

the bile salts. On the other hand, the lactic enzyme, while resistant to bile salts, is wholly destroyed by urea. This recalls the equally striking difference between these enzymes under the influence of glutathione (Hopkins and Morgan)³.

The following table summarizes some typical results of our experiments.

30 mgm. dry weight of enzyme preparation, 0.3 ml. *M/2* succinate, pH = 7.6, temperature 37°.

Methylene blue (400 γ)	Reduction in minutes	Oxygen uptake (μ l/30 min.)	
1. Salt extr. heart muscle	5	1. Salt extr. heart muscle	220
2. Salt extr. heart muscle in presence of desoxycholate ..		2. Salt extr. heart muscle in presence of desoxycholate ..	170
3. Bile extract ..	6	3. Bile extract alone ..	9
4. Final preparation ..	5	4. " " + 1 mgm. MB ..	100
		5. " " + 0.2 c.c. Koch-saft ..	15
		6. Final preparation alone ..	0
		7. " preparation + 1 mgm. MB ..	180

F. GOWLAND HOPKINS.

C. LUTWAK-MANN.

E. J. MORGAN.

Biochemical Laboratory,
Cambridge.

¹ Ahlgren, G., *Skand. Arch. Phys.*, **80**, 16 (1938).

² Hopkins, F. G., Morgan, E. J., and Lutwak-Mann, C., *Biochem. J.*, **32**, 1829 (1938).

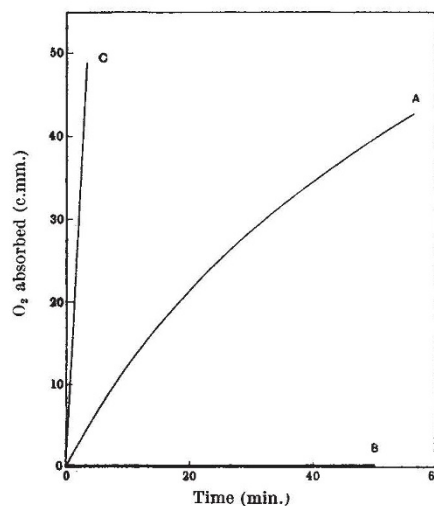
³ Hopkins, F. G., and Morgan, E. J., *Biochem. J.*, **32**, 611 (1938).

Lactic Dehydrogenase and Cytochrome

THE bulk of the respiration of most animal tissues, yeast, etc., takes place through the alternate oxidation and reduction of cytochrome. The reduction of cytochrome is mainly due (a) to a small group of dehydrogenases which are believed to reduce cytochrome directly, and (b) to flavoproteins which transport hydrogen from other dehydrogenases through coenzymes I and II. A typical dehydrogenase of the first class is the lactic dehydrogenase of yeast, which does not depend upon these coenzymes or flavoprotein, but which in the crude state reduces cytochrome *c* readily in the presence of lactate. Some months ago we observed that a partially purified preparation of this dehydrogenase failed to reduce cytochrome, though it still reduced other hydrogen acceptors like methylene blue. We are at present engaged in the purification and isolation of this enzyme. We find that the purified enzyme still reduces methylene blue freely in the presence of lactate, but is completely inactive towards cytochrome. The following experiment shows this very clearly.

Absorption of oxygen was measured in Barcroft manometers in the usual way. Curve *A* shows the oxygen uptake of the purified lactic dehydrogenase + lactate + methylene blue: any methylene blue reduced is, of course, re-oxidized with uptake of oxygen. Curve *B* shows the uptake of the dehydrogenase + lactate + cytochrome *c* + cytochrome oxidase: had any cytochrome been reduced it would have been re-oxidized by the oxidase with uptake of oxygen. Curve *C* shows the uptake of *p*-phenylenediamine + cytochrome + cytochrome oxidase, showing the presence of an excess of oxidase, which was highly active in oxidizing cytochrome reduced by the *p*-phenylenediamine.

These results show that an additional catalyst is necessary for the reduction of cytochrome, possibly a hydrogen-carrier acting between the dehydrogenase and cytochrome. This is present in the crude preparations, but is removed in the purification process. We have shown that this catalyst is not identical with any of the known coenzymes, the flavoproteins of yeast or muscle, or the flavine mono- or di-nucleotides. It is of interest that Haas¹ finds that although



CURVE A: 1 C.C. LACTIC DEHYDROGENASE + 0.3 C.C. *M. LACTATE* + 0.1 C.C. *M/1,000* METHYLENE BLUE + 1.6 C.C. BUFFER.

CURVE B: 1 C.C. LACTIC DEHYDROGENASE + 0.3 C.C. LACTATE + 0.5 C.C. CYTOCHROME *c* + 1 C.C. CYTOCHROME OXIDASE + 0.2 C.C. BUFFER.

CURVE C: 0.2 C.C. *p*-PHENYLENEDIAMINE + 0.5 C.C. CYTOCHROME *c* + 1 C.C. CYTOCHROME OXIDASE + 1.3 C.C. BUFFER.

his flavoprotein reduces cytochrome, it loses this property when highly purified. It is possible that the same or a similar catalyst is involved. If this catalyst is necessary for the reduction of cytochrome, it follows that it must play a very important part in cell respiration.

Biochemical Laboratory,
Cambridge.
Feb. 22.

MALCOLM DIXON.
L. G. ZERFAS.

¹ Haas, *Biochem. Z.*, **298**, 378 (1938).

A New Method of the Determination of Saturation by Vitamin C

THE only reliable method for determination of the saturation of the living body by vitamin C has so far been that of Tillmann-Harris-Ray. It consists essentially of the daily administration of 300 mgm. of ascorbic acid intravenously, until at least half of this amount reappears in the urine. If the body was saturated, the ejection of this quantity takes place within 1-4 days afterwards. If hypovitaminosis dominates, then the ejection is delayed. So far, by examining blood directly only extreme values can be evaluated: above 1 mgm. per cent indicates saturation, while below 0.4 mgm. per cent indicates hypovitaminosis. Concentrations ranging between cannot be evaluated properly, because