

immediately after the operation and continued for 19 days. It was 264 mg. in untreated controls on the 40th day after operation as compared with 285 mg. in the group in which testosterone was given for 19 days after an intermediate period of 21 days between the operation and the initiation of treatment. This is of interest in connection with the publication of Walsh, *et al.*,<sup>6</sup> and Nelson and Gallagher,<sup>2</sup> who conclude that hypophysectomized rats receiving "male sex hormone" prepared from urine show no testicular atrophy and could not be distinguished from the normal even histologically.

*Summary.* Testosterone, which causes mammary gland development in normal rats, has no effect on the mamma after hypophysectomy. The maintenance of the normal testis structure which has been obtained in hypophysectomized rats by the administration of male sex hormone preparations prepared from human urine is not due to testosterone, for the synthetic product does not prevent testis atrophy.

## 9176 P

### Phospholipids as Oxygen Carriers.

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One of the early hypotheses<sup>1, 2</sup> regarding the function of the phospholipids was that they took part in the oxidation processes of the living cell, the belief being based on their well-known ability to take up oxygen. It was assumed, without however any evidence to support the assumption, that the oxidized phospholipids could act as oxidizing agents or oxidases, passing on their oxygen to other substances in the living cell.

The ability of the phospholipids to take up oxygen under approximately physiological conditions has been satisfactorily established.<sup>3-6</sup>

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<sup>6</sup> Walsh, E. L., Cuyler, W. K., and McCullagh, D. R., *Am. J. Physiol.*, 1934, **107**, 508.

<sup>1</sup> Koch, W., *Z. physiol. Chem.*, 1903, **37**, 181.

<sup>2</sup> Fränkel, S., and Dimitz, L., *Wien. klin. Wochschr.*, 1909, **22**, 1777.

<sup>3</sup> Meyerhof, O., *Arch. ges. Physiol.*, 1923, **199**, 531.

<sup>4</sup> Hopkins, F. G., *Biochem. J.*, 1925, **19**, 787.

<sup>5</sup> Tait, H., and King, E. J., *Biochem. J.*, 1936, **30**, 285.

<sup>6</sup> Page, I. H., and Bülow, M., *Z. physiol. Chem.*, 1935, **231**, 10.

The present experiment was directed at the second part of the oxidase hypothesis, *i. e.*, to find out whether the oxidized phospholipids could supply oxygen for the oxidation of other substances. The plan of the experiment was as follows: Reduced methylene blue was subjected under anaerobic conditions to the action of suspensions of purified liver and muscle phospholipids in buffers at various pH, and the resulting color production determined. A typical experiment was as follows:

*Reduced Methylene Blue.* 0.001M methylene blue was reduced by dextrose in hot, faintly alkaline solution in a current of nitrogen (40 cc. of methylene blue, 1-2 drops of 40% alkali, 2-5 drops of 5% dextrose). After centrifugation, the colorless samples were drawn with a fine tube from near the bottom of the solution.

*Phospholipid Suspensions.* Prepared by dissolving 10 mg. of phospholipid in 10 cc. of the different buffer solutions by bubbling a stream of nitrogen through. A control of buffer alone in a matched tube was similarly prepared. After replacement of the air with nitrogen, both tubes were tightly stoppered.

For the experiment, 2 cc. of the reduced methylene blue were introduced into each tube and mixed with a stream of nitrogen. Comparison of the color produced was made in a photoelectric colorimeter similar to that described by Evelyn<sup>7</sup> using a suitable light filter. Typical experiments resulted as shown in Table I.

TABLE I.  
Unoxidized Phospholipid.

|   | Readings | Absorption |
|---|----------|------------|
|   | mm.      |            |
| Buffer alone                              | 200      |            |
| '' + phospholipid                         | 150      | 50         |
| '' + red. m. blue                         | 148      | 52         |
| Total absorption by reagents              |          | 102        |
| Buffer + phospholipid + red. m. blue      | 100      | 108        |
| Difference in absorption due to oxidation |          | +6         |

This difference is within the limit of error of the experiment and therefore inconclusive.

Determinations were made at pH 5, 6, 7, and 8 with purified phospholipids (lecithin and cephalin) from liver and muscle, with no results to indicate oxidation of the reduced methylene blue.

*Oxidized Phospholipid.* The purified phospholipid dissolved in petroleum ether was distributed from a pipette on large sheets of filter paper. After evaporation of the petroleum ether, the sheets were exposed in the laboratory for 48 hours, after which the oxi-

<sup>7</sup> Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

dized phospholipid was extracted by ether and the experiments carried out as before. The material had now markedly oxidizing properties. Moreover it was soluble in water to a clear solution giving a phospholipid + buffer solution which transmitted light without absorption. A typical reading (liver cephalin 10 mg.) is given in Table II.

TABLE II.  
Oxidized Phospholipid.

|   | Readings | Absorption |
|---|----------|------------|
|   | mm.      |            |
| Buffer alone                              | 200      |            |
| " + phospholipid                          | 200      | 0          |
| " + red. m. blue                          | 115      | 85         |
| Total absorption by reagent               |          | 85         |
| Buffer + phospholipid + red. m. blue      | 28       | 172        |
| Difference in absorption due to oxidation |          | +87        |

Lecithin gave similar but somewhat lower values. As before, the pH of the solution in the range 5 to 8 had no effect.

*Summary.* A buffered suspension of the phospholipid from liver and muscle had little or no oxidizing effect on a sensitive preparation of reduced methylene blue. After exposure to air for 24-48 hours, the phospholipid became soluble in water to a clear solution and had marked oxidizing effect on the reduced methylene blue. pH changes of from 5 to 8 were found not to affect the oxidation.

## 9177 P

### Growth-Factors for Propionic and Lactic Acid Bacteria.

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Wood, *et al.*,<sup>1</sup> and Tatum, *et al.*,<sup>2</sup> showed that an ether-soluble acid (obtained from yeast-extract, potato-extract or corn-steep) and vitamin B<sub>1</sub> stimulate growth of the propionic acid bacteria. Snell, *et al.*,<sup>3</sup> have shown that ether-extract of potato stimulates *Lactobacillus delbrückii* but an additional factor present in peptone is

\* Journal Paper No. J. 440 of the Iowa Agricultural Experiment Station. Project No. 421.

<sup>1</sup> Wood, H. G., Tatum, E. L., and Peterson, W. H., *J. Bact.*, 1937, **33**, 227.

<sup>2</sup> Tatum, E. L., Wood, H. G., and Peterson, W. H., *Biochem. J.*, 1936, **30**, 1898.

<sup>3</sup> Snell, E. E., Tatum, E. L., and Peterson, W. H., *J. Bact.*, 1937, **33**, 207.