serum, but remains a possibility. Cross sensitivity to cytochrome C of different manufacture was demonstrated. The possible hazards of its use clinically are emphasized.

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Assay of Anti-Pernicious Anemia Factor with *Euglena.*

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It was noted that massive growth of the algal flagellate *Euglena gracilis* depended on unknown growth factors present in crude casein but absent in certain plant proteins such as edestin and concanavallin A.[1] This factor was removed from casein by repeated isoelectric precipitation. It was subsequently found that alcoholic extracts of crude casein‡ were active, and refined liver extract was recently reported to possess considerable activity.[2] It was found in the present investigation that this growth factor requirement was satisfied by a combination of crystalline anti-pernicious anemia factor (APA)[3] plus thiamine. These findings became the basis of an assay method for APA.

Trial of APA was suggested by the good agreement between the animal protein factor (APF) activity as measured with chicks, and the growth-promoting effect for *Euglena* of injectable liver extracts and of microbial APF concentrates. The latter were found to produce an hematopoietic response in pernicious anemia.[4]

Experimental. The organism used was *Euglena gracilis* var. *bacillaris.* The culture vessels were exposed to "daylight" fluorescent lamps at 25° to 31°C. The optimal temperature was 28° to 31°C; inhibition effects began to appear at approximately 32°C. At first, assays were carried out in 25-ml Erlenmeyer flasks covered with glass caps, and containing 10 ml of medium. It was later found convenient in routine assays to employ 100 x 13 mm tubes containing 2 ml of medium, illuminated from below. The light was supplied by 4, 40-watt lamps mounted side by side, 30 cm from the cultures. Light intensity did not appear to be a limiting factor at the levels of growth reported here. The basal medium is shown in Table I. The thiamine requirement was approximately 0.5 mμg/ml for half

TABLE I.
Composition of Basal Medium.

	Per 1 final medium (pH 6.5)	
$NH_4H_2PO_4$	0.8	g
Potassium citrate, monohydrate	0.2	"
$MgSO_4 \cdot 7H_2O$	0.2	"
Sodium butyrate	2.0	"
Monosodium glutamate	1.0	"
$CaCl_2$	0.1	"
$FeSO_4 \cdot 7H_2O$	20	mg
$MnSO_4 \cdot H_2O$	6	"
$CoSO_4 \cdot 7H_2O$	5	"
$ZnCl_2$	0.8	"
$Na_2MoO_4 \cdot 2H_2O$	1.0	"
$CuSO_4 \cdot 5H_2O$	0.08	"
Thiamine chloride	0.1	"

* Haskins Laboratories. Aided by a grant from Lederle Laboratories Division, American Cyanamid Co.

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‡ Generously supplied by the Nutritional Biochemicals Corporation, Cleveland, Ohio.

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4 Stokstad, E. L. R., Page, A., Pierce, J., Franklin, A. L., Jukes, T. H., Heinle, R. W., Epstein, M., and Welch, A. D., *J. Lab. Clin. Med.*, 1948, **33**, 860.

TABLE II.
Growth of Euglena in the Basal Purified Culture Medium with Various Supplements.

Basal medium used	APA factor added per ml of medium (mμg)	Other additions per ml of medium	Incubation period (hr)	Growth (optical density)
A	None	None	115	.04
,,	.0015	,,	,,	.18
,,	.005	,,	,,	.42
,,	.015	,,	,,	.92
,,	.05	,,	,,	1.34
,,	.15	,,	,,	1.42
,,	.5	,,	,,	1.40
,,	None	0.05 mμl liver extr.	,,	.14
,,	,,	0.15 ,, ,, ,,	,,	.42
,,	,,	1.0 ,, ,, ,,	,,	1.08
,,	,,	3.0 ,, ,, ,,	,,	1.20
,,	,,	10 ,, ,, ,,	,,	1.40
B	None	None	102	.07
,,	.15	,,	,,	.21
,,	,,	0.05 mμg thiamine HCl	,,	.34
,,	,,	0.15 ,, ,, ,,	,,	.52
,,	,,	0.5 ,, ,, ,,	,,	.97
,,	,,	1.5 ,, ,, ,,	,,	1.32
,,	,,	5 ,, ,, ,,	,,	1.30
,,	.5	Vitamin mixture* without thiamine	92	.20
,,	1.0	.05 μg nicotinic acid	,,	.36
,,	,,	0.5 μg pantothenic acid	,,	.35
,,	,,	5 μg pyridoxine HCl	,,	.28
,,	,,	0.05 μg biotin	,,	.30
,,	,,	0.5 μg pteroylglutamic acid	,,	.24
,,	,,	5 μg *p*-aminobenzoic acid	,,	.32
,,	None	None	120	.13
,,	1.0	0.5 μg thiamine HCl	,,	1.40
,,	None	0.5 μg thiamine HCl plus 0.5 μg thymidine	,,	.12
,,	,,	0.5 μg thiamine HCl plus 1.5 μg thymidine	,,	.11
,,	,,	0.5 μg thiamine HCl plus 5 μg thymidine	,,	.12

A—Basal medium as in Table I.
B—Basal medium as in Table I except that thiamine was omitted.
* Riboflavin, niacin, pantothenic acid, pyridoxine, 0.5 μg each, biotin 0.05 μg.

maximum growth and 2.0 mμg/ml for maximum growth. Growth was practically complete in tubes in 4 days when a heavy inoculum was used. The inoculum was prepared by growing the organisms in 50-ml flasks containing 10 ml of basal medium supplemented with sufficient refined liver extract to allow about two-thirds maximum growth; one drop of a dense vigorous culture was then added to each tube or flask. Stock nutrient solutions were preserved by adding a mixture of *o*-fluorotoluene, 1, 2-dichloroethane, and *n*-butyl chloride.[5]

Results and Discussion. The results of some typical experiments are shown in Table II. It was found that about 0.01 mμg of APA factor per ml was required by *Euglena* for "half-maximum growth". This level is only approximately one-tenth as great as that required by *Lactobacillus leichmannii* 313.[6] Thymidine[§] was inactive when tested up to

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

[§] We are indebted to Dr. D. W. Woolley for a sample of thymidine from the Levene collection, to Dr. J. O. Lampen for a sample prepared by him by the method of Klein,[7] m.p. 184.5° to 185.5° (uncorrected), and to Dr. W. Shive for a third sample.

[7] Klein, W., *Z. physiol. Chem.*, 1948, **255**, 82.

10 μg/ml. The lack of response to thymidine, in contrast to the effectiveness of thymidine for lactobacilli[8-10] draws attention to the value of comparative studies of *Euglena* and lactobacilli in exploring the functions of APA. Biochemical generalizations regarding these functions in various species should probably not be made solely on the basis of the observations with lactobacilli.

Experiments now in progress indicate that this preliminary assay medium is capable of further improvements.

Summary. The algal flagellate *Euglena gracilis* var. *bacillaris* was shown to exhibit a quantitative growth response to crystalline antipernicious anemia factor, using a chemically defined medium. Thymidine was inactive.

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Varying Effect of Thyroxine on Oxygen Consumption of Different Tissues.

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Some tissues from hyperthyroid animals preserve their increased metabolism after isolation from the animal. Tachycardia and increased oxygen consumption of the heart persist for hours.[1,2] Similarly, striated muscle and kidney tissue show a greatly increased oxygen consumption.[3] This shows that the increased metabolism is due to biochemical alterations in the cell, and not to nervous influences. It was thought by us that thyroxine might affect all actively metabolizing tissues in the same way. The experiments here reported show that this does not hold for brain cortex slices, which preserve a normal oxygen uptake despite marked hyperthyroidism in the intact animal. Liver, on the other hand, shows a peculiarly variable response.

Adult male albino rats (Donaldson strain) were used in all experiments. Thyroxine was injected subcutaneously in doses from 2.5 to 10 mg per kg every other day. The animals were sacrificed at from 7 to 21 days. Q_{O_2} of slices of gray matter was determined by the Warburg method, in 100% O_2, using phosphate buffer and glucose as substrate. In 26 experiments the Q_{O_2} of brain was found to be between 9.0 and 13.0 in all but 4 instances. In these 4 instances there was moderate increase ranging from 14.9 to 18.7. The mean Q_{O_2} for hyperthyroid brain was 11.5, which is exactly the same as that found in 13 experiments with normal brain (Table I).

In 14 experiments the Q_{O_2} of kidney slices from the same animals was determined. It was found to be greatly increased, averaging 36.1, or almost double the normal value. In 6 experiments the Q_{O_2} of small sheets of diaphragm muscle averaged 8.1, an increase of about 70% over the normal. The high rate of respiration of muscle and kidney tissue confirmed observations made by one of us some years earlier.[3]

In 13 instances the B.M.R. of the animal was followed, up to the time of sacrifice. This was done by the method of Tainter and Rytand.[4] All the animals were markedly hyper-

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3 McEachern, D., *Bull. Johns Hopkins Hosp.*, 1935, **56**, 145.

4 Tainter, M. L., and Rytand, D. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 361.