reabsorption of an Evans blue-plasma protein complex, and, by inference, of normal plasma protein. Such tubular reabsorption possibly involves the mitochondria of the tubular cells.

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Nutritional Value of Plant Materials. II. Prevention of Acute Uremia of the Newborn Rat by Vitamin B_{12}.* (17516)

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A very high incidence of mortality during the first 24-72 hours of the life of young rats born to mothers maintained on rations containing a commercial soybean protein preparation and DL-methionine as the only source of amino acids has recently been reported from this laboratory.(1) In about 50% of the litters cast, from one to all of the young develop a characteristic syndrome terminating in early death. At birth and for the first 24-36 hours these young, on visual inspection, appear to be in every respect normal, indistinguishable from those which survive and develop normally. They are strong and nursed by their mothers until the onset of the crisis. (The support of excellent lactation has been an outstanding feature of these rations). A rapid onset of dyspnea accompanied by cyanosis, weakness and emaciation is typical of the syndrome observed. Milk is always present in the stomachs of the young and very frequently one or two loops of the small intestine contain dark material which is visible through the abdominal wall. At this stage the blood urea as determined by the method of Archibald(2) reaches values of about 150-250 mg per 100 ml as compared to about 35 mg at birth and about 50-70 mg in normal appearing rats of the same age.(3) Death of these rats usually occurs within 4-6 hours after the first symptoms can be observed, although a few individuals have survived as long as 12 hours. Without inference concerning the nature of the biochemical or pathological lesion or the ultimate cause of death the syndrome is conveniently referred to as "acute uremia of the newborn."

Other investigators have reported early deaths of young rats(4-6) and mice(7) that had milk in their stomachs and they may have been dealing with the same syndrome. Zucker and Zucker(8) observed elevated concentrations of NPN and urea in the blood of rats of post weaning age which were fed rations devoid of animal protein. McGinnis *et al.*(9) refer to observation of an increased NPN concentration in the blood of chickens maintained on rations containing plant ingredients plus vitamin free casein. In this laboratory "acute uremia of the newborn" has never been observed on rations containing either alcohol extracted casein,(1) or a com-

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TABLE I.
Effect of Vitamin B_{12} on Acute Uremia of the Newborn.

	Inj. with 0.05 μg vit. B_{12}		Uninjected controls	
	From 13 uremic litters	From 13 normal litters	From 13 uremic litters	From 13 normal litters
Total No. of young	46	54	52	63
Young dead in 48 hr from all causes	7*	4	35	5
Young with acute uremia	1	0	36	0
	Normal appearing young		Uremic young	Normal appearing young
Blood urea mg/100 ml	50.2 ± 11.8 (18)†		207.3 ± 58.2 (9)†	53.7 ± 19.8 (18)†
21 day weaning wt, g	Males 35.4 ± 3.1† (30) Females 32.3 ± 3.7 (18)		Males 32.7 ± 5.4† (26) Females 32.5 ± 5.2 (17)	

Number of individuals given in parentheses.
* Includes 3 young killed by female.
† Standard deviation. Uremic litters refers to those in which one or more rats were observed in acute uremic crisis. The others are referred to as normal litters.

mercial soybean protein supplemented with liver extract (Wilson's 1:20) or condensed fish solubles. Since the ration used in these experiments is presumably low in vitamin B_{12} the experiments reported here were made. They appeared to be further justified by the preliminary observation that subcutaneous injection of an antipernicious anemia liver extract (Abbott) into the newborn rat prevented the development of "acute uremia of the newborn."

Experimental. The mothers of the young used for these experiments were fed the following ration: Commercial soybean protein (Archer Daniels Midland Co., Minneapolis) 274.4 g; DL-methionine, 5.6 g; salts 4(10) (Mn^{++} concentration reduced to 1/10th) 40 g; vegetable fat (Crisco) 70 g; corn oil, 10 g; sucrose, 610 g. Each kg of ration contained in addition thiaminechloride HCl, 5 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 5 mg; calcium pantothenate, 5 mg; cholinechloride, 1000 mg; i-inositol, 400 mg; para-aminobenzoic acid, 10 mg; nicotinic acid, 20 mg; 2-methyl-1,4-naphthoquinone, 5 mg; biotin, 0.2 mg; folic acid, 0.2 mg; d,l α-tocopherol, 100 mg; vitamin A (fish liver concentrate) 10,000 I.U.; vitamin D_3 (Delsterol) 1500 I.U. The mothers of the young used were F_2 or F_3 generation offspring from mothers on the same ration. They were kept on raised screens since weaning at 21 days of age except for a few days before parturition and during lactation. Within 2-3 hours after birth about half the number of live young in each litter were injected subcutaneously on the dorsal side with 0.1 ml of a solution containing 0.05 μg of crystalline vitamin B_{12} (Cobione Merck). Before withdrawal of the needle the site of injection was covered with a small piece of adhesive tape to prevent leakage. The injected young were identified by snipping off the tip of the tail.

Results and discussion. A comparison of the incidence of "acute uremia of the newborn" in injected and uninjected littermates is given in Table I. Only those individuals were counted as having acute uremia which were actually observed in the acute crisis. Litters in which no acute uremia was observed are tabulated as "normal" litters. A few deaths in each group occurred from crushing by the mothers, from unknown causes or probably from unobserved acute uremia in the uninjected control group. The fact that one injected individual was observed in acute crisis suggests that the quantity of vitamin B_{12} injected was not sufficient to prevent the syndrome in all cases. A typical example of the appearance of rats with the acute uremic

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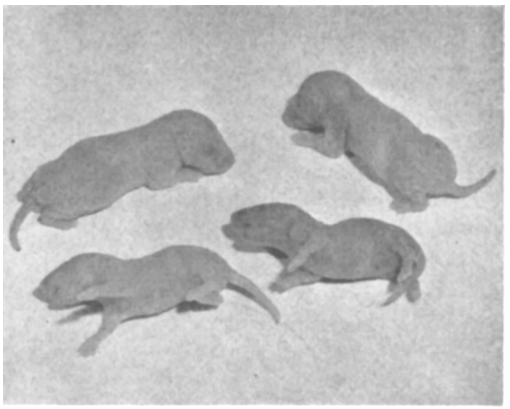

FIG. 1.
Forty-four hour old rats; the 2 rats in the upper portion of the figure were injected 2 hours after birth with 0.05 μg of vit. B_{12}; the 2 rats in the lower portion are their uninjected littermates. The appearance of the latter is typical for "acute uremia of the newborn." Blood urea of these injected rats was 76 and 40 mg per 100 ml; that of the uremic control rats 287 and 240 mg per 100 ml.

syndrome together with their injected littermates is shown in Fig. 1.

The urea concentration in the blood of a few of the rats was determined 40-48 hours after birth. Others were kept to determine weaning weights. The results included in Table I show that rats with "acute uremia of the newborn" have extremely high blood urea values of about 200 mg per 100 ml, whereas normal appearing uninjected young or those injected with 0.05 μg of vitamin B_{12} have a blood urea level of 50-60 mg per 100 ml. These levels fall into the range of values observed in a more extensive series studied in this laboratory(11) which revealed an early transient rise of urea in the blood of normal appearing young rats, even in those born to, and nursing, mothers maintained on the ration described here supplemented with 3% fish solubles or with 2% Wilson's liver extract 1:20. The present data suggest that either a single dose of 0.05 μg of vitamin B_{12} is not sufficient to maintain the blood urea in the range of values found at birth or at weaning or that a transient rise may occur regardless of a vitamin B_{12} supply. The weaning weights of the survivors were not significantly changed by the single injection of vitamin B_{12}.

The observations reported here do not explain the etiology of "acute uremia of the newborn" satisfactorily. Since the condition can be prevented by an early postnatal supply of vitamin B_{12} they suggest that the milk of the females maintained on the ration described here was low in vitamin B_{12} and that the fetal stores of this factor were likewise insufficient to overcome an early postnatal stress which may be associated with the adjustment of the newborn to the sudden supply of nutrients from the alimentary tract. The rapidity of its onset and its prevention by postnatal administration of vitamin B_{12} as well as the normal appearance of the young for the first 24 hours after birth further suggest that "acute uremia of the newborn" is of metabolic origin rather than a pathologic condition established *in utero*. Extensive data accumulated during experiments on reproduction of rats maintained on the ration described here show that often within a litter a few individuals show the acute syndrome while their littermates develop normally.(12) Furthermore, while a mother is maintained continuously on the same ration one litter may develop "acute uremia of the newborn" while the next litter does not, and vice versa. The incidence of the condition does not seem to increase greatly with successive generations. It appears therefore that under the conditions prevailing in these experiments circumstances of a marginal nature determine the incidence of the syndrome. Finally it has been observed(12) that a vitamin B_{12} deficient ration *per se* does not necessarily cause "acute uremia of the newborn." In view of these complex relationships clarification of the causes and of the nature of the syndrome must await further experimental evidence.

The acute uremia in newborn pigs reported by Madsen *et al.*(13) and the "toxemic-uremic syndrome in baby pigs fed on dried skimmilk" observed by Green *et al.*(14) may be related to the syndrome in rats described

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in this paper. In view of the high early mortality observed in swine the prophylactic use of vitamin B_{12} in these conditions should be investigated.

Summary. 1. Acute uremia of the newborn rat observed on rations in which a commercial soybean protein and DL-methionine furnish the only source of amino acids can be prevented by subcutaneous administration of 0.05 μg of vitamin B_{12} shortly after birth.

The assistance of Merck and Co., Abbott Laboratories, Wilson Laboratories, Lederle Laboratories, and DuPont and Co. with various supplies is gratefully acknowledged. Mr. Harlyn O. Halvorson kindly carried out the analyses for blood urea.

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The Volume Distribution and Equilibrium Time of Para-Aminohippurate.* (17517)

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Sodium para-aminohippurate (PAH) is widely used for measurement of effective renal plasma flow and maximal tubular excretory capacity (Tm). Its volume distribution is of importance in studies dealing with rate of change in PAH plasma levels and excretion following single injections,(1-3) or with constant infusions before equilibrium has been established.(4,5) Information concerning volume distribution is also useful in calculating optimum priming doses for rapid achievement of the desired plasma level. The literature provides very few data on the PAH space. It has been stated to be 28% of body weight in the dog(6) and estimates of 4-5 liters(4) and 20 liters(5) have been made for man. We are therefore reporting its determination in man.

Methods. All patients were free of renal, cardiac, or other diseases which might be expected to alter fluid compartments. An intravenous priming dose of PAH was given over a 7-9 minute period and this was followed by a constant intravenous infusion for 1½-3 hours. In three patients the priming dose was omitted. Infusions (both priming and sustaining) were given at an extremely constant and accurately known rate by means of a motor driven worm screw pushing on the barrel of a 50 cc syringe. Urine was collected by catheter with bladder washing. The anticoagulant was heparin powder. Total PAH was determined according to Newman.(4) This method in our hands gives complete hydrolysis of acetylated PAH. Its use, in the cases reported resulted in no destruction of free PAH, as indicated by identical values in infusion solutions analyzed as free and total. The total amount of PAH excreted was subtracted from the total amount injected and this difference divided by the plasma level to arrive at the apparent volume distribution.

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