zone of the salt depleted animal. This may represent the concentration of glycogen in a slightly compressed area rather than an absolute increase.

Discussion. From observations of the control and experimental rat adrenals it is clear that large amounts of stainable glycogen accumulate in the fascicular and reticular zones. Neither the normal glomerulosa nor the glomerulosa stimulated by sodium deprivation appear to accumulate glycogen appreciably. If an important mechanism of ACTH action involves the stimulation of phosphorylase activity it would seem that for this action to be promptly effective stores of glycogen must be present as substrate. The facts that glycogen is accumulated in the inner cortical zones and is not stored in the glomerulosa may provide at least part of the basis for the differing metabolic responses of the zones to stimulation. Without data on glycogen turnover rates no comment can be made on its utilization in a given zone. The data referred to here do not exclude the possibility that glycogen may be actively metabolized in the glomerulosa cells but they do indicate that it is not stored.

Summary. Sodium depletion resulted in widening of the glomerulosa zone of the rat adrenal cortex. The enlarged zone resembled the glomerulosa of the control animal in that no glycogen was stored. This contrasted with the fascicular and reticular zones where abundant glycogen was found. It is suggested that differences in glycogen storage may at least in part explain differences in the immediate metabolic response to stimulation of the zones.

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Nutritional Effects of β-Methyl-Aspartate and Methyl-Malonate on *Euglena gracilis* and *Ochromonas malhamensis.** (27156)

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It was recently reported that the nutritional requirement of *Poteriochromonas stipitata* for Vit. B_{12} could also be satisfied by β-methyl-aspartate(1). The specificity of this organism towards members of the B_{12} group of vitamins is the same as that of *Ochromonas malhamensis* and of mammals (2). Since both *O. malhamensis* and *E. gracilis,* whose specificity is less strict, are widely used to assay Vit. B_{12}, we studied the effect of β-methyl-aspartate and of methyl-malonate, both products of B_{12}-catalyzed reactions (3,4) on the growth response of *O. malhamensis* and *E. gracilis* to B_{12}; full replacement of B_{12} by β-methyl-aspartate or methyl-malonate would mean that the indispensable functions of B_{12} for both protozoa had been identified, and would favor the likelihood that these findings also apply to man.

Results. Euglena gracilis Z strain, ATCC 12716 and *Ochromonas malhamensis,* ATCC 11532 were used. Maintenance methods and media, general experimental procedures, media for *E. gracilis* when grown at pH 3.6, and for *O. malhamensis* when grown at pH 5.0 have been described(5). Euglena was grown in *O. malhamensis* medium for experiments at pH 5.0. Ochromonas was grown in the medium described in Table XI by Nathan

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TABLE I. Inability of Several Compounds to Spare or Satisfy the B_{12} Requirement of *Euglena gracilis.**

		Conc. of B_{12} (mμg %)				
		None	.4	1.4	4.0	13.6
No additions		.01†	.14	.38	1.0	3.3
L-threo-β-methyl-aspartate (BMA)	.1%	.01	.09	.20	.59	1.92
BMA + Ca pantothenate	.1 mg %	.01	.19	.31	.79	2.34
Methyl-malonate (MM)	.1%	.01	.13	.30	.78	1.65
MM + Ca pantothenate	.1 mg %	.01	.12	.34	.79	1.92
BMA + MM + Ca pantothenate	.1 mg %	.01	.13	.38	.94	2.28

* Grown in pH 5.0 medium.

† Growth expressed as optical density units.

and Funk(6) for experiments at pH 6.8.

All compounds used were from commercial sources, unless otherwise specified. All test compounds were added to the media before autoclaving.

Results. To eliminate the possibility that cellular entrance of the compounds being tested is pH-dependent, experiments were done in media which ranged from pH 3.6-6.8.

In our first experiments, L-threo-β-methyl aspartate, ureido-β-methyl aspartate, and N-acetyl-L-threo-β-methyl aspartate (all gifts of H. D. Isenberg), commercial DL-β-methyl aspartate and methyl-malonate were tested over the range of 0.01-0.1%. All of these compounds were inert in either replacing or sparing Vit. B_{12} for either *E. gracilis*, grown at pH 3.6, or *O. malhamensis*, grown at pH 5.0. In subsequent experiments we used only one level, 0.1%, of each of the test compound because this was the level Isenberg *et al.* reported active in replacing B_{12} for *P. stipitata*(1).

To exclude the possibility that our failure to detect B_{12}-replacing or sparing activity resulted from inadequate supplies of coenzyme A in our test organisms, we supplemented all media with Ca pantothenate and also tested the combination β-methyl-aspartate plus methyl-malonate plus Ca pantothenate. The results of a typical experiment are given in Table I. The same experiment was repeated with both organisms at the various pH levels. Similar results obtained in all cases.

Since it is sometimes possible to detect products of vitamin metabolism by ability of the products to annul inhibition of an anti-vitamin, we added toxic amounts of benzimidazole to pH 3.6 and 5.0 *E. gracilis* medium and attempted to reverse the toxicity with β-methyl-aspartate. The toxic effect held: β-methyl-aspartate did not annul the inhibition. Indeed, the addition of β-methyl-aspartate increased the inhibitory effect.

Discussion. For purposes of evaluation of B_{12} assays the series of experiments with *E. gracilis* at pH 3.6 and *O. malhamensis* at pH 5.0 were done using methods recommended for vitamin assays with these organisms(5). The failure of any of the compounds we tested to either replace or spare Vit. B_{12} for either assay organism ensures that assays carried out as directed(5) will measure only vitamin and not a selection of by-pass materials.

The conditions for replacement of Vit. B_{12} by β-methyl-aspartate in *P. stipitata*(1) are not yet clear. For example, the effect is apparent only in some strains of the organism (7) and only in some samples of cultures of one strain sent by ATCC(7). The effective levels of β-methyl-aspartate in the one successful report are high(1). One would expect that activity would be in the range of 1.0-10.0 mg% rather than the reported 100-300 mg%(1) which verges on levels usually reserved for growth substrates. This implies that β-methyl-aspartate is an essential secondary substrate which must either be supplied directly or manufactured by the organism using a B_{12}-mediated reaction. The best growth of *P. stipitata* in a medium containing high β-methyl-aspartate was only half that of a similar medium which contained B_{12}(1) thus pointing to other B_{12}-dependent reactions in this organism which have not yet been identified. In the light of the apparently "unstable" pattern of B_{12} replacement in *P. stipitata*, it is not surprising that β-methyl-aspartate was inert for *O. malhamen-*

sis which has the same specificity towards B_{12}.

Two recent reports suggest that the methyl-malonyl-CoA—succinyl-CoA conversion in *O. malhamensis* is in the direction of succinyl-CoA formation(8,9). Thus if any compound in this pathway is active in replacing B_{12}, one would expect succinate rather than methyl-malonate to be active, but our unpublished experiments exclude this possibility. The importance of the B_{12}-dependent methyl-malonyl CoA → succinyl CoA reaction in *O. malhamensis* has been questioned. In successive notes, the same authors suggested first that succinate formation is not the reaction by which Vit. B_{12} controls growth(8) and then that the major role of B_{12} may be in the propionate → succinate pathway(9). Our results favor the first interpretation.

Our growth experiments indicate that the reactions responsible for the B_{12} growth requirement in *E. gracilis* and *O. malhamensis* are still to be identified. One possible area for investigation is the series of reactions resulting in the formation of lecithin. Lecithin was strikingly effective in reversing benzimidazole-caused growth inhibition in *O. malhamensis* in a medium which contained B_{12} plus all the compounds now recognized as products of B_{12}-catalyzed reactions(6).

Summary. The following compounds do not either replace or spare the growth requirement for Vit. B_{12} of either *E. gracilis* or *O. malhamensis*: DL-β-methyl-aspartate, L-threo-β-methyl-aspartate, ureido-β-methyl-aspartate, N-acetyl-L-threo-β-methyl-aspartate, and methyl-malonate. Inhibition of growth of *E. gracilis* by benzimidazole is not annulled by β-methyl-aspartate.

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Studies on Lipid Metabolism in Mice Treated with β-Hydroxy, γ-Betaine-Butyric Acid. (27157)

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Little is known concerning the physiological or biochemical function of β-hydroxy, γ-betaine-butyric acid (carnitine)(1). Recent reports have shown that carnitine can stimulate the oxidation of palmitic acid by tissue preparations *in vitro*(2-5). It has been suggested that its site of action is in early stages of fatty acid metabolism where it enhances transfer of long chain fatty acids to an enzyme complex(6). The purpose of this study was to evaluate the action of carnitine on fatty acid oxidation by intact mice and fatty acid pattern or quantities of depot fat in non-obese and obese mice respectively.

Materials and methods. Male albino mice weighing 18-23 g, obtained from the Upjohn colony (Rockland Farm Swiss mouse ancestry) were used. For respiratory studies mice were fasted 15 hours (overnight) before use. DL-carnitine-hydrochloride* (DLC) was dissolved in deionized water and either injected intraperitoneally or given orally by stomach tube, ½ or 1 hour, respectively, before C^{14} compounds were injected. The C^{14}-

* International Minerals and Chemical Corporation, Skokie, Illinois.