open methods. The high diffusibility of helium should result in greater accuracy in determining mid-capacity in subjects with pulmonary emphysema or obstruction, since it is more likely to be uniformly distributed in the lung. There should be less disturbance of normal pulmonary function with this method than with others, because the oxygen concentration of the respiratory mixture is atmospheric.

The method should not be regarded as fully developed. Further consideration must be given to the quantity of helium taken up during the rebreathing, to the correction for oxygen concentration, and to the true relation of spirometer helium concentration to alveolar helium concentration.

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Effect of Glutathione on Tyrosinase and the Significance of the Dopa Reaction.*

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Bloch,[1] as a result of his discovery of the "dopa reaction" and because it had been impossible to demonstrate tyrosinase in the skin of any of the slightly pigmented vertebrates, postulated the existence of an enzyme that accelerated the oxidation of dihydroxphenylalanine. He named it "dopa oxidase", and claimed that this enzyme was responsible for melanin formation in human skin. Such an enzyme has not been isolated and its existence has been questioned many times. The controversy in regard to this has been discussed in comprehensive reviews.[2, 3]

Recently it has been shown[4] that the activity of the enzyme tyrosinase is regulated by the oxidation-reduction potential of substances

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† Rockefeller Foundation Fellow, 1940-1941.

1 Bloch, Br., *Z. f. physiol. Chem.*, 1916, **98**, 226.

2 Franke, W., in von Euler's *Chemie der Enzyme*, 1934, part 2, p. 38, J. F. Bergmann, München.

3 Oppenheimer, *Die Fermente und ihre Wirkung*, 5th ed., 1926, vol. 2 and supplement, 1939, George Thieme, Leipzig.

4 Figge, F. H., *J. Cell. and Comp. Physiol.*, 1940, **15**, 233.

added to the substrate. It was evident from this that both the enzyme tyrosinase and its substrate tyrosine could be present in a cell without melanin formation taking place if a sufficiently strong inhibitor such as reduced glutathione was also present. With this in mind, an investigation was started to determine whether the enzyme responsible for the "dopa reaction" was a separate and specific dopa oxidase or tyrosinase associated with an inhibitor. To investigate this possibility chemically the rates of melanin formation were determined in the following series of reaction mixtures:

1. Buffered dopa alone.
2. Buffered dopa plus various reversibly oxidizable substances.
3. Buffered dopa plus tyrosinase.
4. Buffered dopa plus tyrosinase and glutathione.
5. Buffered tyrosine plus tyrosinase.
6. Buffered tyrosine plus tyrosinase and glutathione.

Series 1 was included to study the rate of non-enzymatic oxidation in air. Series 2 was included to see if any of the reversibly oxidizable substances would accelerate or inhibit oxidation in air. The last 4 series were included to determine and compare the effect of glutathione on the rate of enzymatic oxidation of dopa and tyrosine. The pH of the substrate and concentrations of substances are indicated in the tables. The enzyme preparation was filtered potato juice. The methods used were essentially as described in previous papers.[4]

When the rates of melanin formation in Series 1 and 2 were compared, it was found that almost all of the reversibly oxidizable substances accelerated the oxidation of dopa by atmospheric oxygen (Table I). However, cysteine and reduced glutathione inhibited this oxidation slightly. The following substances accelerated the

TABLE I.

Influence of Reversibly Oxidizable Substances on Rate of Oxidation of 2×10^{-3}M Dihydroxyphenylalanine by Atmospheric Oxygen at pH 7.1.

Series No.	Substances added 10^{-4}M	Melanin concentration as indicated by percentage light absorption at end of			
		50 min.	140 min.	360 min.	480 min.
1	Control	0.0	1.5	13.5	28.5
2	Potassium ferricyanide	4.6	22.0	46.5	57.5
	Phenol indophenol	10.5	28.5	46.5	54.0
	Toluylene blue	6.0	29.0	48.0	54.0
	Thionine	9.0	39.0	60.0	65.0
	Methylene blue	5.8	37.0	61.0	68.0
	Indigo disulphonate	2.0	10.0	30.0	42.0
	Phenosafranine	1.4	8.0	26.0	36.0
	Cysteine	0.0	0.0	0.0	9.0
	Glutathione	0.0	0.0	0.0	3.0

non-enzymatic oxidation: potassium ferricyanide, phenol indophenol, toluylene blue, thionine, methylene blue, indigo disulphonate, and pheno safranin. The optimum rate of oxidation shifted during the experiment. At the end of the first period, the optimum rate was found in the phenol indophenol; by the end of the second period, this had shifted to thionine; and finally optimal oxidation of dopa to melanin was observed in the methylene blue mixture. The amount of melanin formed in this mixture was approximately 3 times that in the control. The acceleration of oxidation by thionine was almost as great as that of methylene blue. At present it is thought that this acceleration of oxidation by these reversibly oxidizable systems depends on their ability to accelerate hydrogen transport from the substrate molecules to atmospheric and dissolved oxygen, by acting as carriers in a short chain reaction.

The rates of melanin formation in Series 3 and 4, 5 and 6 respectively were compared (Table II). Small amounts of glutathione (0.1 mg-1.0 mg per 20 cc substrate) definitely inhibited the enzymatic oxidation of tyrosine, but inhibited the rate of oxidation of dopa by tyrosinase only very slightly.

This work does not prove conclusively that Bloch's postulated dopa oxidase does not exist, but it opens the way for at least two more interpretations of a positive dopa reaction not considered by Bloch, namely,

TABLE II.
Effect of Glutathione on Rate of Tyrosinase Oxidation of Tyrosine and Dihydroxyphenylalanine at pH 7.4.

Series No.	Substrate 10-3M	Enzyme, cc	Glutathione in mg	Melanin concentration as indicated by percentage light absorption at end of				
				1 hr	2 hr	4 hr	7 hr	8 hr
	Tyrosine	.0	.0	0.0	0.0	0.0	0.0	0.0
3	"	.2	.0	5.0	7.0	17.5	29.0	33.8
	"	.2	.0	4.5	7.5	17.0	29.0	33.0
4	"	.2	.1	4.0	5.0	12.0	24.0	26.5
	"	.2	.3	3.5	4.0	7.0	16.0	19.0
	"	.2	1.0	2.5	3.0	6.2	6.0	7.5
	Dopa	.0	.0	2.0	3.7	13.0	28.0	34.0
5	"	.2	.0	51.0	73.5	86.0	89.0	90.0
	"	.2	.0	51.0	73.5	86.0	89.0	90.0
6	"	.2	.1	54.6	73.8	86.0	88.0	88.4
	"	.2	.3	54.0	72.0	84.5	86.5	87.2
	"	.2	1.0	54.0	68.5	79.6	82.0	82.8

1. The presence of reversibly oxidizable substances in melanoblasts that accelerate oxidation of dopa by atmospheric and dissolved oxygen.

2. The presence of tyrosinase in melanoblasts along with an inhibitor. Such melanoblasts could oxidize dopa in air rapidly, but tyrosine would be oxidized so slowly as to be imperceptible over a short period of time because of the inhibitor.

That a positive dopa reaction indicates the presence of inhibited tyrosinase seems most likely and experiments are in progress to test this hypothesis.

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Electron Microscopic Studies of Biological Reactions. I. Reduction of Potassium Tellurite by *Corynebacterium diphtheriae.*

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Since the beginning of the science of bacteriology, investigators have pursued the study of the chemical reactions brought about by bacteria. As a result there has accumulated a tremendous mass of knowledge concerning the types of reactions induced by bacteria, and the particular reactions which characterize various species. However, in all this work it has not been shown definitely whether the reactions in question take place exclusively on the cell surface, as some investigators maintain, or inside the cell, or both.

With a powerful new tool, the electron microscope[1] now available, it was thought desirable to attempt to determine the site of some chemical reaction brought about by a bacterial cell. Since the electron microscope detects variations in density, such a reaction should involve as products large particles or crystals resolvable with the microscope; *i. e.*, the particles must have diameters of at least 50 Å (5 mμ). Another possibility would be to select for study reactions which involve substances which are capable of adsorbing particles

* RCA Fellow of the National Research Council.

1 Marton, L., *Phys. Rev.*, 1940, **58**, 57; *J. Bacteriology*, in press; and other references quoted in these papers; Zworykin, V. K., Hillier, J., and Vance, A. W., *Electrical Engineering*, in press.