc., Cincinnati, Ohio.

titrated to pH 7.0 with standard sodium hydroxide.

Plasma was prepared for assay by the procedure of Rosenthal and Sarett (16), modified by adding 2 mg. of potassium cyanide to the original dilution of the plasma.

Oxalated whole blood was prepared for assay by autoclaving 2 ml. of blood with 2.5 mg. of potassium cyanide at 121° C. for 5 minutes, blending with 98 ml. of water, filtering, and adjusting the pH of the filtrate to pH 6.0 (18).

Liver, kidney, pancreas, and fecal samples were prepared for assay by identical procedures. A Waring Blender was used to blend 1 gram of material with 49 ml. of water, and a 10-ml. aliquot of this mixture was adjusted to pH 6.0 and autoclaved with 8 mg. of potassium cyanide at 121° C. for 5 minutes. The sample was then readjusted to pH 6.0, diluted to reach the assay range, and filtered.

Experimental Results

The four experiments summarized in Table II were conducted primarily for the purpose of selecting a diet suitable for studies of controlled vitamin B₁₂ in-

take, rather than a diet which would give optimal growth rate and efficiency. Of the diets tried, the casein ration appeared to be the most satisfactory for the latter purpose, but other workers have indicated that casein is less suitable for the production of a vitamin B₁₂ deficiency state than is soybean protein (2, 3).

Experiment I was conducted to determine the adequacy of isolation facilities and techniques for raising pigs from birth. The only difficulty encountered was with sore feet, and this was corrected by using heavier-gage flooring material and keeping the pen dry with a heat lamp.

Experiment II, comparing two purified rations with a control ration (cow's milk), indicated that newborn pigs could be raised on purified diets but that scouring, apparently noninfectious, was associated with the Alpha-Protein ration. The presence of continuous scouring on an Alpha-Protein ration was reported by Cartwright *et al.* (4), but is contrary to the work of others who have used this product in baby pig studies (11, 13, 14). Curtin and coworkers (6) have reported that Alpha-Protein contains considerable quantities of bisulfite, which can be removed by washing the protein with

water. It was considered possible that the scouring observed in this experiment was due to the presence of bisulfite in the ration.

Experiment III was intended to compare the acceptability of a washed Alpha-Protein preparation with another purified soybean product, Drackett Protein 220, which was found to be low in apparent vitamin B₁₂ content. The Alpha-Protein was autoclaved at 115° C. for 10 minutes, cooled, washed four times with water (6), then once with 95% ethyl alcohol, and dried at 70° C. Two animals received a ration containing this preparation, two received a ration containing Drackett Protein which had been washed in the above manner, and two pigs were on a ration containing untreated Drackett Protein. However, because of three early deaths during this experiment very little could be deduced concerning the acceptability of the Drackett Protein rations. The washing procedure did not appear to modify the scouring resulting from the use of Alpha-Protein.

The diet used for experiment IV (Table I) resulted from several modifications of previous rations, with the object of decreasing the incidence of scouring. A more easily homogenized fat source was selected and the amount of fat in the ration was reduced. Tween 80 was added as an aid in the homogenization process. The solid ration to which the animals were weaned at 11 days of age contained 4.8% nondigestible bulk (cellulose). This ration was found to contain 0.2mγ of vitamin B₁₂ per gram by microbiological assay. Cobalt was omitted from the salt mixture as a possible means of decreasing intestinal synthesis of vitamin B₁₂.

In this experiment two of the pigs, 20 and 21, were given injections of vitamin B₁₂ twice a week at the rate of 8 γ of vitamin B₁₂ per pound of body weight per injection. A total of 1.86 mg. of vitamin B₁₂ was given each of these two pigs over the 8-week period.

The growth progress of these animals is shown in Figure 1. Severe scouring was encountered during the second week, resulting in a temporary cessation of growth. At 28 days of age the pigs were transferred to larger screen-bottomed pens, two animals per pen. At about 6 weeks of age the feed consumption of the four animals receiving no vitamin B₁₂ began to decrease, and by 8 weeks these pigs were eating very little. It is possible that this trend might have been modified had the other B vitamins been forcibly administered. At 8 weeks the animals were killed by stunning and bleeding out. Samples of liver, pancreas, kidney, and feces were obtained for vitamin B₁₂ assay.

The gross symptoms and hematological changes reported by other workers (4, 9, 12, 14) as associated with B₁₂ deficiency

in baby pigs were not observed with these animals, probably because of the shorter duration of this experiment.

The results of the vitamin B₁₂ assays of samples obtained from these experimental animals are recorded in Table III. These assay values were not corrected for nonvitamin B₁₂ activity, although previous work has indicated that such activity is low in blood and liver. More serious, particularly with blood and plasma samples, was the presence of inhibiting substances in the solutions to be assayed. Therefore the blood and plasma values are useful only for comparative purposes, and are considered significant by reason of the consistency of the

difference between the two treatments.

The storage tissues—liver, kidney, and pancreas—all reflect in a similar manner the large difference in intake of vitamin B₁₂ on the two treatments. The average of the liver assay values for four animals receiving no injected vitamin B₁₂, 14mγ of vitamin B₁₂ per gram, is very low. In comparison, the following liver assay values were obtained from non-experimental baby pigs: 190mγ of vitamin B₁₂ per gram for a litter mate of the experimental pigs, sacrificed at birth; an average of 98mγ of vitamin B₁₂ per gram for three litter mates of these experimental pigs raised under herd conditions to 8 weeks of age; and an average

of 170mγ of vitamin B₁₂ per gram for four pigs of similar herd history and ranging from 2 to 14 days of age. It appears therefore that a marked depletion of the vitamin B₁₂ reserves occurred in the animals maintained on the unsupplemented ration, and that an excess amount of vitamin B₁₂ was administered to the two supplemented animals.

A few liver assay values were obtained at the conclusion of experiments II and III and these values, given in Table IV, indicated essentially the same correlation between administered vitamin B₁₂ and vitamin B₁₂ activity present in liver tissue. Pig 8 was given seven injections of the vitamin during the 5th and 7th weeks, and pig 11 was given 12 injections throughout the second half of the experimental period.

The appreciable amounts of fecal vitamin B₁₂ activity shown in Table III indicate that omitting cobalt from the ration does not halt intestinal synthesis of large amounts of vitamin B₁₂ activity. Therefore coprophagy constitutes a potential source of additional vitamin B₁₂ and should be prevented.

In connection with the general problem of raising newborn pigs, it is the authors' observation that the formation of γ-globulin, and therefore presumably antibodies, takes place slowly. It is well known that preparturient transfer of immune globulins does not occur in swine. Electrophoretic analyses of the serum of eight of the pigs in experiments II and III revealed no detectable γ-globulin present during the first 6 weeks of age. Serum samples of the six pigs of the last experiment, taken at 8 weeks of age, contained from 3.4 to 5.7% γ-globulin (of the total serum protein), or about one fourth of the normal amount. It appears necessary, then, to consider the animals as highly susceptible to swine diseases and to provide adequate isolation over the full experimental period.

Conclusions

Baby pigs can be raised from birth on purified diets, without excessive losses, by using moderate means of isolation. The occurrence of scouring has been decreased but not eliminated by a modification of previously described diets. A marked depletion of the vitamin B₁₂ stores of four pigs maintained on this diet for 8 weeks was obtained, as evidenced by the vitamin B₁₂ content of liver, kidney, and pancreas samples.

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Table II. Summary of Experiments with Baby Pigs Obtained at Birth and Fed Experimental Rations

Expt. No.	Method Obtained	Pig No.	Type of Ration	Occurrence of Scouring, Days	Age at Death, Days	Cause of Death
I	Bag	1	Supplemented cow's milk ^a	None	35	Killed
		2		None	35	Killed
		3		None	20	Infected foot
II	Bag	4	Supplemented cow's milk ^a	7	42	Killed
		5		9	56	Killed
		6	"Vitamin-free" casein ration ^b	12	42	Killed
		7		10	42	Killed
		8		Continuously	56	Killed
		9		Continuously	56	Killed
III	Cesarean	10	Washed Alpha-Protein ration ^b	Continuously	42	Killed
		11		Continuously	42	Killed
		12	Washed Drackett Protein ration ^b	2	7	Injured while being bled
		13		4	9	Umbilical infection
		14		21	37	Unknown
15	3	8	Umbilical infection			
IV	Bag	16	Drackett Protein ration ^c	8	56	Killed
		17		9	56	Killed
		18		4	56	Killed
		19		7	56	Killed
		20		8	56	Killed
		21		8	56	Killed

^a Commercial homogenized cow's milk supplemented with egg and salts as described by Young and Underdahl (79).

^b Purified rations prepared according to formulation of Neumann, Krider, and Johnson (73), and varying only in protein constituent used (see text).

^c Purified ration, composition given in Table I.

Table III. Effect of Crystalline Vitamin B₁₂ Supplementation on Vitamin B₁₂ Assay Activity of Selected Tissues from Baby Pigs Maintained on a Purified Ration

Tissue	Age, Weeks	Pig No.					
		16	17	18	19	20 ^a	21 ^a
Vitamin B ₁₂ , mγ per ML.							
Blood	4	0.37	0.33	0.35	0.34	0.63	0.60
	6	0.36	0.34	0.44	0.37	0.81	0.60
	8	0.26	0.23	0.32	0.20	0.66	0.49
Plasma	4	0.11	0.13	0.13	...	0.23	...
	6	0.12	0.13	0.10	0.13	0.27	0.19
	8	0.11	0.10	0.13	0.12	0.59	0.29
Vitamin B ₁₂ , mγ per Gram							
Liver	8	17	13	11	13	660	390
Kidney	8	8.0	6.5	6.5	8.0	380	270
Pancreas	8	10	10	10	9.5	200	140
Feces	8	2500	1300	2300	2300	2600	1400

^a Received 1.86 mg. of vitamin B₁₂ by injection over 8-week period.

Table IV. Effect of Crystalline Vitamin B₁₂ Supplementation on Liver Vitamin B₁₂ Assay Activity of Baby Pigs Fed Experimental Rations

(Experiments II and III)

Expt. No.	Pig No.	Age, Days	Type of Ration	Vitamin B ₁₂ Injected, γ	Liver Activity, m γ B ₁₂ /G.
II	5	56	Supplemented cow's milk	0	97
	8	56	Alpha-Protein ration	165	150
	9	56	Alpha-Protein ration	0	21
III	10	42	Washed Alpha-Protein ration	0	15
	11	42	Washed Alpha-Protein ration	210	250
	14	37	Drackett Protein ration	0	16

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VEGETABLE COMPONENTS

Some Carbohydrate Components of Tomato

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The carbohydrate components of the tomato were separated into free sugars and polysaccharides. The free sugars, representing more than 60% of the solids, were D-fructose, D-glucose, sucrose, and a ketoheptose. The polysaccharides, an araban-galactan mixture, a xylan-rich fraction, pectin, and α -cellulose, were present in comparable amounts.

IMPROVED PROCEDURES for the removal of more and more water from the tomato are constantly being investigated, because more economical methods are needed for the transportation and storage of tomato solids. The production of tomato pastes of high solids content and the preparation of dried tomato flakes or powders have led to problems in processing and storage stability. More detailed information on the composition of the tomato should aid in solving some of these problems. The results of this preliminary study of some of the carbohydrate components of the tomato may contribute such needed information.

Separation of Free Sugars From Polysaccharides

Table-ripe tomatoes were scalded in boiling water to facilitate removal of the skin. The peeled tomatoes were disintegrated in an electric blender. The resulting slurry was poured through a sieve to remove the seeds and then immediately added to sufficient boiling ethyl alcohol to give a concentration of 80% ethyl alcohol. The tomato pulp was extracted with copious amounts of hot 80% ethyl alcohol to remove the last traces of free sugars (17). The pulp containing the insoluble polysaccharides was washed with absolute

ethyl alcohol and anhydrous ethyl ether to remove lipides and pigments and then air-dried.

Free Sugars

Eighty-one per cent of the solids in the peeled, seeded tomato pulp was found in the 80% ethyl alcohol extract. The total and reducing sugars were determined (2) and found to be 63.2 and 59.7%, respectively, of the tomato solids. Examination by paper chromatography (16) of the free sugars in this extract revealed substances indistinguishable from D-fructose, D-glucose, sucrose, and a ketoheptose. A sufficient quantity