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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9 Snell, E. E., Kitay, E., and McNutt, W. S., *J. Biol. Chem.*, 1948, **175**, 473.

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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-

1 Lewis, J. K., and McEachern, D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 504.

2 McEachern, D., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 287.

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.

TABLE I.
Oxygen Consumption of Isolated Tissues from Normal and Hyperthyroid Animals.

	Q_{O_2} tissue (cu mm O_2/mg dry wt/hr)			B.M.R. animal (cc O_2/g/hr)		
	Brain	Diaphragm	Kidney	Initial	Final	Increase
Hyperthyroid series						
Mean	11.5	8.1	36.1	1.69	3.65	+116%
No. of exper.	(26)	(6)	(14)	(13)	(13)	(13)
Normal series						
Mean	11.5	4.8	22.5			
No. of exper.	(13)	(23)	(10)			
T.*	0	8.1	4.5			

* T. is the ratio of the difference to the probable error of the difference. It is only considered to be significant when the ratio is 3:1 or greater.

thyroid, as evidenced by the fact that their basal metabolic rates showed increases of between 84 and 201%. The mean increase for the group was +116%.

It is possible that brain metabolism may be somewhat increased during the first few days of thyroxine administration, and that later an anti-hormone effect may come into play. Of the 4 instances in which we obtained increases of oxygen consumption, 3 occurred in animals given thyroxine for only 7 to 9 days, whereas the majority received it for about 15 days. MacLeod and Reiss[5] found that after treatment of hypophysectomized rats with thyrotrophic hormone there was a moderate increase of oxygen consumption of brain slices between 5 and 8 days after commencement of treatment. After 16 days treatment the effect on brain was no longer apparent. Jandorf and Williams[6] found a reversion to normal in the B.M.R. of rats and the oxygen consumption of their isolated tissues after about 3 weeks treatment with thyrotrophic hormone. Certainly with thyroxine the increased oxygen consumption of muscle and kidney tissue, as well as that of the intact animal, is maintained over the period. This is not so for brain, and it may be an interesting example of homeostasis for a very select organ.

It is not clear why brain tissue fails to show the increase of oxygen consumption so characteristic of other tissues. It fits, however, with the observation of Gross and Leblond[7] who found that both white and gray matter showed minimal take-up of thyroxine labelled with radioiodine.

TABLE II.
Effect of Succinate on Oxygen Consumption of Liver Slices.

Hyperthyroid Q_{O_2}	Mean Q_{O_2} of liver		% Increase
	Glucose	Succinate	
0– 5	3.6	26.1	+625
5–10	7.7	53.2	+590
10–20	13.2	60.4	+357
Normal	11.2	39.4	+251

A curious phenomenon was found with hyperthyroid liver. The Q_{O_2} of normal liver is about 10.0, and liver from some hyperthyroid animals showed increases up to twice normal. Liver from other animals, however, gave Q_{O_2}'s that were practically nil. As shown in Table II, the Q_{O_2} was below 10.0 in 14 instances, in one case being as low as 1.3. In 13 experiments the Q_{O_2} was above 10.0, in one case reaching 21.3. This marked variability was at first attributed to some technical error but it soon became clear that this was not so. The phenomenon of very low Q_{O_2} was only found in the hyperthyroid series, and not with the normal controls which were run at the same time and which always gave Q_{O_2}'s ranging from 9.0 to 12.0. Duplicate runs were made on hyperthyroid liver and

[5] MacLeod, L. D., and Reiss, M., *Biochem. J.*, 1940, **34**, 820.

[6] Jandorf, B. J., and Williams, R. H., *Am. J. Physiol.*, 1944, **141**, 91.

[7] Gross, J., and Leblond, C. P., *J. Biol. Chem.*, 1947, **171**, 309.

they agreed satisfactorily. Furthermore, the very low rates of respiration were only found with hyperthyroid liver and not with other tissues. The phenomenon may be due to liver damage or to the exhaustion of some substrate or enzyme system within the cells.

When M/48 succinate was added at the end of the first hour it was found that the low-respiring liver slices were stimulated to remarkable activity, as shown in Table II. Here the results on hyperthyroid liver are considered in 3 groups, depending on the original Q_{O_2} of the tissue. The mean per cent increase for the lowest respiring group was 625%, considerably greater than for those tissues having a higher initial respiration. Suitably thin slices of tissue were chosen to meet the high O_2 consumption.[8] Increases of respiration were not as great as those obtained by the above author with starved liver since we used M/48 succinate instead of the M/25 optimal concentration. The differential increase between normal and hyperthyroid liver slices is clear, however. Our results show that the low-respiring liver slices may have difficulty in using glucose as substrate but are capable of using succinate.

Numerous experiments were carried out to try to pin down the cause of the low respiration in liver of some hyperthyroid animals, but without success. Three different types of diets were tried, one heavily supplemented with vitamins and minerals. These were Fox Chow, the diet of Sherman and Sandels[9] and that of McEachern.[3] The method of killing the animal was explored, using ether, gas and drowning in different experiments. The effect of fasting or of feeding right up to the time of sacrifice was investigated. None of the above factors appeared to play a role.

Rosenthal[8] found that the intensity of spontaneous respiration and the oxidation of lactate and pyruvate by liver slices is decreased in starved animals, but that the intensity of succinate oxidation is not influenced by starvation. He suggested that a decrease in the concentration of co-enzymes in the cell may be responsible since the oxidations of lactate and pyruvate, in contrast to the oxidation of succinate, are known to require co-enzymes. Peters and Rossiter[10] showed that rapid depletion of co-carboxylase occurs in the livers and other tissues of hyperthyroid rats fed upon a basal diet and that this defect is somewhat repaired by administration of thiamin. It is possible that hyperthyroidism may deplete the liver in some such way even when the animals are offered an adequate diet.

Summary. Cerebral gray matter of hyperthyroid rats does not show the increase of respiration shown by the intact animal or by diaphragm muscle or kidney tissue. Liver tissue shows a highly variable respiration in hyperthyroidism, sometimes being abnormally low. These low-respiring tissues are, however, greatly stimulated by the addition of succinate. It would seem that in hyperthyroid liver there may be a shortage of co-enzyme for the glucose-pyruvate cycle. The cytochrome-oxidase system, as judged by succinate response, remains intact or is enhanced.

8 Rosenthal, O., *Biochem. J.*, 1937, **31**, 1710.

9 Sherman, H. C., and Sandels, M. R., *J. Nutrition*, 1931, **3**, 395.

10 Peters, R. A., and Rossiter, R. J., *Biochem. J.*, 1939, **33**, 1140.