pears that the extreme lability of xanthine oxidase activity is due to several unknown factors. It is difficult to believe that activity of this enzyme is lost because of its lack of necessity to the animal since purine metabolism is believed to be very important in animal functions.

As shown in the table, general endogenous respiration of the livers of the animals receiving the complete ration and those receiving the methionine deficient ration are essentially the same. Stare and Elvehjem[8] had reported that endogenous respiration of chick and rat tissue is practically unaffected by various vitamin deficiencies. It has been recently reported[9] that endogenous respiration of chick liver is unaffected by folic acid or vitamin B_{12} deficiencies. It thus appears that while dietary conditions have little effect upon the total endogenous metabolism of the liver individual enzyme activities may be varied significantly by the diet.

Summary. It has been observed that a methionine deficiency in the rat reduces liver succinic dehydrogenase activity slightly, completely reduces liver xanthine oxidase activity, and has practically no effect upon endogenous respiration of liver tissue ***in vitro.***

8 Stare, F. J., and Elvehjem, C. A., *Am. J. Physiol.*, 1933, **105**, 655.

9 Williams, J. N., Jr., Nichol, C. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **180**, 689.

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Lipotropic Effects of Vitamin B_{12} Concentrate. (17442)

VICTOR A. DRILL AND HARRY M. MCCORMICK

From the Department of Physiology and Pharmacology, Wayne University College of Medicine, Detroit, Mich.

It has recently been reported that crude liver extract exerts a lipotropic effect in rats with dietary induced liver injury.[1] The effect of the liver extract was not due to stimulation of the appetite with a resultant increase in protein intake, nor did the lipotropic effect seem to be related to the choline content of the liver extract. It was also demonstrated that other supplements. with a higher choline content than that of liver extract, exert a lesser degree of lipotropic activity when compared with liver extract.[2] In the present studies the lipotropic effect of a vitamin B_{12} concentrate was determined in rats fed a high-fat diet.

Methods. Male rats of the Sprague-Dawley strain, weighing between 115 and 149 grams were used. The high-fat diet (51% lard) and the control diet (6% lard) as recently described,[3] were fed *ad libitum* for the period listed in Tables I and II. When the animals were placed on the synthetic diets injections of vitamin B_{12} concentrate were begun and were administered subcutaneously 3 times a week.* At the end of the experiment sections were taken from the left lobe of the liver and stained with hematoxylin and eosin. Frozen sections were also made and stained with Sudan III. The remainder of the liver was analyzed for total fat.[4]

Results. In the first study treatment with a vitamin B_{12} concentrate, in doses of 0.2 μg of vitamin B_{12} 3 times a week, reduced the average fat content of the liver from 31.5%

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2 Drill, V. A., and Hall, C. A., *Am. J. Med. Sci.*, in press.

3 Hall, C. A., and Drill, V. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 202.

* The Oleum Percomorphum used in the diet was kindly supplied by Mead Johnson and Company.

The vitamin B_{12} concentrate was supplied by Dr. T. H. Jukes of Lederle Laboratories Division of the American Cyanamid Company.

4 Outhouse, E. L., and Forbes, J. C., *J. Lab. and Clin. Med.*, 1939, **25**, 1157.

TABLE I.
Effect of Vitamin B_{12} Concentrate on Hepatic Changes of Rats Fed High-Fat Diet for 29 Days.

No. of rats	Avg wt change, g	Avg liver wt, g	Avg liver wt g/100 g rat	No. animals with hepatic fatty changes* 0	1+-2+	3+-4+	Avg liver fat, %
			High fat diet, untreated				
6	55 ± 6.12	17.1 ± 1.25	8.1 ± 0.47	0	0	6	31.5 ± 1.18
			High fat diet, + B_{12} conc. ≎ 0.2 μg B_{12} 3 times a wk				
5	48 ± 8.13	10.4 ± 0.66	5.1 ± 0.17	1	4	0	16.3 ± 1.47

* Grading of fatty change.
0 = None or only a rare large droplet.
1+ = Few large droplets in each lobule.
2+ = More than 1+, but less than half of lobule involved.
3+ = Over half of lobule involved, but some cells fat-free.
4+ = Almost no fat-free cells.

TABLE II.
Effect of Vitamin B_{12} Concentrate on Hepatic Changes of Rats Fed High-Fat Diet for 66 Days

No. of rats	Avg wt change, g	Avg liver wt, g	Avg liver wt g/100 g rat	No. animals with hepatic fatty changes* 0	1+-2+	3+-4+	Avg liver fat, %
			Normal diet, control				
4	160 ± 3.14	12.1 ± 1.13	4.0 ± 0.37	3	1	0	5.6 ± 1.08
			High fat diet, untreated				
8	43 ± 9.67	15.7 ± 1.07	8.5 ± 0.62	0	1	7	24.6 ± 0.59
			High fat diet, + B_{12} conc. ≎ 1.0 μg B_{12} 3 times a wk				
10	84.6 ± 8.44	10.7 ± 0.71	4.7 ± 0.22	8	1	1	7.8 ± 0.89

* Fatty changes graded as in Table I.

in untreated animals to 16.3% in treated rats (Table I). Histological examination also showed a partial effect of the treatment. The vitamin B_{12} concentrate also prevented the increase in liver weight that usually occurs in untreated rats fed a high-fat diet. There was no significant difference in the gain in weight between the two groups of animals.

Treatment with a higher amount of vitamin B_{12} concentrate (1 μg of vitamin B_{12} 3 times a week) over a period of 66 days demonstrated a marked lipotropic activity of the concentrate. The fat content of the liver of the treated animals was not significantly different from the amount present in rats fed a normal diet (Table II). Histological examinations of the liver also demonstrated the lipotropic activity of the vitamin B_{12} concentrates. The weight of the liver was similarly affected by the treatment and was in the range of the normal controls. Furthermore, there was a significantly greater weight gain in the treated group as compared with the animals fed a high-fat diet alone.

Discussion. A vitamin B_{12} concentrate administered over a period of 29 and 66 days to rats receiving a high-fat diet showed a significant lipotropic effect. This effect is comparable to the previous results obtained with liver extract.[1,2] Both vitamin B_{12} concentrate and liver extract contained a small amount of choline and methionine. An injection of vitamin B_{12} concentrate, containing 1 gamma of vitamin B_{12}, supplied only 0.5 mg of choline, 0.19 mg of methionine, and 0.06 μg of folic acid. Based on 3 injections of the concentrate per week, each rat received only 0.21 mg of choline and 0.08 mg of methionine per day. These amounts of choline and methionine *per se* are ineffective in preventing the dietary induced fatty livers. The rats injected with 0.2 μg of vitamin B_{12} received only one-fifth of the amount of these added factors. The nature of the lipotropic action of the vitamin B_{12} concentrate is not known. It is possible that the effect of vitamin B_{12} and choline are interrelated, particularly as it has been reported that dietary choline has a specific sparing action on vitamin B_{12} as measured by the growth of the chick.[5] Further studies on the possible lipotropic action of crystalline vitamin B_{12} are in progress.

Conclusion. A concentrate of vitamin B_{12} exerted a marked lipotropic effect when injected into rats receiving a high-fat diet. This lipotropic effect was not due to the small amount of choline present in the concentrate.

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A Micro Version of the Barcroft-Warburg Tissue Metabolism Apparatus.* (17443)

S. B. BARKER

From the Department of Physiology, State University of Iowa, Iowa City.

Often it is desirable to measure oxygen consumption or other gaseous exchange of amounts of tissue too small to yield adequate changes in the usual 15-17 ml Warburg vessel. Several different types of apparatus have been constructed for this purpose: Warburg mentioned one of 4 ml capacity,[1] the Fenn type[2] is well known, and in recent years more diversified models have appeared (cf. evaluation by Tobias[3]).

A miniature form of the standard Warburg type, shown in Fig. 1, was designed of 2.5 to 3.3 ml capacity which has proved so convenient that this brief report is being made to note its availability.† The actual size is about 20 mm bottom diameter and 35 mm height, with the main vessel orifice being standard taper 14/20 and the side-bulb opening 6/15. A lip is placed on the center well, facing the side-bulb, for ease in pouring if the barium hydroxide-hydrochloric acid procedure is used for measurement of CO_2 production in the same vessels in which O_2 consumption is determined. Four hooks, instead of the conventional two, are routinely placed on all of our flasks and manometers in order to avoid the need for repairs when one hook is accidentally knocked off.

The manometer has a graduated portion 150 mm long, made from tubing about 1 mm inner diameter, to increase the total capacity as little as possible. Calibration is carried out by the use of mercury, although it is desirable to

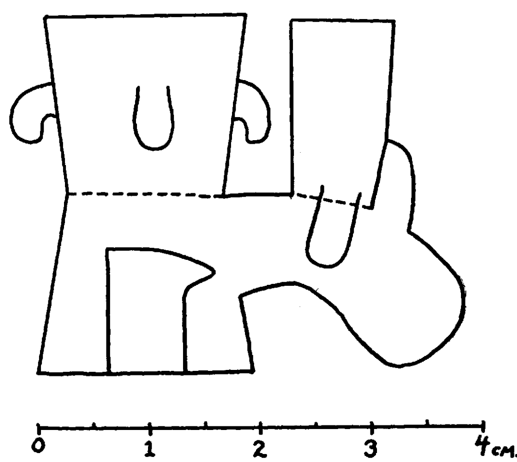

FIG. 1.
Diagram of micro respiration vessel.

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2 Fenn, W. O., *Am. J. Physiol.*, 1928, **80**, 327.

3 Tobias, J. M., *Physiol. Rev.*, 1943, **23**, 51.

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