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4. Hemolysates were prepared from erythrocytes, washed in 0.9 percent saline and preserved in glycerol at —60°C. until use. Starch gels were prepared with a tenfold dilution of a solution containing 0.9*M* tris (primary standard), 0.5*M* boric acid, and 0.02*M* ethylene diamine tetraacetic acid (EDTA). Two milliliters of 0.005*M* diphosphopyridine nucleotide (NAD were added to 500 ml of gel just prior to degassing. A solution of 0.11*M* tris, 0.06*M* boric acid, 0.0024*M* EDTA was used as a bridge solution and 1 ml of 0.005*M* NAD was added to the 500 ml of solution in the anodic bridge vessel. Vertical electrophoresis was carried out at 4°C for 10 to 15 hours with a voltage gradient of 4 to 5 volts per centimeter. Zymograms were developed as described by Vesell and Bearn (see 5).
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Trachoma Agent: Glucose Utilization by Purified Suspensions

Abstract. *Strain TE-55 of trachoma "virus" was extracted from infected chick-embryo yolk sacs, purified with the aid of diethylaminoethyl cellulose, and incubated with C^{14}-glucose. Highly significant amounts of $C^{14}O_2$ were produced from carbon-1, but not from carbon-6 of glucose. This demonstrates independent carbohydrate metabolism in an extracellular environment by a microorganism of this group.*

The agent of trachoma, which is currently placed in the large and poorly-defined group of microorganisms known as the psittacosis-lymphogranuloma-trachoma (PLT) group (*1*) has been successfully cultivated in the yolk sac of the chick embryo (*2*). This intracellular agent has been of interest not only because of the disease it causes but also because of its complexity, as evidenced by its developmental cycle within the host cell (*3*), the formation of conspicuous glycogen-like granules within the intracellular inclusion (*4*), and the susceptibility both to sulfonamides (*5*) and penicillin (*6*), which suggests the possibility of independent enzymatic activity. The closely related agents of the psittacosis group oxidize reduced nicotinamide adenine dinucleotide (NAD) in the presence of cytochrome *c* (*7*), synthesize folic acid (*8*), and decarboxylate diaminopimelic acid to lysine (*9*). Independent carbohydrate metabolism, however, has not been demonstrated. Conclusive evidence for such an activity by the agent of trachoma is presented in this report.

Strain TE-55 (*10*) of the agent of trachoma was selected because of its luxuriant growth in the yolk sac of chick embryos (*11*). For each experiment, approximately 60 yolk sacs were harvested from infected moribund embryos and suspended in an equal volume of solution X (0.1*M* KCl, 0.2*M* sucrose, 0.02*M* phosphate buffer) at *p*H 7.2 and stored overnight at 6°C. The procedure of purification differed principally from that recently developed for psittacosis (*12*) in that one anion exchanger, diethylaminoethyl cellulose [DEAE, (*13*)], was used instead of another, ECTEOLA, because the former possesses a higher absorptive capacity. The yolk sacs suspended in solution X were ground in a Waring blender for 1 minute and centrifuged at 30,000*g* for 30 minutes. The pellet was resuspended in solution X and treated with 0.5 percent trypsin (Difco Bacto) for 45 minutes at room temperature. The suspension was then again centrifuged at 30,000*g* and the pellet resuspended in 20 ml of solution X at *p*H 6.9. To this was added DEAE in a concentration of 1 g per 50 g of original yolk sac. After it had been mixed gently for 5 minutes, the suspension was centrifuged at 800*g* for 5 minutes and the supernatant was again subjected to the cycle of DEAE treatment. The resulting suspension was then centrifuged at 30,000*g* for 30 minutes; the supernatant and upper, brownish portion of the pellet were scraped off and discarded, leaving only the lower whitish layer. This was again resuspended in solution X at *p*H 7.2 and treated with DEAE. The resulting suspension was concentrated by centrifugation which produced a pellet almost homogeneous in appearance and almost entirely white.

Table 1. The production of $C^{14}O_2$ from C^{14}-labeled glucose by purified suspensions of the trachoma agent, strain TE-55. The three preparations of labeled glucose were diluted with unlabeled glucose to a concentration of 1 μmole per flask and a specific activity of 4 mc/mmole, or 1.2×10^6 count/min per flask. Each flask also contained 18 μmoles of ATP, 9 μmoles of NAD, 7.5 μmoles of Mg^{++}, 1.5 μmoles of Mn^{++} and 7.5 mg of bovine plasma albumin. The trachoma and control preparations contributed 3.5 mg and 13.2 mg of protein per flask, respectively. The control preparation consisted of the discard from one of the final steps of purification of *C. burneti* from chick embryo yolk sacs. The total volume in each flask was 3.0 ml. All flasks were incubated for 2 hours at 34.4°C. The CO_2 was trapped by 0.2 ml of 40 percent KOH placed in the center well.

Substrate	$C^{14}O_2$ recovered (count/min per mg of protein)	
	Trachoma suspension	Control preparation
Glucose-*R*-C^{14}*	1990	76
Glucose-1-C^{14}	5240	101
Glucose-6-C^{14}	232	93

* Randomly labeled glucose.

Examinations by light and electron microscopy of suspensions purified by this method revealed typical small and large forms of the agent of trachoma, relatively free from contaminating host-cell debris. Enumeration of the microorganisms by electron microscopy after sedimentation onto collodion-coated grids (*14*) indicated that typical preparations contained 4 to 6×10^9 particles per milliliter. Titrations in yolk sacs of chick embryos yielded a ratio of total particles to infectious particles of approximately 200:1. The yield of purified trachoma particles in terms of total protein (*15*) ranged from 0.2 to 0.5 mg per gram of yolk sac in different preparations.

Concentrated suspensions of the trachoma agent were immediately tested for metabolic activity by conventional manometric techniques in the Warburg respirometer. To each vessel were added the suspension of microorganisms, adenosine triphosphate (ATP), nicotinamide adenine dinucleotide, and bovine plasma albumin (crystalline, Calbiochem). Small but steady uptake of oxygen was observed (1 to 2 μl/mg of protein per hour) under these conditions but oxygen consumption was not increased by the addition of either pyruvate or glucose. However, when C^{14}-labeled glucose was added and the radioactivity in the CO_2 was tested, it became apparent that this substrate was metabolized. The data of a representative experiment are presented in Table 1. Glucose was labeled

in positions 1, 6, and randomly. The CO_2 produced was trapped with KOH, converted to $BaCO_3$ and counted as an infinitely thick layer in a proportional gas-flow counter with an efficiency of 13.8 percent. An appreciable amount of radioactivity was collected in the CO_2 when glucose-1-C^{14} was used (Table 1). With randomly labeled glucose of identical specific activity, the radioactivity of the CO_2 was reduced to approximately 38 percent. With glucose-6-C^{14} the radioactivity in the CO_2 was negligible. These results were confirmed in three additional experiments with randomly labeled glucose alone or with the other two types of labeled glucose. The control preparation shown in Table 1, which gave essentially negative results, consisted of the discard from the last step of the purification of *Coxiella burneti* from yolk sacs of chick embryos. Other control preparations which gave very similar results (not shown in Table 1) consisted of trachoma suspensions inactivated by formalin or heat, and suspensions of *C. burneti* or *Rickettsia prowazeki*, strain E, both purified from infected yolk sacs of chick embryos and both highly active when tested against known substrates, succinate (*16*), and glutamate (*17*), respectively.

The failure to produce $C^{14}O_2$ from glucose-6-C^{14} indicated that the Embden-Meyerhof pathway for glycolysis was not followed, since in this case the yield of $C^{14}O_2$ from glucose-1-C^{14} and glucose-6-C^{14} would have been identical. The yield of $C^{14}O_2$ from randomly labeled glucose amounted to 38 percent of that from glucose-1-C^{14}. This value is reasonably close to that predicted (33 percent) if the Entner-Doudoroff pathway (*18*), in which the yield of CO_2 is identical from carbons 1 and 4 of glucose, were operating. However, the pentose pathway with production of $C^{14}O_2$ from carbon 1 plus one or two additional decarboxylations is a possibility. The amount of glucose utilized, 0.2 to 0.3 μmole in individual experiments, was not sufficient to produce measurable manometric changes in the Warburg respirometer, even if all the oxygen required had been derived from the atmosphere. In view of failure by previous investigators (*7*) to demonstrate a complete cytochrome system in the related microorganism of meningopneumonitis, it appears likely that oxidation was not effected by molecular oxygen.

These data are the first unequivocal evidence for the existence of independent carbohydrate metabolism in trachoma virus and in the PLT group of agents. The data suggest that trachoma "virus" in its metabolic nature is more closely related to bacteria and rickettsiae than to true viruses.

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Reticuloendothelial Function and the Immune Response

Abstract. *Hyperphagocytic activity of the reticuloendothelial system induced in mice by the injection of glucan is associated with a significant increase in the primary and secondary immune responsiveness of these animals to sheep erythrocytes. Conversely, the administration of an agent which reduces endothelial phagocytosis is associated with significant depression of both primary and secondary immune responsiveness.*

That the phagocytic activity of the reticuloendothelial system (RES) may be involved in the response of an organism to antigenic challenge has been suggested by the finding that the intravascular clearance of particulate antigenic material proceeds according to the same laws which govern the phagocytosis of inert colloidal material (*1*). Similarly, stimulation of the phagocytic activity of the RES by zymosan (*2*) and Bacillus Calmette-Guérin (*3*) is associated with marked elevations in antibody response to particulate antigenic material.

Reticuloendothelial (RE) participation in the immune response is also suggested by the fact that animals subjected to procedures which might result in an impairment of the functional state of the RES manifest a marked reduction in antibody formation as demonstrated by administration of cortisone (*4*) and of thorium dioxide (*5*).

Various simple lipid complexes induce a marked depression of the granulopectic activity of the RES (*6*). Methyl palmitate, the methyl ester of palmitic acid, induces a selective depression of the RES by impairing the degree of phagocytic activity of Kupffer cells (*7*). By using the techniques of selective stimulation of the RES by glucan, a highly purified neutral polysaccharide (*8*), and selective depression of the RES by methyl palmitate, we evaluated the relation between the functional state of the RES and the immunological response of mice to sheep erythrocytes.

Male C57BL/6J mice, 8- to 12-weeks-old, were given five daily intravenous injections of glucan (4 mg/100 g of body weight) to stimulate the RES. Control mice received saline injections. Twenty-four hours after the last injection, animals received 0.5 ml of a 2 percent saline suspension of sheep erythrocytes intravenously. Seven days later half of each group were bled for testing the primary immune response. A second antigenic challenge was administered at this time to the remaining