TABLE I. Phosphopyruvic Acid Formation by Tissue Homogenates of Male and Female Rats. (Results expressed in terms of phosphopyruvic acid formed by 1 mg tissue during 15' incubation).

	Phosphopyruvic acid					
	Male		Female			
Organ	Mean	S.E.	Mean	S.E.	% difference	"t" values
Leg muscle	418.0	± 19.80	258.8	± 14.60	—38	6.5
Heart muscle	174.8	± 24.70	100.1	± 16.30	—43	8.55
Kidney	162.8	± 17.6	72.7	± 7.50	—55.5	4.72
Liver	58.1	± 2.24	36.36	± 3.38	—37.5	5.36
Brain	68.7	± 5.55	44.17	± 2.68	—35.7	3.96
Spleen	50.8	± 7.00	31.72	± 2.78	—37.6	6.35
Thyroid	72.4	± 4.45	49.57	± 2.00	—31.4	4.7
Adrenal	54.3	± 9.53	38.92	± 6.20	(—28.4)	1.35
Testis	45.3	± 6.75		—		
Ovary		—	91.0	± 10.5		
Uterus		—	78.0	± 5.7		

S.E. = Standard error.

5% homogenates (0.1 ml) were used for enzyme assays of liver, brain, spleen, thyroid, adrenal, testis, and ovary; 1% homogenates (0.1 ml) for leg and heart muscle, kidney, and uterus.

concerned with storage of readily available energy in the form of energy-rich phosphate bonds. The marked differences found between male and female organs suggests that this energetically important phase of glycolysis might be regulated by hormones. Further data on the effect of endocrine organs on this enzyme reaction will be published in a subsequent paper.

Summary. It was shown that dilute homogenates of rat organs catalyze *in vitro* the conversion of 3-phosphoglycerate to phosphoenolpyruvate as measured by the formation of iodine labile phosphorus. A marked sex difference was observed between the rates of phosphopyruvate formation by male and female organs.

The valuable assistance of Mr. Donald R. McCurley, who carried out part of the enzyme assays, is gratefully appreciated.

13. Bucher, T., *Biochem. et Biophysica Acta,* 1947, v1, 292.

14. Lipmann, F., *Adv. Enzymol.,* 1941, v1, 99.

Received July 21, 1950. P.S.E.B.M., 1950, v75.

Properties of the Methanol Soluble Factor Required by the Mink.* (18104)

S. B. TOVE,† R. J. LALOR, AND C. A. ELVEHJEM

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Previous work has shown that mink given a purified diet require a heat stable methanol soluble liver factor, and preliminary results indicated that a methanol insoluble (residue) factor was also required(1). The methanol soluble factor was found in liver, whole milk and whey, but not in tomatoes, alfalfa or yeast. Similar results have been obtained with the fox(2). Further studies on these

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were supported by Project 614 of the Wisconsin Agricultural Experiment Station and grants from the Borden Co., New York, New York and Fur Farm Food and Supplies, St. Paul, Minn.

† Present address, Department of Animal Industry, North Carolina State College, Raleigh, N. C.

1. Schaefer, A. E., Tove, S. B., Whitehair, C. K., and Elvehjem, C. A., *J. Nutr.,* 1948, v35, 157.

TABLE I. Effect of Supplements to Basal Ration on Body Weight of Mink.

	Before supplement			After supplement		
Mink No.	Ration	Total wk on exp.	Body wt, g	Supplement	Total wk on exp.	Body wt, g
85	Basal	34	575	2% EtOH ext. of fish solubles	39	870
110	"	17	1055	4% $(NH_4)_2SO_4$ ppt of fish solubles	21	1260
111	"	23	1030	2% BuOH insoluble portion of fish solubles	26	1175
99	"	11	425	2% Acetone insoluble portion of MeOH ext.	19	710
142	" + 4% residue	5	595	4% $(NH_4)_2SO_4$ ppt of MeOH ext.	8	900
Avg for 117 140 143	" + 6% yeast + 8% residue	25	935	1% Phenol ext.	31	1230
Avg for 205 235 199 203*	Basal	24	1060	10-20 μg vitamin B_{12}/day	29	1290
Avg for 155 107 178 183	Basal + 1% MeOH ext.	36	905	4% Residue	50	1320

* High casein basal diet.

two factors are presented in this report.

Experimental and results. The mink were given the same basal ration used in the earlier work(3), with the exception that pteroylglutamic acid was included in the diet at a level of 100 μg per 100 g ration. The results obtained when various supplements were added to the basal ration are presented in Table I. It was found that the methanol extract factor was also present in fish solubles but not in 4% dried distillers solubles, dried whey or 2% desiccated hog intestinal mucosa. Fish solubles had been shown to contain a growth factor for chicks(4). Since the methanol extract factor is not present in dried distillers solubles it is unlikely that this factor is identical with vitamin B_{13}(5).

The chemical and physical properties of the methanol extract factor were studied further. An acetone precipitate of the methanol extract was found to be active. The methanol extract factor is insoluble in 3/4 saturated ammonium sulfate. Since an ammonium sulfate preparation was inactive at a 2% level, it would appear that at least 50% of the activity is lost by this procedure. A butanol soluble fraction obtained by continuous extraction of an 80% ethanol extract of fish solubles did not show activity. However, the butanol insoluble portion of a similar extract (extracted at pH 3 and 7) did produce a body weight response.

Phenol extraction of a methanol extract was accomplished in the following manner. Equal portions of the methanol extract and 90% phenol were shaken in a separatory funnel, the phenol layer removed, an equal volume of water added, and the phenol removed by

2. Schaefer, A. E., Whitehair, C. K., and Elvehjem, C A., *J. Nutr.*, 1948, v35, 147.

3. Tove, S. B., Schaefer, A. E., and Elvehjem, C. A., *J. Nutr.*, 1949, v38, 469.

4. Robblee, A. R., Nichol, C. A., Cravens, W. W., and Elvehjem, C. A., *Poultry Sci.*, 1947, v27, 442.

5. Novak, A. F., and Hague, S. M., *J. Biol. Chem.*, 1948, v174, 647.

ether extraction. This preparation proved as active as the methanol extract.

Previous work in this laboratory(3), has shown that the daily intramuscular injection of 1 μg crystalline vitamin B_{12} did not replace the methanol extract factor. Recently responses have been obtained by the daily injection of 10 μg of crystalline vitamin B_{12}, and by the oral administration of 20 μg per day of this vitamin. In addition, a vitamin B_{12} concentrate obtained from a streptomycin fermentation proved active when fed at a level equivalent to 10 μg per day.

The results as shown in Table I confirm the earlier observation that a factor present in the methanol insoluble portion of liver (residue) is required by the mink. Poor fur development is one of the most prominent symptoms noted in animals which received only the basal ration. Animals which were given the residue fraction of liver as the only supplement were observed to have dense luxuriant underfur, equal to those receiving liver. Conversely the underfur of those mink which received only the methanol extract was extremely thin or nonexistent.

Discussion. It is apparent that striking similarities exist between the methanol extract factor required by mink and vitamin B_{12}. It will be noted that there is a good correlation between the products containing methanol extract factor activity and their vitamin B_{12} content (Table II). Furthermore the properties of the methanol extract factor and vitamin B_{12} are very similar. The methanol extract factor is soluble in water, phenol and 60% methanol, but insoluble in acetone, n-butanol and 3/4 saturated ammonium sulfate. Vitamin B_{12} has been shown to have similar properties(6). The classical symptoms of pernicious anemia, namely, anemia and neurological disorders, are seen in mink deficient in the methanol extract factor. Vitamin B_{12} has been shown to be effective in preventing fatty degeneration of the liver and kidneys in the chick(7), and rat(8). The methanol extract cures a similar condition in the mink.

TABLE II. Comparison of Products Tested for Methanol Extract Factor Activity and Their Content of Vitamin B_{12}.

Product	Methanol ext. factor activity	Min. vit. B_{12} content(9), μg/100 g
Liver	+	15
Fish solubles	+	20
Milk	+	2.5*
Hog intestinal mucosa	—	Trace
Pork spleen	—	†
Yeast	—	†
Distillers solubles	—	†
Alfalfa	—	†
Tomatoes	—	†

* Whole milk powder.
† No measurable quantity.

In spite of these similarities the methanol extract fraction must have an effect in addition to the actual amount of vitamin B_{12} present as measured by rat assay(9). The methanol extract (which is active at an intake of less than 1 ml per day) contains only 2 μg vitamin B_{12} per ml while 10 to 20 μg crystalline vitamin B_{12} are needed to give the same activity. From the foregoing considerations, it would seem that the methanol extract factor may be another form of vitamin B_{12}.

Summary. The methanol extract factor required by the mink has been found to be soluble in 90% phenol and insoluble in acetone, n-butanol and 3/4 saturated ammonium sulfate.

Fish solubles contain this factor, but dried distillers solubles, dried whey, pork spleen, and hog intestinal mucosa were found to be inactive.

The relationship between the methanol extract factor and vitamin B_{12} is discussed.

The original observation that the mink requires a methanol insoluble factor as well as

6. Smith, E. L., *British Med. J.*, 1949, v2, 1367.
7. Schaefer, A. E., Salmon, W. D., and Strength, D. R., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 202.
8. Schaefer, A. E., Salmon, W. D., and Strength, D. R., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 193.
9. Lewis, U. J., Register, U. D., Tappan, D. V., and Elvehjem, C. A., unpublished data.

a soluble factor has been confirmed by the data presented.

We wish to acknowledge our indebtedness to Merck and Co., Rahway, N. J., for the crystalline vitamins; to Lederle Laboratories, Pearl River, N. Y., for the pteroylglutamic acid.

Received July 24, 1950. P.S.E.B.M., 1950, v75.

Utilization of L-, D- and DL-Forms of Asparagine and Aspartic Acid by *Leuconostoc mesenteroides* P-60.* (18105)

MERRILL N. CAMIEN† AND MAX S. DUNN

From the Chemical Laboratory, University of California, Los Angeles.

A microbiological method for the determination of L-aspartic acid with *Leuconostoc mesenteroides* P-60 was reported by Hac and Snell(1). These authors showed that L-asparagine had only a fraction of the activity of L-aspartic acid for this organism, but although their method has been widely used it does not appear that the activities of D-aspartic acid and D-asparagine for *Leuconostoc mesenteroides* have been reported previously.‡ It seemed desirable, therefore, to determine the response of this organism to the optically active and racemic forms of aspartic acid and asparagine.

Experimental. The culture of *Leuconostoc mesenteroides* P-60 (American Type Culture Collection catalogue No. 8042) was the same as that described previously(2). The inoculum was prepared as described in Paper 47(3). The experimental medium and assay technics were the same as those described previously for experiments with phenylalanine(4) except that 1 g of DL-phenylalanine was included in the amino acid mixture of the basal medium and the L-asparagine was omitted. In some of the experiments the medium was modified by additions of suboptimal amounts of L-asparagine or D-aspartic acid. Response to L-, DL-, and D-asparagines and aspartic acids§ was determined in the various modifications of the basal medium with incubation periods from 24 to 72 hours.

Discussion. Experiments with L-asparagine gave results which closely resembled those obtained previously by Hac and Snell(1). D-asparagine was entirely inactive in the present experiments (tested at final concentrations as high as 20 mg %), and DL-asparagine had 50% of the activity of L-asparagine.

Experiments with DL-aspartic acid gave essentially the same results (Fig. 1) as those obtained previously by Steele, *et al.*(5), however D-aspartic acid (Fig. 1) elicited a unique response. At concentrations up to about

* Paper 74. For Paper 73, see Eiduson, *et al.*(4). This work was aided by grants from the American Cancer Society through the Committee on Growth of the National Research Council. The authors are indebted to Samuel Eiduson, Gene Molene, and Ruth B. Malin for technical assistance.

† Present address: Université de Liége, Laboratoire de Chimie Physiologique, Institut Léon Fredericq, 17 Place Delcour, Liége, Belgium.

1. Hac, L. R., and Snell, E. E., *J. Biol. Chem.*, 1945, v159, 291.

‡ Steele, *et al.*(5) found that the activity of DL-aspartic acid was essentially the same as that of L-aspartic acid for *Leuconostoc mesenteroides*, but apparently these authors did not test D-aspartic acid.

2. Dunn, M. S., Shankman, S., Camien, M. N., and Block, H., *J. Biol. Chem.*, 1947, v168, 1.

3. Dunn, M. S., Camien, M. N., Malin, R. B., Murphy, E. A., and Reiner, P. J., *Univ. Calif. Publ. Physiol.*, 1949, v8, 293.

4. Eiduson, S., Camien, M. N., and Dunn, M. S., Paper 73, *Archiv. Biochem.*, in press.

§ The L- and DL-asparagines and aspartic acids were A.P. and C.P. products of Amino Acid Manufacturers. The D-asparagine was a gift from M. Palmer Stoddard, Gerber, Calif. and D-aspartic acid ($[\alpha]_D^{25.0} = -25.45°$ in 1.008 *N* HCl) was obtained by acid hydrolysis of this product.

5. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., *J. Biol. Chem.*, 1949, v177, 533.