literature regarding endogenous ACTH titers in the blood. However, it is certain that the concentration of ACTH in blood is very low, even under stressful circumstances. In this connection the authors would like to suggest to clinical investigators that the bioassay of kidney rather than of blood for ACTH may provide a useful tool for elucidating the etiology of adrenocortical dysfunction. For example, kidney biopsy could be performed during the course of an exploratory laparotomy for the purpose of determining the status of the adrenals. In hypercorticism with primary involvement of the adrenals, one would expect to find a lower than normal concentration of ACTH in the kidney because of suppression of ACTH release by the high concentration of cortical hormone in the body fluids. In contrast, in hypercorticism secondary to pituitary over-activity, one would expect to find a higher than normal concentration of ACTH in the kidney if it proves to be true that the renal material is of pituitary origin.

Summary. Rat ACTH is a dose equivalent to 100 μg La-1-A per 100 g body weight, injected intravenously into rats, disappears rapidly from the circulating blood of intact and adrenalectomized rats. Five minutes after injection of the hormone into intact rats about 40% of the injected dose is present in extracellular fluid and 20% in the kidneys; after 15 minutes a negligible quantity is present in extracellular fluid and 15% in the kidneys. The adrenals have a relatively high concentration of the hormone although the concentration is lower than in the kidney. It is estimated that less than 1/500th and 1/2000th of the injected dose is present in the adrenals at 5 and 15 minutes after injection, respectively. Liver contains no detectable quantity of the injected ACTH either 5 or 15 minutes after injection of hormone. Injected ACTH could not be detected in the urine in these experiments.

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Observations on a Relationship Between Vitamin B_{12}, Folic Acid and the Citrovorum Factor.* (18688)

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The presence of a growth factor for *Leuconostoc citrovorum* 8081, present in natural products was reported by Sauberlich and Baumann(1). That this factor is closely related to folic acid (PGA) has been established beyond all reasonable doubt. It has been shown that *Leuconostoc citrovorum* factor (LCF) possesses *Streptococcus faecalis* R activity(2,3). Broquist *et al.*(3) have reported that LCF may replace the PGA requirement of the chick. Drysdale *et al.*(4) have observed that the administration of vit. B_{12} enhances the urinary excretion of LCF in

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TABLE I.
Effect of Different Levels of Vitamin B_{12} on Liver Storage of PGA in the PGA-deficient Chick.*

Treatment†	PGA‡ γ/g fresh wt	PGA‡ Storage§ equivalent
None	.88	3.26 ± .22
Vit. B_{12} (.1 γ/day)	.92	3.90 ± .25
(.3 ")	1.41	4.08 ± .08
(.5 ")	.85	2.85 ± .44
(1.0 ")	.78	2.32 ± .18
(.3 " orally)	1.72	4.45 ± .28

* 6 observations per group, including the standard error $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$.
† Injected unless otherwise stated.
‡ Total *S. faecalis* activity.
§ Storage equivalent = γ per liver per 100 g of body weight.

the hyperthyroid rat. In this paper we wish to present certain observations pertaining to the interrelationship of vit. B_{12}, PGA, and LCF.

Experimental. Straight run (New Hampshire ♂♂ X Single Comb White Leghorns ♀♀) crossbred chicks, the progeny of hens fed diet B-1(5) were used in all studies. The chicks were housed in electrically heated batteries with raised screen floors. Feed and water were supplied *ad libitum*. The chicks were wing banded and weighed at one day of age. Weights were recorded at weekly intervals. The experimental procedure for Exp. 1 (Table I) was as follows: All birds were placed on a PGA-deficient ration containing sucrose 61 g, alcohol extracted casein 18 g, gelatin 10 g, salts V(6) 6 g, soybean oil 5 g, L-cystine 0.3 g, thiamine hydrochloride 0.3 mg, riboflavin 0.6 mg, nicotinic acid 5.0 mg, pyridoxine hydrochloride 0.4 mg, calcium pantothenate 2.0 mg, choline hydrochloride 150 mg, biotin 0.03 mg, inositol 100 mg, 2-methyl-1,4-naphthoquinone 0.05 mg, and α-tocopherol 0.3 mg. Fortified haliver oil (60,000 U.S.P. units of vit. A, 6000 U.S.P. units of vit. D_3 per g) was given by dropper (2 drops per bird per week). After the chicks had been on this PGA-deficient ration for a 2-week period, the different vit. B_{12} supplements shown in Table I were administered. In Exp. 2 (Table II) all birds were placed on the basal ration containing 200 γ % PGA at 1 day of age. Supplementation was started immediately and continued for 4 weeks. Exp. 3 (Table III) was similar to Exp. 1, except that the supplements were given for the entire 4-week period.

In all cases, 12 chicks were used per group. Crystalline vit. B_{12} was injected into the pectoral muscle using a 1 ml calibrated syringe. Oral administrations were given by pipette. At the termination of the test period, the birds were sacrificed, and the livers removed, frozen immediately, and stored at freezing temperatures until assayed. PGA and vit. B_{12} were extracted and determined by methods previously described(7). The LCF activity was determined on the PGA extract using the medium of Sauberlich and Baumann(1). The procedure used for measuring the conversion of PGA to LCF was similar to that of Nichol *et al.*(8). Liver slices were prepared, using the method of Deutch(9). Samples of 0.5 g,

TABLE II. Effect of Vit. B_{12} on Storage of PGA, Vit. B_{12} and LCF in the Liver of Chicks Receiving Ample PGA.*

Treatment	Vit. B_{12} γ/g	PGA† γ/g	LCF γ/g	LCF / PGA
None	.17 ± .03	2.26 ± .14	1.67 ± .15	.74
Vit. B_{12} (.5 γ/day inj.)	.29 ± .01	.44 ± .02	1.59 ± .52	3.62
Vit. B_{12} (.5 γ/day orally)	.26 ± .05	4.72 ± .50	2.60 ± .51	.55

* 6 observations per group including the standard error $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$.
All values are on a fresh weight basis.
† Corrected PGA value—observed values minus *S. faecalis* R activity due to the LCF present.

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TABLE III. Effect of Vit. B_{12} on Conversion of PGA to LCF by Liver Slices of PGA-Vit. B_{12} Deficient Chicks.*

Treatment	Vit. B_{12}, γ/g	γ LCF per g liver slices fresh wt, present after incubation	
		PGA, 5 γ/flask	PGA, 5 γ/flask + ascorbic acid, 10 mg/flask
None	.01 ± .01	.54 ± .16	1.35 ± .06
Vit. B_{12}, 1.0 γ/day, inj.	1.02 ± .42	.15 ± .09	1.06 ± .35
Vit. B_{12}, 1.0 γ/day, orally	.83 ± .26	1.01 ± .29	1.84 ± .31

* 5 observations per group, including the standard error $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$.

weighed to the nearest mg, were used. In all cases freshly neutralized ascorbic acid was added immediately before the flasks (25 ml Erlenmeyers) were agitated in a constant temperature bath, at 37°C for 2 hours. Duplicate determinations were made in all cases.

Results and discussion. The injection of vit. B_{12} at a level of 0.1 γ or 0.3 γ per day (Table I) increased the PGA stored in the liver, in accordance with previous results(7). However, when the level of vit. B_{12} injected was increased to 0.5 γ per day a depression in the PGA liver storage resulted. The injection of 1.0 γ of vit. B_{12} per day produced a still greater depression of the liver PGA. The oral administration of vit. B_{12} at a level of 0.3 γ per day, produced results similar to those obtained by the injection of 0.3 γ of vit. B_{12} per day.

The second experiment (Table II) differed in that the vit. B_{12} deficient chicks received ample PGA (200 γ %). Under these conditions the injection of 0.5 γ of vit. B_{12} per day resulted in a definite depression of the corrected PGA stored in the liver while no change in the LCF was observed. On the other hand, the oral administration of 0.5 γ of vit. B_{12} per day produced a marked increase of both the LCF and PGA levels as measured by liver storage. The oral administration of 0.5 γ of vit. B_{12} per day produced liver stores of vit. B_{12} slightly lower than those obtained by the injection of 0.5 γ of vit. B_{12} per day. It is of interest to note that although there was no increase in LCF upon the injection of vit. B_{12}, the ratio of LCF to PGA was increased about 6 fold over that of the control group and the group receiving orally administered vit. B_{12}. This indicates that the injected vit. B_{12} displaced the equilibrium between LCF and PGA in favor of LCF. In the case of the oral administration of vit. B_{12} no change in the LCF-PGA ratio was observed. This shows that although there was an increase in both LCF and PGA levels, there was no disruption in the LCF-PGA equilibrium, as in the case of the injected vit. B_{12}.

In Exp. 3, as in Exp. 1, PGA-vit. B_{12} deficient chicks were employed. The administration of vit. B_{12} at the level of 1.0 γ per day produced results similar to those observed in Exp. 2, *i.e.*, the liver levels of vit. B_{12} were similar regardless of the route of administration. Furthermore, the injection of vit. B_{12} resulted in a significant lowering of the liver tissue level of PGA, while the LCF level remained unchanged. The oral administration of vit. B_{12} increased the liver levels of both PGA and LCF. Liver slice studies (Table III) were made on the same birds. The administration of vit. B_{12} by injection significantly decreased the amount of PGA converted to LCF. The addition of ascorbic acid overcame this inhibition. The oral administration of vit. B_{12} significantly increased the amount of PGA converted to LCF either with or in the absence of ascorbic acid.

These data demonstrate that vit. B_{12} plays some role in the conversion of PGA to LCF. Whether this action of vit. B_{12} is that of a specific vitamin action or an indirect action is unknown. The problem is complicated by the action of ascorbic acid, the intricacies of intestinal synthesis and the fact that there is

a significant difference in the effect of vit. B_{12} when fed or injected. The action of ascorbic acid does not appear to be specific, since it can be replaced by glucoascorbic acid(8). It must furthermore be remembered that the natural means of receiving nutrients is by absorption from the alimentary canal. It is also well known that the intrinsic factor(9) has a definite effect on the utilization of vit. B_{12}. With these facts in mind, it is possible that the vit. B_{12} absorbed from the tract is altered in such a manner that it may then act as a precursor to a coenzyme participating in an enzyme system which converts PGA to LCF. If this is the case, it is possible that the injected vit. B_{12} is similar enough to the coenzyme to compete for the apoenzyme, thus producing the inhibition noted. However, the effect of ascorbic acid in overcoming this inhibition is more obscure.

Summary. Vit. B_{12} injected at low levels increased the PGA stored in chick liver tissue. Higher levels of injected vit. B_{12} suppressed the liver storage of PGA. Vit. B_{12} administered orally, increased the level of both PGA and LCF as measured by chick liver storage.

Vit. B_{12} injected at the level of 1 γ per day significantly inhibited the conversion of PGA to LCF in PGA-vit. B_{12} deficient chicks. This inhibition was overcome by the *in vitro* addition of ascorbic acid. The oral administration of vit. B_{12} at the level of 1 γ per day significantly increased the conversion of PGA to LCF with or in the absence of ascorbic acid, in the PGA-vit. B_{12} deficient chick.

The possible relationships and interactions of vit. B_{12}, PGA, LCF, and ascorbic acid have been discussed.

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Essential Amino Acids in Mature Human Milk.* (18689)

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The amounts of the essential amino acids in colostrum and transitional milk from healthy nursing mothers were given in a preceding paper(1). This report presents the values for the essential amino acids in whole human milk as consumed by infants of the same group of mothers after lactation was established.

From 61 healthy mothers (47 white, 14 Negro) who were successfully nursing their infants, 147 samples of milk were collected at intervals ranging from 15 to 362 days postpartum. The mothers were 18 to 42 years old and resided in the Detroit area. Five women receiving known diets varying in quantity according to size and appetite but not in quality, provided 18 samples, each sample being a composite of all milk obtained by completely emptying both breasts at regular intervals during 24 hours. Fifty-six women whose diets were self-chosen provided 129 samples, each consisting of the entire amount of milk obtainable from one or both breasts, 4-8 hours after nursing. Samples were usually collected at the mid-morning feeding period, but in some instances were not obtained until early afternoon. Preceding papers have described in detail the method of manual expression used in the collection of samples(2,3), the preparation and storage of samples(4,5),

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