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)

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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

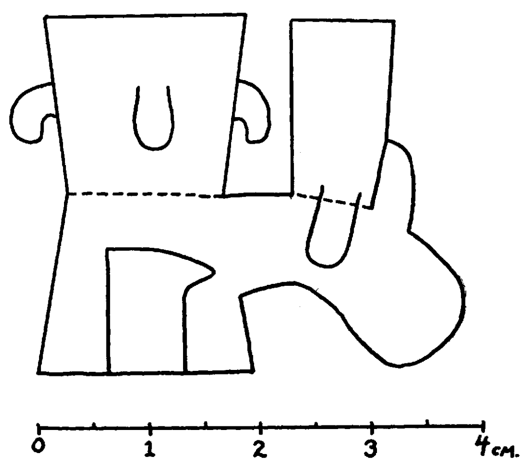

FIG. 1.
Diagram of micro respiration vessel.

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service.

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

† The author wishes to thank the E. Machlett Company, 220 East 23rd St., New York City, for its cooperation in designing and fabricating these vessels.

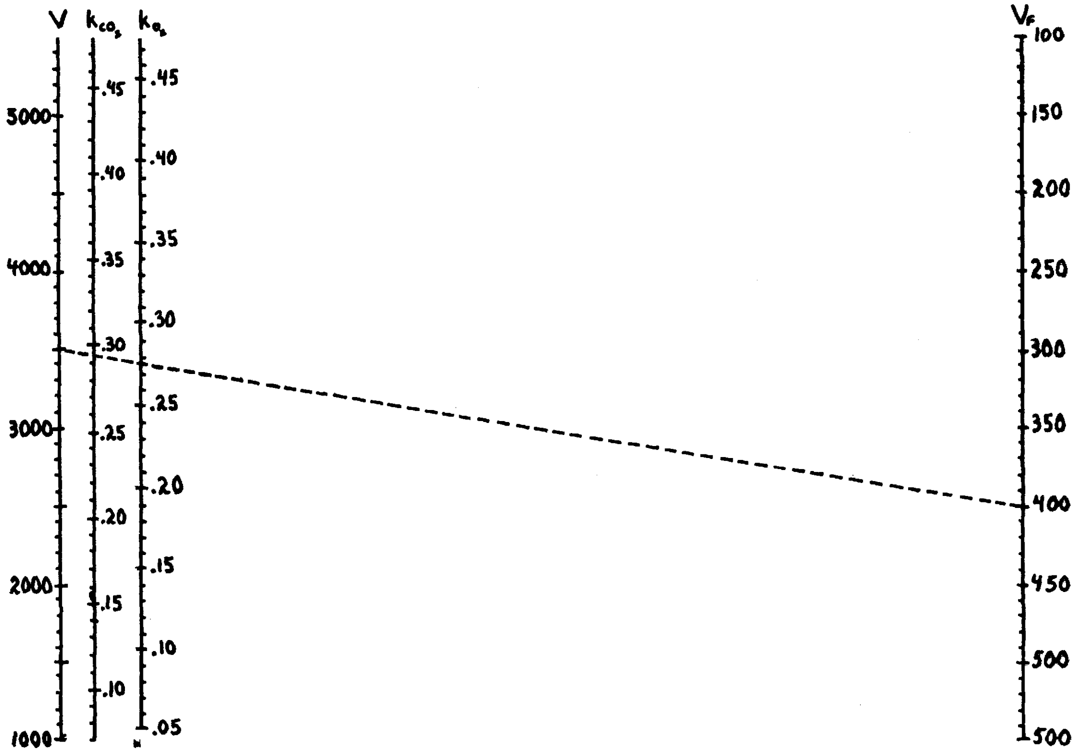

FIG. 2.
Nomogram for determining k_{O_2} and k_{CO_2} values for micro respiration vessels.

fill the vessel and upper part of the manometer down to the 75 mark to be used as zero with one filling of mercury. This technic avoids the second stage of filling from a scratch mark to the zero with its possible errors.

In operation, the movement of fluid in the open limb of the manometer is quite rapid because of the small gas volume in the vessel for expansion or contraction. Tipping over substrate from the side-bulb requires a scrupulously clean glass surface, lest a grease layer accentuate the difficulty of pouring from such a small cup. Volumes of solution which have been found convenient to use are 300 μl of buffered medium, 50 μl of alkali (usually 10% KOH) in the center well and 50 μl of substrate solution in the side-bulb. For complete CO_2 and O_2 determinations, 75 μl of $Ba(OH)_2$ may be placed in the side-arm and 100 μl of HCl in the center well. These small volumes of fluid may be introduced into the desired parts of the vessel by means of a tuberculin syringe and needle, Kirk micro-pipette and delivering device, or glass Mohr pipette with drawn-out tip.

A nomogram constructed according to Dickens[4] is presented in Fig. 2 to facilitate obtaining vessel constants at 37°C for vessels from 1 to 5.5 ml in volume. It should be emphasized that V is the total capacity of vessel and manometer to the midway mark used as the zero for the constant volume readings. In the example shown, the total volume of vessel and manometer limb is 3500 μl and the fluid volume in the vessel is 400 μl. The k_{O_2} for this experiment is 0.277 and the k_{CO_2} is 0.294. Thus the change in manometer reading due to gaseous exchange would be approximately 3.5 times as great as the actual volume changes.

Table I lists Q_{O_2} comparisons for several rat tissues obtained in this laboratory between these vessels and the conventional 17 ml size. The metabolic activities correspond closely. The sensitivity difference is about the 4- to

[4] Dickens, F., *Biochem. J.*, 1945, **39**, 427.

TABLE I.
Comparison of Amounts of Rat Tissue and Q_{O_2} Values in Standard and in Micro Vessels.

Tissue	Micro vessel		Macro vessel	
	Tissue wt, mg*	Q_{O_2}†	Tissue wt, mg*	Q_{O_2}†
Diaphragm	40	0.98	150	1.02
Heart	40	0.76		
Skeletal muscle	60	0.57	300	0.45
Kidney	15	3.32	80	3.25
Liver	40	1.29	200	1.34
Thyroid	20	0.95		

* Wet weights are used throughout.
† cu mm O_2/mg wet wt/hr.

5-fold one would anticipate from the difference in constants. There is little reason for using the micro-vessels for a tissue as metabolically active as kidney, because of the great care required in weighing the approximately 15 mg samples. The real value is in such cases as skeletal muscle, with a low rate of metabolism, or thyroid gland, with so little total tissue available.

Summary. A micro modification of the standard Warburg vessel for the study of gaseous exchange of tissues has been described. As would be anticipated from the decrease in size from 17 ml to about 3 ml, the amounts of tissue required for satisfactory results can be reduced to about one-fifth those usually used.

An advantage over other more sensitive apparatus is that the manometer supports are interchangeable with the standard Barcroft-Warburg supports, so the same water-bath and shaking device can be used for both.

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Administration of Folic Acid to Patients Without Anemia.* (17444)

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In view of reports[1-11] showing the appearance of neurologic disease following folic

TABLE I.
Distribution of Cases Treated for More Than One Year. ♂ 24 ♀ 21.

Age	No.
10-19	1
20-29	3
30-39	8
40-49	8
50-59	8
60-69	9
70-79	7
80-89	1

* The folic acid used in this study was furnished by the Lederle Laboratories Division, American Cyanamid Company.

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