By extending this work in the methanol system to a wide variety of additives, we intend to test this apparent correlation between ionization potential and the ability of the solute to affect the radiolytic yields.

G. M. Meaburn
F. W. Mellows
A. Reiffsteck

Service de Chimie physique,
Commissariat à l'Énergie Atomique,
Centre d'Études Nucléaires de Saclay,
Gif-sur-Yvette,
Seine-et-Oise, France.

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BIOCHEMISTRY

Electron Microscope Investigation of Lactic Dehydrogenase Agent

The lactic dehydrogenase (LDH) agent, detectable by increased levels of plasma or serum LDH in inoculated test mice, was found in association with a variety of transplanted murine tumours by Riley *et al.*[1]. Various estimations of its size by filtration methods have been suggested. These include diameters of 2 mμ, approximately 45 mμ, and 55 mμ (refs. 2, 3 and 4, respectively). In an attempt to resolve these conflicting observations, electron microscopic investigations of the LDH agent were initiated, and this report describes preliminary findings.

Three pools of heparinized blood were obtained from *C57BL/Fg* mice 24–36 h after an intraperitoneal injection of 0·1 ml. of a preparation containing $10^{3\cdot5}ID_{50}$/ml. of the LDH agent. Plasma was collected by centrifugation at 2,000 r.p.m. for 20 min, diluted with an equal volume of Eagle's basal medium, and centrifuged for 5 min at 9,000 r.p.m. The supernatants were then spun at 105,000*g* for 2 h in a Spinco 'Model L' ultracentrifuge and the pellets removed. For controls, plasma was obtained from non-treated *C57BL/Fg* mice and a pellet prepared as described. A pellet was also prepared from a filtrate (100 mμ 'Millipore' filter) of a human carcinoma of the breast which does not contain the LDH agent[5]. Ice was used throughout and each centrifugation was at 0° C. One pellet, prepared from infected mouse plasma, was resuspended to its original volume; the infective titre was determined to be $10^{10}ID_{50}$/ml. Pieces cut from the remaining four pellets were fixed in 1 per cent osmic acid with veronal buffer (*p*H 7·4), dehydrated, and embedded in methacrylate. Thin sections were cut with glass knives on a Porter–Blum microtome and mounted on 'Formvar'-coated grids. These were stained for 5–10 min in lead citrate and examined in an RCA '*EMU-3C*' electron microscope.

Fig. 1 illustrates the typical appearance of particles found in thin sections from pellets of infected mouse plasma. They are oval-shaped and measure approximately 15 mμ in width and 45 mμ in length. As evidence that these particles are the LDH agent, it should be noted that: (*a*) their length, 45 mμ, is identical with the diameter calculated by Rowson *et al.*[3] from filtration data; and (*b*) similar particles were not seen in preparations of control pellets.

The above observations, and other findings derived from investigations of its epizootiology and properties[4,6], suggest that the LDH agent is a virus. Since this communication was completed, Bladen and Notkins[7] have reported an electron microscope investigation which demonstrates that the LDH agent is an essentially spheroidal particle measuring 69 mμ × 76 mμ in diameter. The techniques used by these investigators were similar to ours with the exception that their material was examined by negative staining. This variable could account in part for these discrepancies, but it is of interest to consider the possibility that the confusion which now exists as to the size of the LDH agent may be due to a failure to recognize that there is more than one agent.

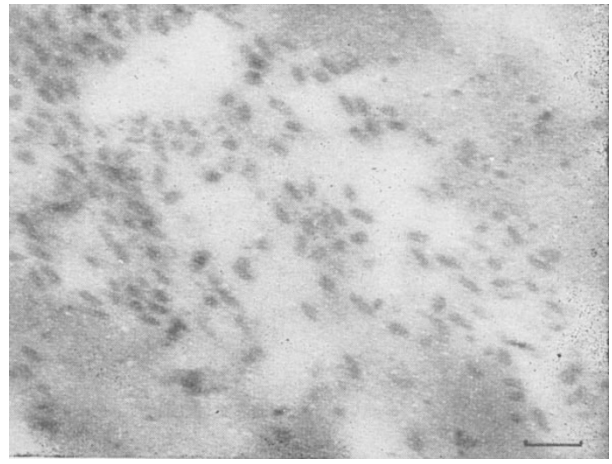

Fig. 1. Electron micrograph of pellet prepared from mouse plasma infected with the LDH agent showing oval-shaped particles (× *c.* 22,700). Line at lower right represents 100 mμ

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C. G. Crispens, jun.
T. A. Burns

Department of Anatomy,
University of Maryland School of Medicine,
Baltimore.

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Interaction of Cytochrome *c* with Yeast D(−) Lactic Oxidase

In 1961 Gregolin and Singer[1] reported that yeast respiratory particles, prepared by mechanical disruption of cells in 1 per cent NaCl, do not oxidize D(−) lactate with oxygen as the terminal electron acceptor. However, the particles exhibit strong D(−) lactic dehydrogenase activity, and oxidize D(−) lactate with oxygen uptake on addition of external cytochrome *c*. More recently, Gregolin and D'Alberton observed[2] that D(−) lactate is oxidized at high rate by oxygen when respiratory particles are prepared by disintegration of the cells in sucrose solution. When tested in a medium known to give maximal oxidase activity, the oxidation is not stimulated by addition of cytochrome *c*. In the presence of the same mixture (plus NaN_3), D(−) lactate is not oxidized by this preparation with cytochrome *c* as artificial electron acceptor.

In simultaneous and independent work, Roy[3] noticed an analogous ineffectiveness of cytochrome *c* on a D(−)