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Effect of *Streptococcus pneumoniae* Infection on the Synthesis of Rat Liver Plasma Membranes[1] (41165)

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Abstract. The incorporation of [^{3}H]leucine into plasma membranes isolated from liver homogenates of control and *Streptococcus pneumoniae*-infected rats was studied. Rats were injected with [^{3}H]leucine and at selected times after injection, specific and total activities of the label were determined in isolated plasma membranes. During this pneumococcal infection, there was a significant increase in the incorporation of label into plasma membranes. These results suggest increased synthesis of liver cell plasma membranes during *S. pneumoniae* infection. The possible significance of these results is discussed.

A *Streptococcus pneumoniae* infection in rats has been shown to induce marked alterations in systemic host metabolism (1–3). These changes include altered hepatic nucleic acid (4, 5), carbohydrate (6), lipid (7, 8), and protein metabolism (2, 4, 9–11), as well as increased uptake of zinc (12) and amino acids (10, 13). Plasma insulin and glucagon are also elevated during this infection, and may affect hepatic carbohydrate metabolism (14, 15). *S. pneumoniae* infection also alters the hepatic enzyme composition (7, 16) as well as the number (17) and equilibrium density (18) of hepatic cellular organelles.

During bacterial infection in rats, hepatic synthesis of secretory proteins is increased (9, 10, 19). In addition, there is increased transport, packaging, and secretion of these proteins into the circulation (20). A significant increase in liver weight during *S. pneumoniae* infection in rats also occurs (20, 21). This increase results from an increase in both hepatic water content and hepatic dry weight (22). It was hypothesized that the increased synthesis, transport, packaging, and secretion of plasma proteins which occurs during *S. pneumoniae* infection (20) required increased synthesis of intracellular organelles involved in these processes. As an initial test of this hypothesis, the effect of *S. pneumoniae* infection on the synthesis of hepatic plasma membranes was determined because of the known involvement of hepatic plasma membranes in the secretory process (23). *S. pneumoniae* was chosen because it has no known toxin and is generally not considered to produce liver damage.

Materials and Methods. *Chemicals.* L-[4,5-^{3}H(*N*)]Leucine (5 Ci/mmole) and Liquifluor were obtained from New England Nuclear (Boston, Mass.). Scintisol complete was obtained from Isolab, Inc. (Akron, Ohio). All other chemicals were of analytical grade when available and were obtained from commercial sources.

Animals. Male Sprague–Dawley rats (260–280 g) were obtained from Charles River and maintained on Wayne LabBlox (Peoria, Ill.) and tap water *ad lib.* All rats were acclimatized to a 12-hr day–night cycle for 12 days prior to experimentation to standardize circadian variations and to permit convenient sampling of the animals during the dark or active phase. Rats were inoculated sc with 3×10^5 to 6×10^5 heat-killed (control) or virulent (infected) colony forming units (CFU) of *S. pneumoniae*,

[1] In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

[2] The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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type I. This dose routinely killed approximately 80% of the inoculated rats by 5 days postinoculation. Following inoculation, control and infected rats were fasted for 40 hr prior to injection with radioisotope and killed at the times indicated in the figure legends. All rats were killed between 8:00 AM and 10:00 AM, a time roughly corresponding to the midpoint of the night cycle.

Administration of radioactive isotope. Rats were anesthetized with halothane and injected iv in the dorsal vein of the penis with 100 μCi [^{3}H]leucine in 0.3 ml of 0.9% NaCl. At various time intervals after injection of radioisotope, rats were killed. Livers were removed, weighed, and placed in ice-cold buffer.

Plasma membrane isolation. Plasma membranes from control and infected rats were isolated and characterized as previously described (16).

Preparation of plasma membranes for the determination of protein and radioactivity. Radiolabeled plasma membranes isolated from control or infected rats were washed by suspension and recentrifugation in 0.25 *M* sucrose containing 10 m*M* L-leucine and 20 m*M* Tris–chloride (pH 7.5). The cold leucine was added to remove any absorbed radioactive leucine. The Tris–chloride wash was included to remove adsorbed cytoplasmic proteins (24). It was shown previously (20) that greater than 90% of the radioactivity in these hepatic homogenates was precipitated in 10% trichloroacetic acid and that less than 1% was solubilized when either lipid (25) or RNA (26) was extracted. Therefore, lipid or RNA extraction from labeled plasma membranes prior to the determination of radioactivity was not necessary. Washed plasma membranes were suspended in water and assayed as described below for protein and radioactivity.

Antibody studies. Golgi, plasma membranes, and plasma from control or infected rats were labeled to maximal specific activity with [^{3}H]leucine and isolated as previously described (20). Plasma ($\leq$15.0 mg protein/ml) and Golgi ($\leq$1.0 mg protein/ml) were adjusted to 1% (v/v) Triton X-100 by the addition of equal volumes of 2% (v/v) Triton X-100. Plasma membranes ($\leq$0.5 mg protein/ml) were adjusted to 1% Triton X-100, 0.5% (w/v) sodium deoxycholate in a similar manner. Samples were then sonicated three times for 10 sec each time at a setting of 4.5 with a W185D cell disruptor (Heat Systems, Ultrasonics Inc., Plainview, N.Y.) and then incubated at 37° for 30 min. This treatment completely solubilized all samples.

Antigen–antibody reactions were carried out in Beckman Model 152 microfuge tubes (Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.) in a total volume of 400 μl. Labeled Golgi, plasma, or plasma membranes were added to each tube such that each tube contained a total of 2000 counts per minute (cpm). Anti-rat serum prepared in rabbits (Microbiological Associates, Bethesda, Md.) was added in increasing amounts as shown in the figure legends. Control rabbit serum in 1% (v/v) Triton X-100 was added to each tube to keep the protein concentration constant. In control experiments, anti-turkey serum prepared in rabbits (Microbiological Associates) replaced the anti-rat serum. After addition of antibody the mixture was incubated with shaking for 2 hr at 37°. Following incubation, the tubes were centrifuged at maximum speed for 10 min in a Beckman Model 152 microfuge. The resulting pellets were washed twice with 1% (v/v) Triton X-100 and recentrifuged. The tips of the microfuge tubes containing the antigen–antibody precipitates were cut off and incubated overnight in 0.5 ml of NCS tissue solubilizer (New England Nuclear) and radioactivity was determined as described below.

Scintillation spectrometry. All samples were counted in a Mark II liquid scintillation spectrophotometer (Searle, Des Plaines, Ill.). Plasma membranes were counted in 5.0 ml of Scintisol complete. Antigen–antibody complexes were counted in 20 ml of toluene containing 0.84 ml Liquifluor. Counting efficiency for all samples was approximately 40%.

Analytical procedures. Protein was assayed by the method of Lowry *et al.* (27) using bovine serum albumin as a standard.

Statistics. Group mean values were compared by the Student's *t* test; the difference

between the two means was considered significant at $P < 0.05$.

Results. Figure 1A shows the effect of *S. pneumoniae* infection on the incorporation of [^{3}H]leucine into plasma membranes isolated from rat liver homogenates. As can be seen, there was a significant increase in the specific activity of the isotope incorporated into the membranes isolated from infected rats, compared to uninfected controls.

Figure 1B shows the same results when total liver plasma membrane activity is plotted rather than specific activity. The total radioactivities in Fig. 1B were calculated by multiplying the specific activities (cpm/mg plasma membrane protein) shown in Fig. 1A by the plasma membrane yield (mg plasma membrane protein isolated/total wet weight of liver. These results, therefore, represent the total amount of radioactivity associated with hepatic plasma membranes which could be isolated per liver.

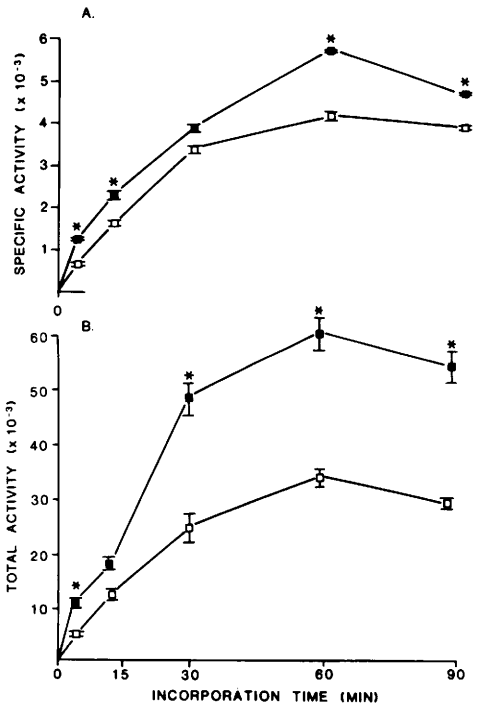

FIG. 1. Incorporation of [^{3}H]leucine into plasma membrane isolated from control (□) and infected (■) rats. Each value is the mean of 6 rats ± SE. (A) Specific activity is expressed as cpm/mg plasma membrane protein. (B) Total activity is expressed as specific activity × plasma membrane yield (mg plasma membrane protein isolated/total wet weight of liver). *$P < 0.05$.

It was next shown that radioactivity in the plasma membrane preparations was due to labeled plasma membrane proteins and not to contaminating absorbed serum proteins. To accomplish this, plasma membrane preparations from control and infected rats were treated with anti-rat serum to determine if any radioactivity could be precipitated. It was first necessary, however, to show that labeled plasma from both control and infected rats could be precipitated with anti-rat serum. Results in Fig. 2 show that approximately 80% of the radioactivity was precipitated when labeled control or infected plasma was treated with anti-rat serum, but none when anti-turkey serum was used as a control. It should be noted that equal amounts of radioactivity were added to each assay system (2000 cpm/assay) for both control and infected plasma. Equal amounts of plasma protein were not added, because of the observation that infected plasma had a much higher specific activity (20).

Since plasma membrane preparations contain both sheets and membrane-bound vesicles, they were treated with detergent to completely solubilize all protein. This was necessary to permit antibody access to antigen contained within membrane-bound vesicles. To determine if the detergent concentrations used affected the precipitation of antigen by antibody, rat plasma labeled and prepared as previously described (20)

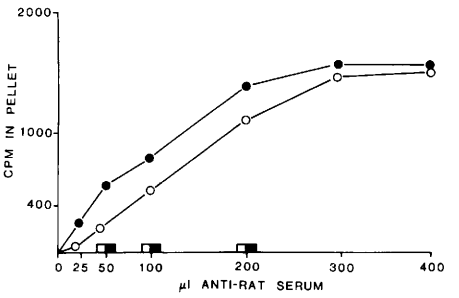

FIG. 2. Treatment of control (○) or infected (●) plasma with anti-rat or anti-turkey serum (■). A total of 2000 cpm for control or infected plasma membranes was incubated with anti-rat serum as described under Materials and Methods. Results are those of one representative experiment.

was treated with increasing amounts of anti-rat serum in the presence or absence of detergent. Treatment with sodium deoxycholate (DOC) and Triton X-100 had no significant effect on the precipitation of antigen by antibody.

An additional control was necessary to determine whether possible contaminating serum proteins were antigenic by the time they reached the plasma membrane. The intracellular secretory route of serum proteins is from the Golgi secretory vesicles to the plasma membrane to the circulation (19, 23, 28). Therefore, if serum proteins could be precipitated from Golgi and plasma with antibody, then any contaminating serum proteins in the plasma membrane preparations should be precipitated.

When radiolabeled Golgi (20) were treated with anti-rat serum (Fig. 3), approximately 50% of the radioactivity was precipitated. However, when radiolabeled plasma membranes were solubilized and treated with anti-rat serum, no radioactivity was precipitated (Fig. 3).

Discussion. There is a significant increase in the incorporation of [^{3}H]leucine into plasma membranes isolated from rat liver during *S. pneumoniae* infection (Fig. 1A). There is also a significant increase in the weight of the liver during *S. pneumoniae* infection (20, 21). Since the same amount of plasma membranes could be isolated per gram of liver (yield) from control and infected rats (16), significantly more isotope was incorporated into total liver plasma membranes isolated from infected rats (Fig. 1B). Comparisons between control and infected plasma membrane preparations are valid, since the yield, purity, and recovery of plasma membranes isolated from control and infected rat liver homogenates are the same (16).

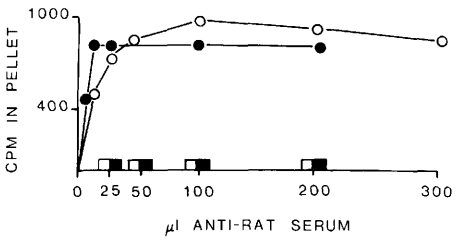

FIG. 3. Precipitation of control (○) or infected (●) Golgi and control (□) or infected (■) plasma membranes by anti-rat serum. A total of 2000 cpm for control or infected Golgi or plasma membranes was incubated with anti-rat serum. Results are those of one representative experiment.

However, before it can be concluded that this increased incorporation of label represents increased synthesis of the plasma membrane, it is necessary to know the effect of infection on the hepatic leucine pool size. The observed increased specific activity of plasma membranes during infection could result from a decreased hepatic free amino acid pool size. There is no change, however, in the leucine or free amino acid pool size of the liver 28 to 52 hr after inoculation with *S. pneumoniae* (10, 13). These results argue against the possibility that the increased incorporation of [^{3}H]leucine during infection is a consequence of a decreased leucine pool size and support the hypothesis of increased liver cell plasma membrane synthesis during *S. pneumoniae* infection.

It is also possible that the results simply reflect increased turnover of plasma membrane proteins and reutilization of label during infection. Unfortunately, it is not possible to test this hypothesis, since infected rats die before turnover studies can be completed. However, the average turnover value for control plasma membrane proteins is between 2 and 3 days (27). Since our incorporation studies continued for only 90 min after the injection of isotope, it seems unlikely that turnover of plasma membrane proteins and reutilization of isotope would present a serious problem.

The plasma membrane preparations employed in these studies were relatively pure by both biochemical and morphological criteria (16). However, since secretory proteins have a tendency to adsorb to membranes (30, 31), it could be argued that some of the radioactivity in the preparations was due to plasma proteins either adsorbed to, or in small vesicles coisolating with the plasma membranes. Since increased synthesis of acute-phase globulins occurs during infection (9, 10, 20, 32, 33), the increased incorporation of label into plasma membranes during infection could be explained on the basis of acute-phase

globulin contamination. It should be noted, however, that all plasma membrane preparations were washed in 20 m*M* Tris–chloride (pH 7.5) which has previously been shown to remove adsorbed proteins (24).

In addition, anti-rat serum precipitated labeled proteins from Golgi and plasma but not from the labeled plasma membrane preparations (Figs. 2 and 3). In both control and infected rats, serum proteins are synthesized on the rough endoplasmic reticulum, transported through the smooth endoplasmic reticulum to the Golgi, make their way to the plasma membrane, and finally to the circulation (23, 28). Since anti-rat serum precipitated serum proteins in the Golgi and also in the plasma, any contaminating serum proteins in the plasma membrane preparations would also be expected to be precipitated. It was not anticipated that all the radioactivity would be precipitated from the Golgi preparations, since a portion of the radioactivity in this fraction is expected to be in the Golgi membranes rather than in the secretory content. Therefore, the results demonstrated that the radioactivity in the isolated plasma membrane preparations from both control and infected rats was due to label incorporated into plasma membrane proteins and not to contamination by plasma proteins.

It could also be argued that antibody to infected rat serum should have been used to titrate infected Golgi, plasma, and plasma membranes. However, it was shown that control anti-rat serum precipitated 80% of the radioactivity from either control or infected plasma. It is unlikely that 100% of the radioactivity would be precipitated from infected plasma even if antibody were prepared to infected serum, since only 80% of the label could be precipitated from control plasma with control anti-rat serum. Since conclusions drawn from the antibody studies would be the same whether 80 or 100% of the radioactivity were precipitated, commercially prepared control anti-rat serum was used.

Finally, it should be noted that hepatic plasma membrane preparations are heterogeneous, since liver contains both parenchymal and reticuloendothelial (Kupffer) cells. Since the liver is heterogeneous with respect to cell type, the results reported here could be due to alterations in parenchymal cells, Kupffer cells, or both. However, as previously reported (16), the contribution of plasma membranes from Kupffer cells is expected to be less than 7%. Therefore, it is anticipated that the majority of the plasma membranes are derived from parenchymal cells. Also, examination of these plasma membrane preparations by electron microscopy revealed many intercellular specializations (16) which are derived from parenchymal cells. In addition, when control and infected livers were examined histologically, a significant increase in the diameter and volume of the parenchymal cell was observed. Comparable changes were not observed in Kupffer cells (22). Further, protein synthesis by the Kupffer cells constitutes only about 5% of liver protein synthesis (34). Although the observed changes in the incorporation of isotope into plasma membrane could be due to Kupffer cell plasma membranes, it seems more likely that they were due primarily to changes in parenchymal cells.

The reason for the increased synthesis of the plasma membrane during infection has not been determined. There is, however, an increase in synthesis, transport, packaging, and secretion of plasma proteins during infection (20). This phenomenon may require increased synthesis of the intracellular organelles involved in the process. Since Golgi secretory vesicles fuse with plasma membranes (23, 35) and release their contents into the circulation, it may be necessary to synthesize more plasma membrane to accommodate for this process during periods of enhanced secretory activity. Experiments are in progress to determine if the synthesis of other intracellular organelles involved in secretion is altered during *S. pneumoniae* infection.

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