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.

RICHARD A. ORMSBEE
Naval Medical Research Institute, Bethesda, Maryland, and *Rocky Mountain Laboratory, Hamilton, Montana*

EMILIO WEISS
Naval Medical Research Institute, Bethesda, Maryland

References and Notes

1. F. B. Gordon, Ed., "The biology of trachoma agent," *Ann. N.Y. Acad. Sci.* **98**, 1–382 (1962).
2. Isolations reviewed by P. Thygeson, *ibid.*, p. 6.
3. L. Halberstaedter and S. von Prowazek, *Arb. Gesundh.-Amte (Berlin)* **26**, 44 (1907); P. Thygeson, in *Virus and Rickettsial Diseases of Man*, T. M. Rivers and F. L. Horsfall, Jr., Eds. (Lippincott, Philadelphia, ed. 3, 1959), p. 729.
4. C. E. Rice, *Am. J. Ophthalmol.* **19**, 1 (1936).
5. F. Loe, *J. Am. Med. Assoc.* **111**, 1371 (1938).
6. E. W. Hurst, *Ann. N.Y. Acad. Sci.* **98**, 275 (1962).
7. E. G. Allen and M. R. Bovarnick, *J. Exptl. Med.* **105**, 539 (1957).
8. J. I. Colón, *J. Bacteriol.* **79**, 741 (1960).
9. J. W. Moulder, D. L. Howard, I. C. Tribby, *ibid.* **85**, 701 (1963).
10. F. F. T'ang, H. L. Chang, Y. T. Huang, K. C. Wang, *Chinese Med. J.* **75**, 429 (1957).
11. Our strain of TE-55 was received in 1960 from Dr. L. H. Collier of the Lister Institute, London, and has been carried by us in embryonated chicken eggs since that time.
12. R. Silberman and P. Fiset, *Nature* **198**, 699 (1963).
13. E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.* **78**, 751 (1956).
14. D. G. Sharp and J. R. Overman, *Proc. Soc. Exptl. Biol. Med.* **99**, 409 (1958).
15. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
16. R. A. Ormsbee cited by E. J. Bell and C. B. Philip, *Ann. Rev. Microbiol.* **6**, 91 (1952).
17. M. R. Bovarnick and J. C. Snyder, *J. Exptl. Med.* **89**, 561 (1949).
18. N. Entner and M. Doudoroff, *J. Biol. Chem.* **196**, 853 (1952).
19. We thank the following members of the Division of Microbiology, Naval Medical Research Institute for their help in these investigations: Dr. F. B. Gordon, Dr. N. A. Vedros, Dr. A. L. Terzin, Dr. E. C. Suitor, Jr., H. R. Dressler, A. Quan, L. W. Newman, H. E. Smith, R. B. Clarke, and J. E. Metzler.

14 August 1963

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

mice and the secondary response was evaluated 7 days later.

Suppression of phagocytic activity was accomplished by the intravenous injection of a suspension of methyl palmitate in a 0.1 percent Tween 20 and 5 percent dextrose solution, 35 mg per animal daily for 2 days. Control mice received 0.1 percent Tween in 5 percent glucose. Twenty-four hours later 0.5 ml of the sheep-cell suspension was injected intravenously and the primary immune response was evaluated 7 days later. A second injection of sheep cells was administered to the remaining mice and the secondary response was evaluated 7 days later. The weights of various organs were determined at 7 and 14 days in both experiments.

The functional state of the RES was evaluated 24 hours after the cessation of glucan and methyl palmitate treatments, by determining the intravascular clearance of colloidal carbon as described by Halpern *et al.* (*9*). The amounts of hemolysin were determined by the twofold serial dilution method and all values were converted to the $\log_2$ titer for statistical analysis (*10*).

The marked increase in phagocytic activity induced by glucan is indicated by the mean $t/2$ of 1.8 minutes as opposed to 10.5 minutes in the control group (Table 1). The glucan-induced hypertrophy of those organs containing a large population of RE cells, is manifested by significantly greater weights of liver (35 percent), lung (32 percent), and spleen (150 percent). The primary hemolysin response of RE-stimulated mice was increased 315 percent as the reciprocal of the mean titer or 37 percent of $\log_2$ titer. Weights of liver, lung, and spleen were increased 45, 32, and 240 percent, respectively, in animals receiving glucan and sheep erythrocytes. The marked increase in RE organ weights during the primary response in the glucan-injected group, as compared to organ weights one day after the cessation of glucan treatment, demonstrates the continued proliferation of RE cellular elements.

When compared to the primary response, the secondary immune response of saline-treated mice expressed as the reciprocal titer was increased 170 percent and the $\log_2$ titer 31 percent. In marked contrast, a 2700 percent increase in actual titers and a 62 percent increase in $\log_2$ titers above saline control values was observed in RE-stimulated mice in the secondary response. When compared to the primary immune response in glucan-treated mice the secondary response of the glucan group was increased approximately 1900 percent. In agreement with the increases in organ weight during the primary response, the weight of liver, lung, and spleen was increased 26, 12, and 149 percent, respectively, in the RE hyperfunctional group over the values observed in the saline control group (Table 1). However, the degree of hypertrophy of liver, spleen, and lung at the 14-day period was not as great as during the primary response and reflects the reversible nature of glucan-induced RE hypertrophy.

The marked depression of phagocytic activity induced by methyl palmitate is manifested by the mean $t/2$ of 39 minutes in the treated group as opposed to 11.2 minutes in the Tween control group (Table 2). Organ weights of mice with a depressed RES were similar to control values. Mice with a depressed functional state of the RES at the time of antigenic challenge manifested a profound depression of the primary immune response amounting to 90 percent of the reciprocal titer and 54 percent of the $\log_2$ titer.

In contrast to the increased secondary response in saline-treated mice (Table 1), no elevation in the secondary response was observed in the Tween control group (Table 2). In agreement with the finding of a depressed primary response after administration of methyl palmitate, the secondary antibody response of RE-depressed mice was markedly reduced: 73 percent of the reciprocal titer and 37 percent of $\log_2$ titer. No alteration in the weight of liver, lung, and spleen was observed during the secondary response.

The glucan-induced hypertrophy of RE organs of mice is in agreement with previous observations of marked increases in liver, lung, and spleen weight after zymosan treatment (*8*). The decrease in weight of these organs during the secondary response indicates that

Table 1. Reticuloendothelial (RE) stimulation with glucan and the primary and secondary immune response in RE-hyperfunctional mice. Values are expressed as mean ± standard error.

Group and number	Percentage of body weight			$\log_2$ titer	$t/2$ (min)
	Liver	Lung	Spleen		
		Before treatment			
Saline (6)	5.20 ± 0.36	0.77 ± 0.04	0.36 ± 0.04		10.52 ± 1.53
Glucan (6)	6.33 ± 0.19	0.89 ± 0.03	0.81 ± 0.04		1.85 ± 0.06
		*Primary response**			
Saline (9)	5.23 ± 0.08	0.64 ± 0.02	0.47 ± 0.03	5.34 ± 0.49	
Glucan (9)	8.64 ± 0.53	0.98 ± 0.04	1.75 ± 0.20	7.43 ± 0.43	
		Secondary response			
Saline (17)	5.05 ± 0.08	0.59 ± 0.02	0.42 ± 0.01	7.01 ± 0.17	
Glucan (11)	6.68 ± 0.15	0.70 ± 0.02	1.08 ± 0.05	11.37 ± 0.36	

* Primary response evaluated 7 days after the initial injection of sheep cells. At this time the remaining mice in each group received a second injection of sheep cells and the secondary response was evaluated 7 days later.

Table 2. Reticuloendothelial (RE) depression in mice treated with methyl palmitate and the primary and secondary response in "RE hypofunctional animals." Values are expressed as mean ± standard error.

Group and number	Percentage of body weight			$\log_2$ titer	$t/2$ (min)
	Liver	Lung	Spleen		
		Control			
Tween (7)	5.34 ± 0.24	0.75 ± 0.05	0.35 ± 0.03		11.21 ± 0.40
Methyl palmitate (8)	6.03 ± 0.40	0.98 ± 0.08	0.33 ± 0.03		39.00 ± 6.11
		*Primary response**			
Tween (10)	5.45 ± 0.11	0.68 ± 0.03	0.48 ± 0.02	7.17 ± 0.29	
Methyl palmitate (8)	5.46 ± 0.27	0.71 ± 0.02	0.44 ± 0.03	3.27 ± 0.61	
		Secondary response			
Tween (11)	5.41 ± 0.12	0.76 ± 0.01	0.46 ± 0.03	7.23 ± 0.18	
Methyl palmitate (9)	5.90 ± 0.18	0.76 ± 0.03	0.42 ± 0.03	4.53 ± 0.75	

* Primary response evaluated 7 days after the initial injection of sheep erythrocytes. At this time the remaining annmals received a second injection of sheep cells and the secondary response was evaluated 7 days later.

glucan-induced RE organ hypertrophy is of a reversible nature. Indeed, RE organ hypertrophy induced by zymosan and *Myocobacterium phlei* Halpern, both excellent RE-stimulatory agents, has been shown to be reversible (*11*).

The exact role of the RES in antibody formation has not yet been clearly defined. It has been suggested that RE stimulation enhanced the period of antigen recognition (*12*) and presumably hastens antibody formation. However, studies have indicated that the induction period of antibody formation is not reduced by RE stimulation (*2, 3*).

Stimulation of the RES markedly increases the phagocytosis of inert colloidal material and enhances the intracellular metabolism of I^{131}-labeled denatured serum albumin (*13*). Since particulate antigenic material is known to be phagocytized, it is likely that RE stimulation increases the intravascular clearance of the antigen and the subsequent intracellular digestion of the phagocytized antigen.

It is well established that the spleen is a major site of antibody formation. The marked hypertrophy of the spleen induced by RE stimulation is the result of proliferation of RE cellular elements which are derived from undifferentiated mesoderm (*14*). Among these newly formed reticular cells may be a number of cells which can mature into plasmocytic cells or cells with antibody-forming potential, and under these conditions more cells are available to react with the antigen (*15*). Possibly these newly formed, relatively undifferentiated cells can develop either as cells with phagocytic or antibody-forming capacity or both and only in the presence of antigen do these cells develop as plasmocytic cells. Thus RE stimulation increases antibody formation by enhancing the solubility of the antigen and by increasing the population of potential antibody-forming cells.

The degree of depression of the phagocytic activity of the RES induced by methyl palmitate is comparable to that reported for other simple lipid complexes (*6*). However, such depression, induced by ethyl stearate, was associated with destruction of hepatic and splenic RE cellular elements (*16*). Histological observations of our mice treated with methyl palmitate have indicated a selective depression of phagocytosis by a resulting impairment in the phagocytic activity of hepatic RE cells with no alteration in the uptake of colloidal carbon by splenic or pulmonary RE cellular elements (*7*). No losses of hepatic and splenic RE cellular elements were observed in mice treated with methyl palmitate.

The liver, which is not directly involved in the formation of antibody, participates in the phagocytosis and subsequent intracellular digestion of particulate antigenic material (*17*). The solubilized antigen is then made available to the antibody-forming cells (*18*). If the process of phagocytosis and intracellular solubilization is reduced by methyl palmitate, little or no solubilized antigen is available to react with antibody-producing cells and the hemolysin titers would be correspondingly reduced.

Although the underlying mechanisms of the effects of glucan and methyl palmitate are as yet unknown, there is a unique relation between the functional state of the RES and the immune response to a particulate antigen. A decisive factor in the early acceptance or rejection of marrow grafts in lethally irradiated mice is the functional state of the RES of the host (*19*). Moreover, from the results reported here this rejection of allogenic and xenogenic bone marrow in RE hyperfunctional mice could be due to hyperphagocytosis, a hyperimmune response, or a combination of both factors.

W. R. Wooles
N. R. Di Luzio

Department of Physiology, University of Tennessee Medical Units, Memphis 3

References and Notes

1. B. N. Halpern, G. Biozzi, B. Benacerraf, C. Stiffel. *Am. J. Physiol.* **189**, 520 (1957).
2. J. C. Cutler, *J. Immunol.* **84**, 416 (1960).
3. B. N. Halpern, *J. Pharm. Pharmacol.* **4**, 321 (1959).
4. K. Berglund, *Acta Pathol. Microbiol. Scand.* **38**, 311 (1956).
5. L. Hektoen and H. J. Cooper, *J. Infect. Diseases* **26**, 320 (1920).
6. A. E. Stuart, G. Biozzi, C. Stiffel, B. N. Halpern, D. Mouton, *Brit. J. Exptl. Pathol.* **41**, 599 (1960).
7. N. R. Di Luzio and W. R. Wooles, *J. Reticuloendothelial Soc.* (Academic Press, New York, in press), vol. 1.
8. W. R. Wooles, E. E. Elko, N. R. Di Luzio, *Radiation Res.* **16**, 546 (1962).
9. B. N. Halpern, G. Biozzi, G. Mene, B. Benacerraf, *Ann. Inst. Pasteur* **80**, 582 (1951).
10. N. Gengozian, T. Makinodan, C. C. Congdon, R. T. Owen, *Proc. Natl. Acad. Sci. U.S.* **44**, 560 (1958).
11. F. J. Di Carlo, L. J. Haynes, S. G. Malament, G. E. Phillips, *Can. J. Biochem. Physiol.* **41**, 731 (1963).
12. H. Balner, L. J. Old, D. A. Clarke, *Proc. Soc. Exptl. Biol. Med.* **109**, 58 (1962).
13. B. Benacerraf, B. N. Halpern, G. Biozzi, C. Stiffel, D. Mouton, *Brit. J. Exptl. Pathol.* **38**, 35 (1957).
14. O. Fusen, in *Reticuloendothelial Structure and Function*, J. Heller, Ed. (Ronald, New York, 1960), p. 3.
15. G. J. Thorbecke and B. Benacerraf, *Progr. Allergy* **6**, 559 (1962).
16. A. E. Stuart and G. N. Cooper, *J. Pathol. Bacteriol.* **83**, 245 (1962).
17. L. L. Miller and W. F. Bale, *J. Exptl. Med.* **99**, 125 (1954).
18. D. H. Campbell and J. S. Garvey, *Lab. Invest.* **10**, 1126 (1961).
19. W. R. Wooles and N. R. Di Luzio, *Am. J. Physiol.* **203**, 404 (1962).
20. Supported by Atomic Energy Commission and U.S. Public Health Service H-05367 and U.S. Public Health Service predoctoral fellowship award (W.R.W.).

8 August 1963.

Gamma-Globulins: Quantitative Relationships in Human Serum and Nonvascular Fluids

Abstract. *Three types of γ-globulins, γ_2, γ_{1A}, and γ_{1M}, are present in certain body fluids and secretions in proportions significantly different from those of normal human serum. Although γ_{1A}-globulin is present in only small amounts in serum, it represents a major fraction of the gamma globulin of tears, bile, saliva, colostrum, and fluid of the small intestine.*

Three types of gamma globulin, designated γ_2, γ_{1A}, and γ_{1M}, are present in normal human serum (*1*) in the approximate proportions of 85:10:5. While the majority of serum antibodies are γ_2 and γ_{1M}, certain antibodies have been identified as γ_{1A}-globulins (*2*). Studies by immunoelectrophoretic and gel-diffusion techniques with antiserums to gamma globulin and to whole human serums have shown that gamma globulin is present in many fluids and secretions (colostrum, aqueous humor, bile, parotid, synovial, bronchial, nasal, amniotic, and cerebrospinal fluids). While γ_2 and γ_{1M} were demonstrated in many of these fluids, γ_{1A} has not been clearly distinguished from the other globulins and the relative amounts of the various globulins present have not been measured quantitatively.

Recently, Tomasi and Zigelbaum (*3*) noted that γ_{1A} was the predominant gamma globulin of parotid saliva. They postulated a specific and highly selective transport mechanism in the passage of γ_{1A} from serum to secretions or, alternately, the local synthesis of γ_{1A}.

Fluids and secretions free from contamination with blood were collected from a variety of noninfected sources. The types of gamma globulin present were measured by a two-dimensional gel-diffusion technique (*3*). The test fluids diffuse from a well into an agar gel containing an antiserum specific for the component to be measured. The concentration of gamma globulin is