tered only in very low dilutions, and, quantitatively, the amounts of rat serum protein appearing (calculated by difference) are within the order of magnitude for normal protein excretion.

The data presented here cannot answer whether rat serum protein is excreted along with bovine albumin as the result of diminished tubular reabsorption or the result of increased glomerular permeability or filtration rate. Such information might be obtainable from further investigation under different conditions, varying the rates of protein excretion over a wider range. However, the data given do demonstrate a gross difference in the mechanism of proteinuria following administration of bovine albumin and egg albumin, proteins that differ widely in structure. This difference is consistent with differences found in the effect produced by intraperitoneal injections of these proteins upon hemoglobin excretion in the rat,[2,3] and is of special significance in that the data given here were obtained by an independent experimental approach.

Summary. 1. Parenteral injection of egg albumin in the rat produces proteinuria composed of egg albumin, with no appreciable excretion of rat serum protein.

2. After parenteral injection of bovine albumin, large quantities of rat serum protein are excreted in addition to bovine albumin.

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17252. Some Observations on Growth Factors Required by *Leuconostoc citrovorum*.*

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The presence of a growth factor for *Leuconostoc citrovorum* 8081 in various natural materials was reported by Sauberlich and Baumann.[1] They noted that the organism would respond to thymidine but it was concluded that some other active factor was present in a liver concentrate. The "citrovorum factor" in liver extract was differentiated[2] from a factor active for *Lactobacillus leichmannii* in liver extract by observing that the two factors migrated in opposite directions in an electric field. In the present report, further observations of the characteristics of the "citrovorum factor" are described.

Experimental. Lactobacillus leichmannii 313 (ATCC 7830) and *Leuconostoc citrovorum* 8081 were used in this study. Methods for the use of *Lactobacillus leichmannii* have been previously described;[3-5] the assay technic with *Leuconostoc citrovorum* followed that of Sauberlich and Baumann.[1]

The data of Table I indicate the response of the test organism to concentrated liver extract (15 U.S.P. units per cc), vitamin B_{12} and thymidine before and after heating with alkali. *Lactobacillus leichmannii* gave heavy growth with only 0.03 μl of untreated liver extract, but after treatment with alkali much higher levels of liver extract were required to promote good growth of the organism. As noted elsewhere this alkali treatment destroys the growth-promoting action of vitamin B_{12}

* We are indebted to Dr. J. O. Lampen for thymidine, to Dr. E. E. Snell for hypoxanthine desoxyriboside, and to Dr. E. Hoff-Jorgensen for guanine desoxyriboside.

1 Sauberlich, H. E., and Baumann, C. A., *J. Biol. Chem.*, 1948, **176**, 165.

2 Lyman, C. M., and Prescott, J. M., *J. Biol. Chem.*, 1949, **178**, 523.

3 Snell, E. E., Kitay, E., and McNutt, W. S., *J. Biol. Chem.*, 1948, **175**, 473.

4 Hoffmann, C. E., Stokstad, E. L. R., Franklin, A. L., and Jukes, T. H., *J. Biol. Chem.*, 1948, **176**, 1465.

5 Stokstad, E. L. R., Dornbush, A. C., Franklin, A. L., Hoffmann, C. E., Hutchings, B. L., and Jukes, T. H., *Fed. Proc.*, 1949, **8**, 257.

TABLE I.
Effect of Alkali on Growth Promoting Action of Concentrated Liver Extract, 15 U.S.P. Units per cc. for *Lactobacillus leichmannii* and *Leuconostoc citrovorum*.

Additions to 2 ml medium	Optical density*			
	Lactobacillus leichmannii		*Leuconostoc citrovorum*	
	(a)	(b)	(a)	(b)
None	0.28	0.28	0.03	0.03
0.03 μl liver extract	1.15	0.46	0.08	0.08
0.1 " " "	1.33	0.66	0.20	0.19
0.3 " " "	1.32	0.90	0.42	0.42
1.0 " " "	1.39	1.25	0.94	0.92
3.0 " " "	1.40	1.42	1.45	1.50
10.0 " " "	1.50	1.50	1.90	1.90
10 mγ vitamin B_{12}	1.40	0.40	0.03	0.03
10 γ thymidine	1.20	1.00	0.32	0.34

* Determined after 20 hr incubation.
(a) Untreated supplements.
(b) Supplements steamed for 30 min. with 0.2 N NaOH.

for *L. leichmannii*, although thymidine appears to be resistant.[6] The small residual effect of liver extract for *L. leichmannii* after alkali treatment is presumably due to thymidine and other desoxyribosides. In contrast to the results with *L. leichmannii*, the response of *Leuconostoc citrovorum* 8081 to liver extract was unchanged following treatment with alkali and it was also noted that *L. citrovorum* did not respond to vitamin B_{12}. In accord with the results of Sauberlich and Baumann[1] it was found (Table I) that *L. citrovorum* gave only a partial growth response with thymidine as compared to a marked response with liver extract, thus supporting the view that there is an unknown "citrovorum factor", in addition to thymidine, in liver extract. The data of Table I demonstrate that this factor is not identical with the factor required by *Lactobacillus leichmannii*, and show that the "citrovorum factor" is stable to steaming with 0.2 N NaOH for 30 minutes. Lyman and Prescott[7] have noted that the "citrovorum factor" is stable to alkali.

The report that high levels of pteroylglutamic acid (PGA) could in the presence of purine bases replace the "citrovorum factor,"[8] and the finding that 4-amino pteroylglutamic acid, a PGA antagonist, produces an inhibition of the growth of *L. citrovorum* which is reversed by natural materials containing the "citrovorum factor,"[8] prompted further study of the role of PGA in the nutrition of *L. citrovorum*. The data of Table II show that although after 36 hours incubation thymidine or PGA when assayed singly can only partially replace the factor required by this organism, the simultaneous addition of thymidine and PGA to the medium resulted in marked growth after only 18 hours incubation. These results give additional indication of a relationship between PGA, thymidine and the "citrovorum factor." In other experiments, it was found that the addition of 10 γ of vitamin B_{12} per 2 ml medium to tubes containing thymidine and PGA had no additional effect on growth. The addition of *p*-aminobenzoic acid, pteroyltriglutamic acid, pteroic acid or xanthopterin to tubes containing thymidine (Table II) in no instance gave the marked effect on growth produced by the mixture of thymidine and PGA. It was also noted that after a 12-hour incubation period the response of the organism to liver extract was much greater than the response to thymidine *plus* PGA. This finding suggests that the pre-formed factor was present in liver extract, but that in the tubes containing thymidine and PGA some additional transformation must have occurred. However, it is apparent that the presence of thymidine and PGA in natural materials could have a marked

[6] Hoffmann, C. E., Stokstad, E. L. R., Hutchings, B. L., Dornbush, A. C., and Jukes, T. H., *J. Biol. Chem.*, in press.

[7] Lyman, C. L., and Prescott, J. M., *Fed. Proc.*, 1949, **8**, 220.

[8] Sauberlich, H. E., *Fed. Proc.*, 1949, **8**, 247.

TABLE II.
Relationships Between Thymidine, Pteroylglutamic Acid, and Related Compounds for Growth of *Leuconostoc citrovorum*.

Additions to 2 ml medium	Optical density after incubation time of 12 hr	18 hr	36 hr
None	0.04	0.04	0.06
10 γ thymidine	0.08	0.17	0.98
10 γ pteroylglutamic acid (PGA)*	0.04	0.04	0.38
10 γ thymidine + 10 γ PGA	0.40	1.40	1.70
10 γ thymidine + 10 γ p-aminobenzoic acid	0.04	0.15	0.94
10 γ " + 10 γ pteroyltriglutamic acid	0.04	0.20	1.40
10 γ " + 10 γ pteroic acid	0.04	0.15	1.02
10 γ " + 10 γ xanthopterin	0.04	0.15	0.96
10 μl liver extract	1.10	1.80	1.80

* Purified PGA containing at least 98.8% PGA (moisture free basis) was used in these experiments.

TABLE III.
Determination of R_f Values of Fractions of a Liver Extract, 15 Units per cc Separated by Paper Chromatography Using *Lactobacillus leichmannii* and *Leuconostoc citrovorum* as Indicators.

Substance chromatographed	Zones	R_f values of growth factors: *Lactobacillus leichmannii*	*Leuconostoc citrovorum*
Liver extract	1	0.05	—
	2	0.45	—
	3	—	0.55
	4	0.60	0.64
	5	0.72	—
Vitamin B_{12}	1	0.07	—
Thymidine	1	0.63	0.64
Mixture of guanine desoxyriboside and hypoxanthine desoxyriboside	1	0.42	—

effect on the response of *L. citrovorum*.

Liver extract was separated into a number of fractions by paper-strip chromatography following a technic similar to that of Winsten and Eigen.[9] A mixture of 9 parts n-butanol: 1 part acetic acid was used as the mobile phase. The strip chromatograms were laid on a nutrient agar suitable for the growth of the test organisms, the agar was seeded with the appropriate organism, and incubated. The zones of growth indicated the positions of the growth factors present in the liver extract. Chromatograms of known compounds gave R_f values of aid in identifying the fractions separating in liver extract. Table III summarizes the R_f values found on chromatographing liver extract using *Lactobacillus leichmannii* and *Leuconostoc citrovorum* as test organisms. In accord with recent investigations[4,10,11] *L. leichmannii* responded to fractions in liver corresponding to vitamin B_{12} (R_f 0.05), guanine and hypoxanthine desoxyribosides (R_f 0.45), thymidine R_f 0.60), and an as yet unidentified component (R_f 0.72). Of great interest in the present investigation was the finding that *L. citrovorum* not only responded to thymidine (R_f 0.64) but also to another component presumably the "citrovorum factor" (R_f 0.55) in the liver extract. No zones of growth of *L. citrovorum* were observed in the positions on the chromatogram corresponding to vitamin

[9] Winsten, W. A., and Eigen, E., *J. Biol. Chem.*, 1949, **177**, 989.

[10] Kitay, E., Snell, E. E., and McNutt, W. S., *J. Biol. Chem.*, 1949, **177**, 993.

[11] Hoff-Jorgensen, E., *J. Biol. Chem.*, 1949, **178**, 525.

B_{12} and the desoxyribosides of guanine and hypoxanthine. These observations are in full accord with direct growth experiments (Table I).[10] *L. leichmannii* showed no zone of growth on the chromatogram in the position where the "citrovorum factor" was located. Thus the data of Table III provide additional evidence for the separate identities of the factors required by *L. leichmannii* and *L. citrovorum* and demonstrate the existence in liver of an unknown component, not identical with thymidine, that promotes the growth of *L. citrovorum*.

Liver extract, treated with alkali as described in Table I gave results in a paper strip chromatogram identical with those shown in Table III except that vitamin B_{12} was destroyed as indicated by lack of growth of *L. leichmannii* in the region appropriate to vitamin B_{12}.

Summary. 1. *Leuconostoc citrovorum* was found to respond to a growth-promoting factor in the concentrated liver extract, but this organism did not respond to vitamin B_{12}. The alkali-stable nature of the "citrovorum factor" further contrasts it with vitamin B_{12}. 2. Two fractions were separated from liver extract by paper strip chromatography; one of these fractions was presumably thymidine and promoted growth of *Lactobacillus leichmannii* and *Leuconostoc citrovorum*. The other fraction was inactive for *L. leichmannii* but active for *L. citrovorum*.

3. Although thymidine or high levels of pteroylglutamic acid (PGA) when tested singly were only partially effective in promoting growth of *L. citrovorum*, the simultaneous addition of thymidine plus PGA produced marked growth of the organism. This finding suggests a functional relationship between thymidine, PGA and the "citrovorum factor."

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17253. The Lipotropic Effect of Estrogenic Hormones in Inbred Rats.*

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It has been shown[1,2] that estrogenic hormones exert in rats distinct lipotropic activity. In particular, estrogenic compounds (estrone, estradiol benzoate, ethinyl estradiol) allow a more efficient use of methionine as a lipotropic agent. In these studies, various possible sources of error were taken in consideration, such as difference in food intake, weight, sex and genetic identity. Further, it has been found necessary to run control and treated rats simultaneously in all experiments. This latter precautionary measure was prompted by the observation that experimental groups when not run simultaneously often differed in their absolute response even under identical experimental conditions.

With the exception of a few experiments in which unidentified strains obtained from a local dealer were employed, the studies were carried out on rats of the Sprague-Dawley strain.[1,2] In view of the observation that various inbred strains may vary more than 500% in their ability to inactivate estrogen[3] it became necessary to extend the observations on the lipotropic activity of estrogens to other strains beyond those used in previous studies,[1,2] especially to inbred strains, such as the Fischer strain, which shows "both a high threshold for vaginal estrus and an im-

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1 György, P., Rose, C. S., and Shipley, R. A., *Arch. Biochem.*, 1947, **12**, 125.

2 György, P., Rose, C. S., and Shipley, R. A., *Arch. Biochem.*, 1949, **22**, 108.

3 Segaloff, A., and Dunning, W. F., *Endocrinology*, 1946, **39**, 289.