hepatic glycogen retention in amino acid deficient rats poses many problems for which there is no satisfactory explanation at this time. It is believed that the tissue alterations observed in the deficient rats were not the result of changes in water content.

The tissues of the 2 isoleucine-deficient rats which were placed on the recovery diet showed no abnormalities. Thus, the effects of the total lack of isoleucine were not permanent and were correctable by the addition of adequate dietary isoleucine.

Summary. The total lack of isoleucine in the diet of rats resulted in regression in the size of the pituitary acidophils, deletion of the pituitary gonadotrophic cells, atrophy of the testes and accessory sex glands, thymic involution, interference with somatic growth, liver glycogen accumulation and degenerative changes in skeletal muscle. Replacement of adequate isoleucine to the diet of isoleucine-deficient rats resulted in complete recovery.

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Vit. B_{12}: Correlation of Serum Concentrations and Pregnancy. (22411)

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Vit. B_{12} occupies a significant place in the nutrition of microorganisms, lower animals and man. It seems purposeful to seek clinical situations in which a deficiency of vit. B_{12} may be important, either as the sole factor or a contributing factor to the causation of the signs or symptoms which are observed. There is general agreement that a deficiency of vit. B_{12} exists in pernicious anemia (P.A.) and in this clear-cut clinical example of the deficiency state, the serum concentration of vit. B_{12} is a good index of the situation, being less than 100 $\mu\mu$g/ml and approaching zero in frank untreated P.A. It is fully recognized that there is the possibility of a relatively normal serum concentration in the presence of deficiency in the total body stores and conversely the possibility of a lowered serum concentration in the absence of a measurable total body deficiency(1). Caution in interpreting serum vit. B_{12} concentrations is further enjoined on the basis of the accumulating evidence that when patients with P.A. in remission have had their vit. B_{12} serum concentrations assayed, the values may be below 100 $\mu\mu$g/ml despite the fact that clinically the

patient is in remission(2). In view of the wide variation of vit. B_{12} serum concentrations from individual to individual when a presumably "normal" population is studied and, consequently, the probability that relatively large numbers of any "abnormal" group will have to be studied in order for a trend to achieve statistical significance, we have chosen to employ the vit. B_{12} serum concentration as the single criterion of potential deficiency in order to accelerate the "screening" of large numbers of people. It is hoped that the identification of populations found to be "abnormal" with respect to vit. B_{12} serum concentrations will contribute to the further investigation of these states and the establishment of whether the low serum vit. B_{12} is a direct measure of deficiency or of some other conditioning factor.

As a preliminary to studies of the type reported here, we have tried to ascertain something of the degree of variation between "normals" as well as the absolute values obtained. It was found and previously reported(3) that in a group of 528 individuals values below 200 $\mu\mu$g/ml or above 1000 $\mu\mu$g/ml were uncommon. The average was 560 $\mu\mu$g/ml with 95% confidence bands at 70 and 1,060 $\mu\mu$g/ml. By personal communication and subsequent publication(4) we learned of a tendency to lowered serum concentrations of vit. B_{12} having been observed during pregnancy in patients studied in Germany. Because of the possibility of therapeutic or prophylactic implications of such an observation, if this were related to the pregnant state independent of dietary or other variables, we were interested to attempt to corroborate and extend these data in the United States.

Methods and materials. Methods for the determination of vit. B_{12} where a titrimetric response is employed, such as the *L. leichmannii* method(5) will allow the direct assay of turbid and colored solutions. Previous studies have indicated that, whereas vit. B_{12} added to serum prior to "clarification" could not be recovered quantitatively, dilution without clarification did not affect recovery. Accordingly, we have employed the method previously described(4) of a microbiological assay with *L. leichmannii* and a dilution technic, which we believe represents "total serum vit. B_{12}." Patients from whom blood specimens were obtained were seen at one of two hospitals, namely, Abington Memorial Hospital (Hospital A) and Chestnut Hill Hospital (Hospital B). The method of handling specimens was essentially the same at each of these hospitals. Approximately 10 ml of blood were drawn from an antecubital vein and placed in a test tube which was allowed to stand in the ice box while clotting occurred and the serum separated. Later the same day the serum was transferred to a sterile 10 ml rubber-stoppered specimen vial and held in the deep freeze at –10°C until submission for assay. Specimens were coded at the time of freezing and decoded after the assays were reported. Beyond the routine dietary guidance given to pregnant patients, no attempt was made to regulate the diet or the vitamin supplementation of it in any of these patients. We recognized the vagaries of appetite of pregnant women as well as the sheer impossibility of rigidly controlling the intakes of so large a group of ambulatory individuals. Many of these patients received supplementary polyvitamin preparations by mouth, however, any patient who, in answer to questioning, indicated that she was receiving vit. B_{12} by injection, was excluded from the study. It was our feeling that vitamins by intramuscular injection represented a sufficient departure from the "average" of the middle class group of female patients who were followed in this study that inclusion of such patients would not be consonant with the objective of this study. None of these patients was toxemic at the time of blood collection. It was hoped that by sampling such a large group of women, the influence of variations in dietary intake and age would be minimized, and the duration of pregnancy became the only significant variable in terms of serum vit. B_{12}.

Results. The designation "days prior to delivery" represents the difference between the expected date of confinement (EDC), as calculated by the obstetrician, and the date of blood collection, with the exception of those

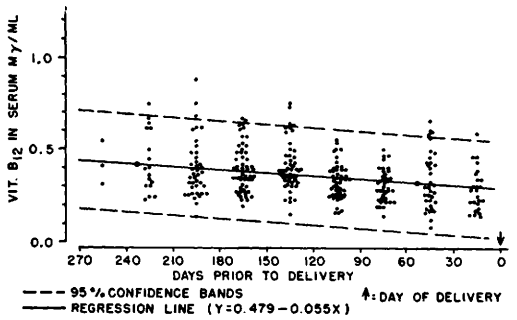

FIG. 1. Serum concentration of vitamin B_{12} as a function of duration of pregnancy in hospital A (318 females).

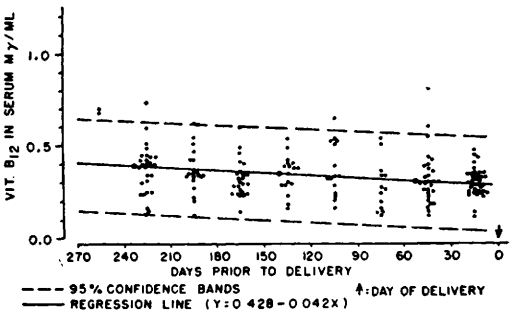

FIG. 2. Serum concentration of vitamin B_{12} as a function of duration of pregnancy in hospital B (184 females).

patients on whom blood was drawn at the time of delivery. Because of the considerable error involved in calculating the EDC, it seemed to us deceptively accurate to attempt to analyze the data according to the specific day of pregnancy. Accordingly, the individual values have been graphed according to 30-day periods and the statistical evaluation has been done according to 90-day periods.

Hospital A. Fig. 1 presents the individual serum concentrations and demonstrates the decline of the average value for serum vit. B_{12} in successive periods as the pregnancy approached term. Table I below presents the essential data from which the regression equation $Y = 0.479 - 0.055x$ was calculated where x is the period (1, 2 or 3). The slope, –0.055 mγ/ml per period is highly significant statistically ($P<0.001$).

Hospital B. Fig. 2 presents individual values and averages for 90-day periods and shows a decline of serum vit. B_{12} concentrations with duration of pregnancy similar to that seen in Hospital A. Table II below presents the essential data from which the regression equation $Y = 0.428 - 0.042x$ was calculated. The slope, –0.042 mγ/ml per period is highly significant statistically ($P< 0.001$).

Discussion. The objective of this investigation was to correlate serum vit. B_{12} concentrations with duration of pregnancy. It seems evident from these results that a significantly lower serum vit. B_{12} level has been observed in the later stages of pregnancy. The lack of serum vit. B_{12} values within the "deficiency" range (under 100 μμg/ml) is noteworthy. It is also of interest that the average serum B_{12} values for this entire group, even in the first trimester of pregnancy, was lower than the average established for "normal" males and females(3).

Many auxiliary determinations which might elucidate the mechanism involved were considered at the inception of the study but were deferred, because the extreme variation in serum vit. B_{12} concentration from individual to individual had been established and made

TABLE I. Summary of Data on 318 Pregnant Women in Hospital A.

	Period and days to delivery		
	1 (181–270)	2 (91–180)	3 (0–90)
No. of pregnant women	61	157	100
Avg B_{12} blood level (mγ/ml)	.417	.377	.311
Expected or calculated level	.424	.369	.314
95% confidence limits for line of regression	.162–.686	.109–.629	.053–.575

TABLE II. Summary of Data on 184 Pregnant Women in Hospital B.

	Period and days to delivery		
	1 (181–270)	2 (91–180)	3 (0–90)
No. of pregnant women	51	57	76
Avg B_{12} blood level (mγ/ml)	.384	.347	.300
Expected or calculated level	.386	.344	.302
95% confidence limits for line of regression	.133–.639	.093–.595	.050–.554

it likely that large numbers of patients would have to be studied. It seemed purposeful to confirm the existence of a lowered serum vit. B_{12} concentration in the pregnant state before more elaborate studies were undertaken.

It is well known that fluid retention and an expansion of plasma volume may occur in the later stages of pregnancy(6). It is possible that this may account for part or all of the decrease in the serum vit. B_{12} concentration which has been observed. It would seem necessary, however, to test the hypothesis that serum vit. B_{12} binding capacity might keep pace with hemodilution before concluding that hemodilution is the responsible factor. On the other hand, the increased requirement for many other vitamins of women during pregnancy is well established(7) and repeated pregnancies on a vit. B_{12} deficient diet have been the most effective way of inducing vit. B_{12} deficiency in animals(8).

The lowered serum vit. B_{12} concentrations that have been observed in this group of 502 pregnant women merits further study in order to ascertain the mechanisms involved.

Summary. 1. Serum vit. B_{12} estimations were made on sera obtained from 502 women after varying durations of pregnancy. 2. This work has confirmed previous suggestions that lower than normal serum vit. B_{12} concentrations are observed during pregnancy. It has also demonstrated a direct and statistically significant ($P<0.001$) correlation between serum level of vit. B_{12} and duration of pregnancy.

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Effect of Local Anesthetics on Motility of Upper Gastrointestinal Tract.* (22412)

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Radiologists have reported that the intragastric administration of local anesthetic drugs results in relaxation of the pyloric sphincter. Bayer(1) gave peptic ulcer patients a 0.25% solution of larocaine orally. This was followed by relaxation of the previously closed pylorus and ready fluoroscopic visualization of the duodenum by contrast material passing from the stomach. Roka and Lajtha(2) used 100 cc of 1% solution of procaine in the same fashion and likewise demonstrated the opening of the pylorus by

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