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Biochemical and cytological aspects of liver cell function during infection

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I. INTRODUCTION

The primary emphasis of this chapter is on the effects of bacterial infection on the liver only because it is about this model that we have the most information. It should be noted that no attempt is made to review all the literature in this field. We do, however, attempt to present a general picture of what effects infection has on hepatic constituents, components and metabolism.

The production of microbial exotoxins, the presence of lipopolysaccharide endotoxins and differences in distribution of microorganisms among different target tissues can modify the generalized host response and make difficult the differentiation between changes which are pathophysiological and those which are typically part of the host defense mechanisms. For these reasons many of the studies focused on in this chapter were carried out in *Streptococcus pneumoniae*-infected rats. This experimental model represents an extracellular infection characterized by the absence of lipopolysaccharide and protein toxins so that changes in liver functions more closely represent nonspecific host adaptations to infection and inflammation.

It is not certain how the myriads of changes observed in the liver during such a model infection are mediated. It is believed, however, that phagocytosis of invading microorganisms by host phagocytes results in the release of endogenous mediators which subsequently initiate a wide range of hepatic metabolic alterations. These endogenous mediators may produce their effects by acting directly on the liver (Rupp and Fuller, 1979; Wannemacher et al., 1979), indirectly by stimulating the release of insulin, glucagon (George et al., 1975) and other hormones, or via the central nervous system (Bailey et al., 1976).

II. EFFECTS OF INFECTION ON HEPATIC CONSTITUENTS

A. *Water content and dry weight*

S. pneumoniae is a highly virulent pathogen for the rat. Within 12—24 h after exposure to the organism, rats become anorectic and lose significant amounts of body weight. In spite of this, the liver increases in weight by more than 30% in 48 h, both water and dry weight contributing to the increase (Table 1). The accumulation of water is limited to the intracellular compartment with no significant change in the extracellular component (Wannemacher et al., 1974). When expressed on the basis of per gram of liver, the concentration of water is found to increase, whereas that of the dry weight decreases significantly (Table 1). The increase in hepatic water appears to result from a shift of Na^+ and K^+ across the cell membrane, possibly as a result of a change in the integrity of the liver cell plasma membrane.

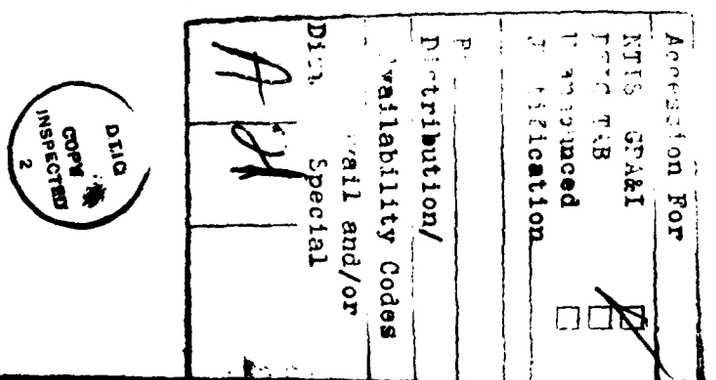
Dry weight accounts for about 16% of the weight increase of the liver during

TABLE 1

EFFECT OF *S. PNEUMONIAE* INFECTION ON RAT LIVER PARAMETERS

Group	Body weight		Liver wet weight	Liver dry weight			Water content			Hepatocyte	
	0 h	48 h	(g)	g	%[a]	mg/g[b]	g	%	mg/g	Diameter (μm)	Volume (μm³)
Pair-fed control	268 ±1.03	228 ±0.81	9.58 ±0.23	2.27 ±0.04	23.7 ±0.85	237 ±3	7.32 ±0.20	76.4 ±0.32	764 ± 3	17.68 ±0.30	3039 ±153
Infected	265 ±5.84	229 ±2.02	12.25 ±0.36	2.68 ±0.07	21.9 ±0.42	219 ±4	9.56 ±0.32	78.0 ±0.52	783 ±5	21.07 ±0.25	5259 ±207
$P <$	n.s.	n.s.	0.001	0.001	0.001	0.005	0.005	0.001	0.025	0.01	0.001

[a]Percent of total liver weight.
[b]mg/g wet weight.
Parameters were assessed 48 h after inoculation with $3 \cdot 10^6$ heat-killed (control) or live (infected) bacteria. P values are for comparison of infected to control rats by unpaired Student's t-test. Data from Little et al. (1981).
n.s., not significant; $n > 6$.



infection. A portion of this increased mass may be contributed by an increased concentration of intracellular secretory proteins in transport through the liver cell (Little, 1978). The weight gain may also be due in part to an increase in the biosynthesis of the liver cell. The enhanced synthesis, transport, packaging and secretion of plasma proteins by the liver during sepsis appear to require accelerated biogenesis of intracellular organelles involved in these processes (Little, 1978).

B. *Ions*

Little et al. (1981) have shown that the total, intracellular, and extracellular Na^+ and total Cl^- concentrations in the liver are not significantly altered in *S. pneumoniae*-infected rats when these are expressed as milliequivalents of electrolyte per kilogram of fat-free wet tissue (Table 2). However, when the data are expressed as milliequivalents per kilogram of fat-free dry weight, significant increases in total Na^+ and Cl^- concentrations are noted. Total and intracellular liver K^+ concentrations, on the other hand, decrease markedly. As previously mentioned, the increased hepatic water appears to result from this shift in Na^+ and K^+ across the liver cell plasma membrane, since Cl^- and water are expected to follow passively with Na^+ according to the principles of bioelectrical balance and osmosis. The movement of water, therefore, helps maintain constant intracellular concentrations of Na^+ and Cl^-, while the total hepatic content of these ions increases.

C. *Macromolecules*

Of all the chemical constituents, only the concentration of hepatic RNA increases significantly during sepsis (Table 3). However, when the total content of chemical constituents is determined for the entire liver, significant increases are noted in all constituents except DNA (Table 3). This suggests that cell division or mitotic activity is not altered by infection. In fact, histological studies reveal that the frequency of mitotic activity in control and infected hepatocytes is equal.

Elevated production of RNA by the liver has been observed during infection and will be discussed in the next section. Increases in hepatic lipid synthesis are equally well documented. For example, the synthesis of triglycerides from acetate increases markedly in liver slices from rabbits infected with *S. pneumoniae* (Shieh, 1976). Hepatocytes isolated from *S. pneumoniae*-infected rats incorporate significantly more [^{14}C]acetate into cholesterol and various lipid fractions than do hepatocytes from control rats (Canonico et al., 1977a). Enhanced cholesterogenesis is found in rhesus monkeys infected with either *S. pneumoniae* or *Salmonella typhimurium* (Fiser et al., 1971). Increased hepatic lipid synthesis also accompanies rickettsial infection. Total liver triglycerides, cholesterol and free (unesterified) fatty acids increase during Q fever in guinea-pigs (Paretsky et al., 1964; Bernier et al., 1974).

TABLE 2

ELECTROLYTE CONCENTRATIONS (meq/kg) IN LIVER OF CONTROL AND *S. PNEUMONIAE*-INFECTED RATS

	Na^+				Cl^-		K^+			
	Wet weight[a]			Dry weight[b]	Wet weight	Dry weight	Wet weight			Dry weight
Group	T	I	E	(T)	(T)	(T)	T	I	E	(T)
Pair-fed control	29.64 ±0.58	2.84 ±1.07	26.85 ±0.78	104.1 ±3.22	27.7 ±0.80	97.0 ±3.38	99.12 ±1.70	98.4 ±1.69	0.71 ±0.02	346.7 ±3.92
Infected	31.01 ±0.77	2.20 ±0.76	29.14 ±1.37	123.9 ±3.19	27.4 ±0.89	109.5 ±3.75	93.35 ±1.77	92.4 ±1.79	0.99 ±0.07	313.4 ±7.30
$P <$	n.s.	n.s.	n.s.	0.005	n.s.	0.05	0.05	0.05	0.005	0.01

[a]Fat-free wet liver weight.
[b]Fat-free dry liver weight.
See legend to Table 1. T, total; I, intracellular; E, extracellular; n.s., not significant; $n = 6$. Data from Little et al. (1981).

TABLE 3

EFFECT OF *S. PNEUMONIAE* INFECTION ON RAT LIVER CHEMICAL CONSTITUENTS

	Protein		Lipid		RNA		Carbohydrate		DNA	
Group	g[a]	mg/g[b]	g	mg/g	g	mg/g	g	mg/g	g	mg/g
Pair-fed control	1.26 ±0.03	132 ±3	0.377 ±0.029	39 ±3	0.117 ±0.008	12.2 ±0.8	0.067 ±0.004	1.4 ±0.03	0.014 ±0.0004	9 ±0.8
Infected	1.60 ±0.08	130 ±3	0.493 ±0.024	40 ±3	0.176 ±0.004	14.4 ±0.8	0.11 ± 0.008	1.4 ±0.03	0.015 ±0.0007	10 ±0.6
$P <$	0.001	n.s.	0.01	n.s	0.001	0.025	0.001	n.s.	n.s.	n.s.

[a]per total liver.
[b]mg/g wet tissue.
See legend to Table 1. Data from Little et al. (1981).

The higher content of hepatic protein, lipid, RNA and carbohydrate during infection may be the result of increased synthesis, decreased degradation, or decreased secretion of these macromolecules. Since increased incorporation of labeled precursors into all these constituents occurs during sepsis (Thompson and Wannemacher, 1973; Wannemacher et al., 1974; Canonico et al., 1977a), and secretion appears to be increased rather than decreased (Little, 1979), it is likely that the observed accumulation of these chemical constituents is the result of increased synthesis.

D. *Enzymes*

The enzymatic complement of the liver is markedly altered by the infectious process. Changes in activity for representative enzymes of nearly all cellular organelles testify to the major adaptation which the liver undertakes in order to meet physiological and biochemical demands made by the disease process (Table 4). These changes are not limited to enzymatic constituents, but apply to physical properties of cellular organelles as well. Unraveling and characterizing these complex changes have been attempted by applying tissue fractionation techniques to dismantle the cellular machinery, isolate its various components and establish their functions and properties by physical and chemical methods. Subsequent sections of this chapter will deal in some detail with the changes in enzyme complement and physical properties of hepatic organelles during infection.

E. *Trace metals*

Infection-induced alterations in trace metal metabolism during infection are discussed in Chapter 6 of this volume. With respect to the liver, both iron and zinc appear to be taken up by the liver (Pekarek et al., 1972a), iron into ferritin (Konijn and Hershko, 1977), and zinc, at least in part, into metallothioneins (Sobocinski et al., 1978). Copper appears to be associated with a serum protein, ceruloplasmin, synthesized by the liver (Markowitz et al., 1955; Pekarek et al., 1972b).

It is not clear why alterations in trace metal metabolism occur so abruptly and so consistently at the onset of infection. It is possible that these changes contribute in some manner to host defense. Zinc appears to be involved in wound healing (Sandstead et al., 1970; Rahmat et al., 1974; Frommer, 1975) and may also be sequestered in the liver in an attempt to protect the liver from harmful toxins (Snyder and Walker, 1976; Sobocinski et al., 1976). Also, since zinc is required for both hepatic RNA polymerase activity (Terhune and Sandstead, 1972) and for the synthesis of proteins (Hsu et al., 1969), zinc may move to the liver during infection for the accelerated synthesis of nucleic acids and proteins (cf. Section IV). Since iron is required for optimal growth of a number

TABLE 4

HEPATIC ENZYMATIC RESPONSES TO INFECTIONS

Enzyme (EC No.)	Observed change[a]	Organism or illness	Host species	Reference[b]
N-Acetyl-ß-glucosaminidase (3.2.1.30)	0	*S. pneumoniae*	rat	Foulke[c]
N-Acetylglucosaminyltransferase	+	*S. pneumoniae*	rat	12
Acid phosphatase (3.1.3.2)	0	*S. pneumoniae*	rat	8, 10, 25
Aconitate hydratase (4.2.1.3)	–	*F. tularensis*	rat	48
Adenylate cyclase (4.6.1.1)	–	*S. pneumoniae*	rat	52, 53
Adenosinetriphosphatase (3.6.1.3)	+	*M. tuberculosis*	mouse	46
Alkaline phosphatase (3.1.3.1)	+	*S. pneumoniae*	rat	25
	0	*S. pneumoniae*	rat	11
	–	*S. pneumoniae*	mouse	27
	–	Rinderpest	rabbit	19
Arginase (3.5.3.1)	+	Salmonellosis	chick	36
	+	*Fascioliasis*	rabbit	35
Aspartate aminotransferase (2.6.1.1)	0	*S. pneumoniae*	rat	10
	+	*F. tularensis*	rat	47
Catalase (1.11.1.6)	–	*S. pneumoniae*	rat	10
Cathepsin D (3.4.23.5)	+	*S. pneumoniae*	rat	10
Cholinephosphotransferase (2.7.8.2)	0	*S. pneumoniae*	rat	Hauer[c]
3′:5′-Cyclic-AMP phosphodiesterase (3.1.4.17)	0	*S. pneumoniae*	rat	52, 53
Cytochrome oxidase (1.9.3.1)	0	*S. pneumoniae*	rat	10
	–	*M. tuberculosis*	mouse	20
Esterase	–	*S. pneumoniae*	rat	11
Fructose-bisphosphatase (3.1.3.11)	0	Endotoxemia	mouse	28
	0	Endotoxemia	rat	24
	0	*P. pseudomallei*	rat	23
α_2-Fucosyltransferase	+	*S. pneumoniae*	rat	12
α_3-Fucosyltransferase	+	*S. pneumoniae*	rat	12
Fumarate hydratase (4.2.1.2)	–	*F. tularensis*	rat	49
Galactosyltransferase	+	*S. pneumoniae*	rat	12
Glucose-6-phosphatase (3.1.3.9)	–	*S. pneumoniae*	rat	11, 25
	–	*P. pseudomallei*	rat	49
	–	*M. tuberculosis*	mouse	47
	+	*M. tuberculosis*	guinea-pig	38—40, 42
	+	Canine distemper	dog	30
	–	Endotoxemia	rat	24
	–	Endotoxemia	mouse	22, 28
Glucosephosphate isomerase (5.3.1.9)	+	*F. tularensis*	rat	49
	+	Salmonellosis	rat	49
ß-Glucuronidase (3.2.1.31)	+	*S. pneumoniae*	rat	10
	+	*Mycoplasma* sp	mouse	18
Glutamate dyhydrogenase (1.4.1.2)	–	*S. pneumoniae*	rat	Foulke[c]
	0	*S. pneumoniae*	rat	25
α-Glycerophosphate dehydrogenase (3.1.3.1)	+	*S. pneumoniae*	rat	17
Glycogen phosphorylase (2.4.1.1)	+	*S. pneumoniae*	rat	13. 14, 16
	0	Salmonellosis	rat	15

Glycogen synthetase *a* kinase (2.7.1.37)	+	Q fever	guinea-pig	31, 32, 44
	−	*S. pneumoniae*	rat	13, 14, 16
	−	Salmonellosis	rat	15
	−	Q fever	guinea-pig	31, 32, 44
Hexokinase (2.7.1.1)	−	*M. tuberculosis*	guinea-pig	7
Hydroxymethylglutaryl-CoA reductase (1.1.1.88)	+	*S. pneumoniae*	rat	11
Inosine 5′-diphosphatase	0	*S. pneumoniae*	rat	11
Isocitrate dehydrogenase (1.1.1.41)	+	*F. tularensis*	rat	49
	+	Salmonellosis	rat	49
Lactate dehydrogenase (1.1.2.3)	−	*M. tuberculosis*	guinea-pig	50
	0	*S. pneumoniae*	rat	8,10
Malate dehydrogenase (1.1.1.37)	0	*S. pneumoniae*	rat	25
Monoamine oxidase (1.4.3.4)	−	*S. pneumoniae*	rat	33
NADH dehydrogenase (1.6.99.3)	±	*M. tuberculosis*	mouse	20
NAD^+glycohydrolase (3.2.2.5)	+	*M. tuberculosis*	mouse	20, 45
	+	Listeriosis	mouse	28, 29
NADH cytochrome *c* reductase	+	*S. pneumoniae*	rat	11
NADPH-cytochrome *c* reductase (1.6.4.6)	−	*S. pneumoniae*	rat	11
NAD(P)H dehydrogenase (1.6.99.2)	±	*M. tuberculosis*	mouse	20, 46
5′-Nucleotidase (3.1.3.5)	−	*S. pneumoniae*	rat	8, 10, 11, 25
Phenylalanine hydroxylase (1.14.16.1)	−	*F. tularensis*	rat	34
Phosphoglucomutase (2.7.5.1)	−	*M. tuberculosis*	guinea-pig	42
Pyruvate kinase (2.7.1.40)	0	*Pseudomonas* sp.	rat	23
	0	Endotoxemia	rat	24
RNA nucleotidyltransferase (2.7.7.6)	−	VEE virus	mouse	21, 26
Sialyltransferase (2.4.99.1)	+	*S. pneumoniae*	rat	12
Succinate dehydrogenase (1.2.99.1)	+	*M. tuberculosis*	rat	45, 50
	−	Endotoxemia	guinea-pig	24
Succinic oxidase (1.3.1.6)	+	*M. tuberculosis*	mouse	20, 37
Tryptophan oxygenase (1.13.1.11.11)	±	Salmonellosis	mouse	3, 4, 43
	+	*S. fecalis*	rat	51
Tyrosine aminotransferase (2.6.1.5)	+	*S. pneumoniae*	rat	41
	+	Endotoxemia	mouse	1, 2, 4—6
	+	Endotoxemia	mouse	1, 2, 4—6
UDPglucose dehydrogenase (1.1.1.22)	−	*M. tuberculosis*	guinea-pig	40
UDPglucose-glycogen glucosyltransferase (2.4.1.11)	−	*M. tuberculosis*	guinea-pig	40, 42
	−	Q fever	guinea-pig	32
UDPglucose pyrophosphorylase (2.7.7.9)	±	*M. tuberculosis*	guinea-pig	40,42
Urate oxidase (1.7.3.3)	−	*S. pneumoniae*	rat	8, 10

[a] Change from normal activity: +, increase; −, decrease; 0, no change; ±, variable.
[b] References: (1) Berry, 1964; (2) Berry and Mitchell, 1954; (3) Berry and Smythe, 1960; (4) Berry and Smythe, 1964; (5) Berry and Smythe, 1966; (6) Berry et al., 1968; (7) Bhargava and Venkitasubramanian, 1965; (8) Canonico and White, 1974; (9) Canonico et al., 1975a; (10) Canonico et al., 1975b; (11) Canonico et al., 1977a; (12) Canonico et al., 1980; (13) Curnow and Pekarek, 1972; (14) Curnow and Rayfield, 1973a; (15) Curnow and Rayfield, 1973b; (16) Curnow et al., 1976; (17) DeRubertis and Woeber, 1972; (18) Gabridge et al., 1975; (19) Garg and Sharma, 1965; (20) Kato, 1966; (21) Kehoe and Lust, 1969; (22) Klimova, 1965; (23) LaNoue et al., 1968a; (24) LaNoue et al., 1968b; (25) Little, 1977; (26) Lust, 1966; (27) Lust and Beisel, 1967; (28) McCallum and Berry, 1972; (29) McCallum and Sword, 1972; (30) Newberne, 1966; (31) Paretsky and Stueckmann, 1966; (32) Paretsky et al., 1964; (33) Popenenkova, 1966; (34) Powanda et al., 1974; (35) Rijavec and Kurelec, 1966; (36) Ross et al., 1956; (37) Segal, 1966; (38) Shah, 1967; (39) Shah et al., 1967a; (40) Shah et al., 1967b; (41) Shambaugh and Beisel, 1968; (42) Singh et al., 1963; (43) Snyder, 1971; (44) Stueckmann and Paretsky, 1971; (45) Toida, 1966; (46) Windman et al., 1965; (47) Woodward and Mayhew, 1956; (48) Woodward and Miraglia, 1961; (49) Woodward et al., 1969; (50) Wyszomirska et al., 1967; (51) Yuwiler et al., 1966, (52) Zenser and DeRubertis, 1973; (53) Zenser et al., 1974.
VEE, Venezuelan equine encephalomyelitis.

of bacteria (Kirkpatrick et al., 1971; Weinberg, 1974), the redistribution of this trace metal to the liver during infection may make bacterial proliferation more difficult.

III. EFFECTS OF INFECTION ON HEPATIC COMPONENTS

A. *Nucleus*

Many of the alterations in nucleic acid metabolism reported to accompany infection involve the nucleus, but most studies done to date have been on rat liver homogenates rather than on isolated nuclei. Consequently, the involvement of the nuclei in these metabolic alterations is unclear. Hauer and Little (unpublished observations) have isolated and partially characterized liver cell nuclei from *S. pneumoniae*-infected rats. In these studies, control and infected nuclei appear morphologically similar when examined by electron microscopy but several biochemical differences are noted. For example, in an in vitro system developed to study RNA synthesis, it was shown that hepatic nuclei from infected rats incorporated significantly more labeled precursor into RNA than nuclei from control rats. Furthermore, the addition of insulin stimulates infected nuclei to synthesize RNA to a much greater extent than controls. These nuclei also bind labeled insulin with a greater affinity and have a higher number of insulin-binding sites than do control nuclei. Although speculative, these results suggest that there may be a causal relationship between the increased circulating plasma insulin levels which accompany *S. pneumoniae* infection (George et al., 1975) and increased hepatic RNA synthesis (Thompson and Wannemacher, 1973).

B. *Endoplasmic reticulum*

A number of biochemical and functional changes occur in the endoplasmic reticulum as a result of infection. First, there is an increase in the synthesis of acute-phase proteins (Koj, 1974). These secretory proteins are synthesized on the rough endoplasmic reticulum, transported through the smooth endoplasmic reticulum, packaged in the Golgi and secreted into the circulation (Palade, 1975; Little, 1978). It is not clear, however, if the increase in protein synthesis is due solely to an increase in precursors and substrates or if the capacity of the protein-synthesizing machinery of the liver is actually enhanced. In vitro studies have shown that liver microsomes isolated from *S. pneumoniae*-infected mice incorporate significantly more precursor into protein than microsomes from control mice (Lust, 1966). Hence, it appears that the endoplasmic reticulum acquires an increased capacity to synthesize proteins. Alternatively, other factors such as increased synthesis of messenger RNA could be involved. Increased RNA synthesis does occur during infection (Kehoe and Lust, 1969;

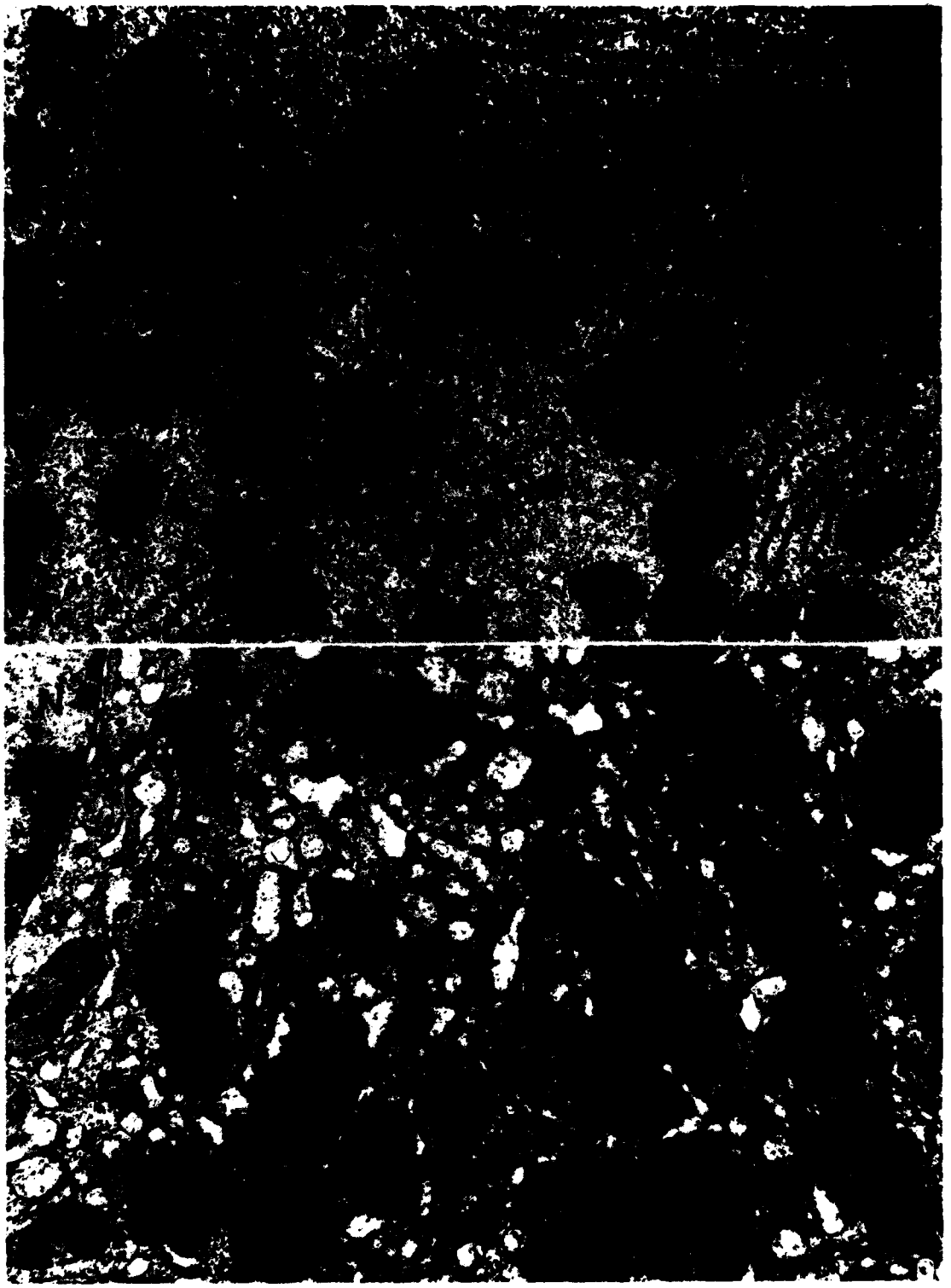


Fig. 1. Electron micrographs of control and experimental rat hepatic parenchymal cells. N, nucleus; M, mitochondria; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum. Calibration bar is 1 μm. (A) Control, after injection of physiological saline. Long slender profiles of RER tend to be arranged in parallel arrays; SER appears as small vesicular images. ×15 000. (B) Experimental, 24 h after subcutaneous injection of 0.5 ml oil of turpentine per 100 g body weight. Compared with controls, there are increased numbers of vesicular images. SER and RER appear considerably dilated and the characteristic parallel arrays of RER are largely absent. ×15 000. Data from Turchen et al., 1977.

Thompson and Paretsky, 1973; Thompson and Wannemacher, 1973), but the amount of this RNA which is messenger RNA has not been determined.

The endoplasmic reticulum is also involved in lipid and carbohydrate metabolism, both of which are altered dramatically during infection and are discussed in some detail in Section IV. It is worth noting here, however, that the activity of a number of enzymes involved in lipid and carbohydrate metabolism is altered during infection and that many of these enzymes are constituents of the endoplasmic reticulum (Table 4).

Biochemical and ultrastructural changes also occur in the endoplasmic reticulum of the liver during experimental inflammation elicited by the injection of sterile turpentine (Turchen et al., 1977). In this experimental model, there are increases in sialyltransferase and galactosyltransferase activities, protein-bound sialic acid and galactose, protein, RNA and phospholipid of the liver microsomal fraction. Morphological changes include dilatation of the endoplasmic reticulum and the presence of numerous circular and oval vesicles (Fig. 1). The parallel arrays of rough endoplasmic reticulum so distinctive of normal rat liver are absent in the experimental tissue. There also appears to be a proliferation of the smooth endoplasmic reticulum. These changes are similar to those observed after steroid hormone administration (Horvath et al., 1971) or phenobarbital intoxication (Orrenius et al., 1965). In both cases, similar ultrastructural changes in the endoplasmic reticulum of the liver are observed. These changes are accompanied by induced synthesis of enzymes found mainly in the endoplasmic reticulum. It is hypothesized that the biochemical and ultrastructural changes which occur in the endoplasmic reticulum of the liver during experimental injury may occur in response to an increased demand for liver-synthesized acute-phase glycoproteins (Turchen et al., 1977).

In summary, infection and experimental inflammation in the rat induce a number of biochemical and morphological changes in the endoplasmic reticulum. These include changes in protein, lipid and carbohydrate metabolism. Other functions of the endoplasmic reticulum, such as drug detoxification and metabolism, have not been studied during infection.

C. *Lysosomes*

Modest increases in lysosomal enzyme activities accompany the acute stages of illness (Canonico et al., 1975b). It is unclear to what extent hepatic parenchymal cells contribute to these increases. Activation of lysosomal enzymes in reticuloendothelial cells of the liver may account for the 10—30% increase in acid hydrolase activities during pneumococcal sepsis. Equilibrium density distribution patterns of lysosomal enzymes obtained by isopycnic centrifugation of liver homogenates on sucrose gradients show characteristically broad patterns with a mean density of 1.21. These patterns are affected only slightly by infection, even in severely ill animals (Fig. 2). Unlike other acid hydrolases, the equilibrium density pattern of β-glucuronidase is characterized by a bimodal distribution, where the less dense minor peak represents that portion of the

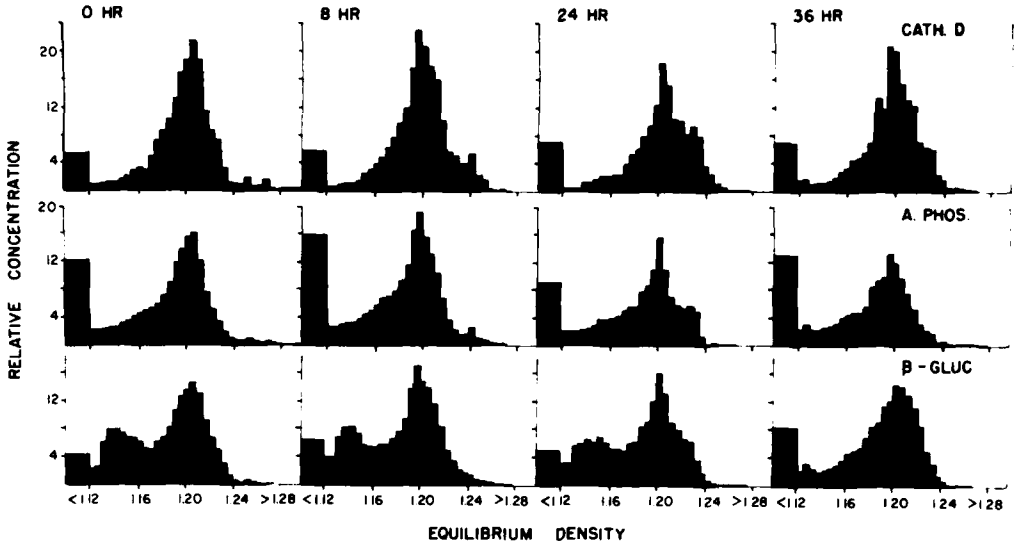


Fig. 2. Equilibrium density distribution of rat liver lysosomal enyzmes following inoculation of 10^6 *S. pneumoniae*. The block having an equilibrium density of less than 1.12 represents nonsedimentable enzyme activity solubilized during tissue homogenization. The areas delineated by experimental histograms are adjusted so as to reflect changes in the total activity of the enzyme with respect to controls. Each frequency histogram represents the average of 3 experiments.

enzyme associated with the smooth endoplasmic reticulum. In infected rats, the disappearance of this minor peak of β-glucuronidase suggests a change in the enzymatic complement of the liver endoplasmic reticulum which accompanies infectious illness.

D. *Mitochondria*

The enzymatic complement of mitochondria is not appreciably affected by acute infections. Cytochrome oxidase, glutamate and malate dehydrogenases, and glutamic-oxaloacetic and glutamic-pyruvic transaminases show little change during pneumococcal sepsis in the rat (Canonico et al., 1975a; Little, 1977). Physical characterization of mitochondria by isopycnic centrifugation of liver homogenates reveals the homogeneous character of liver mitochondria, having a narrow distribution pattern and a mean equilibrium density of 1.19 (Fig. 3). The equilibrium density of these organelles, however, is significantly altered during infection and results in a marked broadening of the distribution pattern. As will be discussed later, attempts to relate these physical changes to altered mitochondrial functions during infection have not been successful.

E. *Peroxisomes*

Marked changes in the composition and properties of peroxisomes accompany

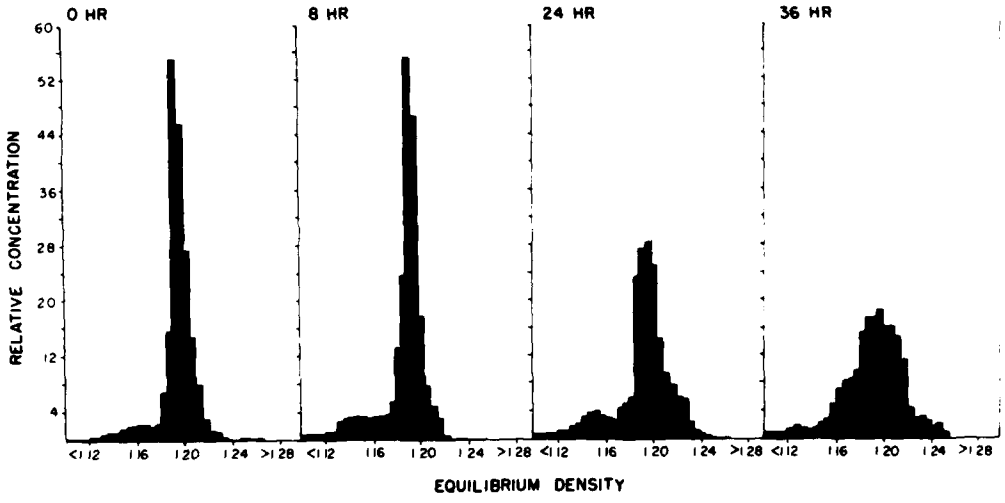


Fig. 3. Equilibrium density distribution of rat liver cytochrome oxidase activity. See legend to Fig. 2 for other details.

both inflammatory and infectious insults (Canonico et al., 1975b, 1977b). These changes are characterized by a decrease in the size of the peroxisomal protein pool and in the number of peroxisomes and appear to be a general response of the host to infection and inflammation. Morphometric analysis of the peroxisomal content of liver parenchymal cells shows a substantial decrease in peroxisome-like organelles by 24 h postinfection. This reduction ranges from $52.9 \cdot 10^9$ in pair-fed controls to $25.4 \cdot 10^9$ peroxisomes/mm^3 of liver in infected rats. Two peroxisomal marker enzymes, catalase and urate oxidase, decrease to 16 and 28%, respectively, of normal values by 48 h postinfection (Fig. 4). In spite of this marked loss in activity, the intracellular distribution of these enzymes as revealed by fractionation studies is not altered (Fig. 5). The mean equilibrium density of the sedimentable activity of both enzymes increases only slightly from 1.23 to 1.24 by 36 h.

The activity of a number of other peroxisomal enzymes also decreases in other models of infection and inflammation. The decrease has been attributed to a major reduction in de novo synthesis of these enzymes as well as to an increase in catabolism. Using a sterile lesion model induced by subcutaneous injection of turpentine, Canonico et al. (1977b) demonstrated that the half-life of catalase decreases by 25%, from 1.23 to 0.95 days. The rate of de novo catalase synthesis following the administration of turpentine is temporally dependent. During the first 65 h, the rate of synthesis is equivalent to 7 U/h, which when compared to control rats represents a 60% reduction in de novo synthesis. This decreased rate can account for 76% of the loss of catalase activity during sepsis or inflammation (Canonico et al., 1977b). Subsequent to this period, the

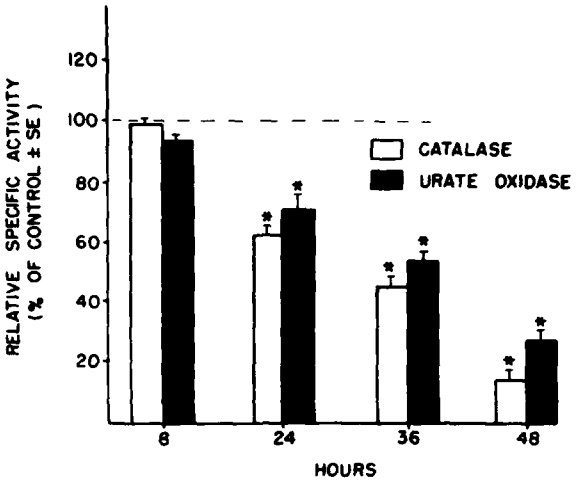


Fig. 4. Specific activity of rat liver peroxisomal enzymes following inoculation of 10^6 *S. pneumoniae*. Values shown are the mean of livers per group in comparison to control (100%) values. (*) $P < 0.01$. Data of Canonico et al., 1975b.

rate of synthesis becomes equivalent to that of the uninfected group and corresponds to the same point in time as the change in the concentration of the acute-phase serum proteins. This suggests that the accelerated rate of acute-phase serum protein synthesis during acute infectious and inflammatory reactions may occur at the expense of peroxisomal protein synthesis.

Peroxisomes appear to occur in interconnected clusters on specialized sections of the endoplasmic reticulum (Novikoff and Shin, 1964). Their protein components probably constitute a single common pool within which there is a rapid exchange. The size of the protein pool appears to determine the size, shape and number of peroxisomes (Svoboda and Reddy, 1972). Hence, the decrease in the number of hepatic peroxisomes during infection may result from atrophy in response to a decrease in the rate of peroxisomal protein synthesis and in the size of the peroxisomal protein pool.

F. *Golgi*

Glycosyltransferases that add sugars to nascent glycoconjugates are especially abundant in the Golgi vesicles of hepatic parenchymal cells. Pneumococcal infection in rats brings about marked increases in specific activity of a number of these enzymes. Sialyltransferases, galactosyltransferase, α_2- and α_3-fucosyltransferases increase 3—4-times in activity within 24—48 h after infection (Canonico et al., 1980). These elevated glycosyltransferase values in livers of septic rats are not unexpected, considering the markedly increased rates of hepatic synthesis and secretion of acute-phase serum glycoproteins which

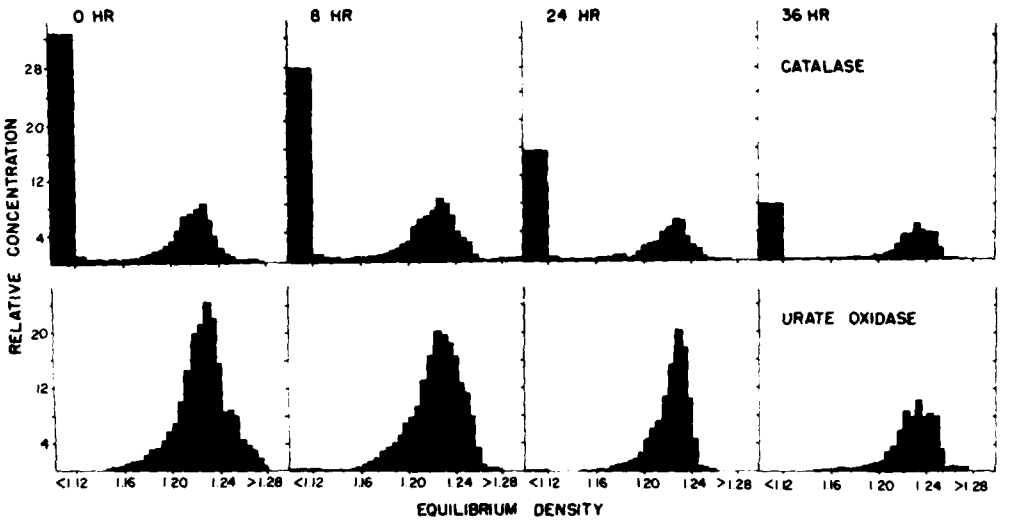


Fig. 5. Equilibrium density distribution of rat liver peroxisomal enzymes following inoculation of 10^6 *S. pneumoniae*. See legend to Fig. 2 for other details. Data of Canonico et al., 1975b.

characterize the infectious process (Williams et al., 1963; Powanda et al., 1972; Bostian et al., 1976; Little, 1978). The increased activities of liver glycosyltransferases appear to represent a nonspecific host response to inflammation engendered by the greater demand for glycosylation of newly synthesized acute-phase globulins within the Golgi complex of liver cells.

Elevated levels of glycosyltransferases are also observed in phagocytic cells. Cultured peritoneal macrophages have been found to secrete glycosyltransferases in appreciable quantities (Canonico et al., 1978). Stimulated rabbit alveolar macrophages have elevated levels of sialyltransferase and an α_3-fucosyltransferase. Therefore, the activation of phagocytic cells of the liver during infection may also contribute to the increased hepatic glycosyltransferase activities.

G. *Plasma membranes*

Although much is known about the effect of infection on plasma membranes of cells (Kaaden and Dietzschold, 1974; Dubois-Dalcq and Reese, 1975; Takacs and Rosenbusch, 1975), there is little specific information on the effects of infection on hepatocyte plasma membranes. Plasma membranes isolated from livers of control or infected rats have a similar density in alkaline sucrose, but differ significantly in their enzyme complement. For example, the specific activity of the plasma membrane marker, 5′-nucleotidase (Riemer and Widnell, 1975), decreases significantly in infection. On the other hand, the specific activity of alkaline phosphatase, another plasma membrane marker enzyme, is increased.

The nature of the changes in specific activity of plasma membrane enzymes during infection is unknown. Altered affinity of the enzymes for their respective substrates is not a contributing factor, since kinetic analysis demonstrates that the K_m values of 5′-nucleotidase and alkaline phosphatase are the same in plasma membranes isolated from livers of both control and infected rats (Little, 1977).

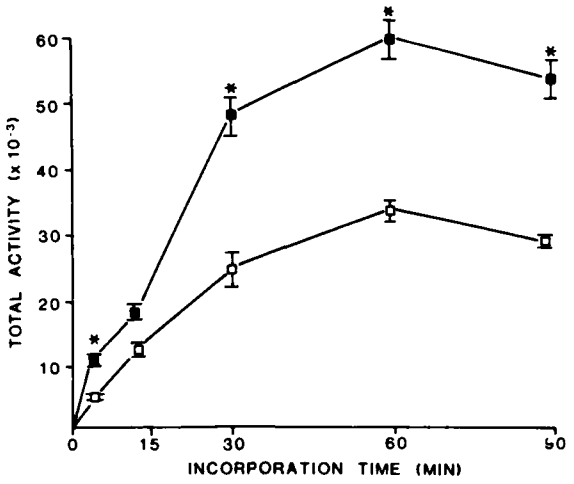


Fig. 6. Incorporation of [^{3}H]leucine into plasma membranes isolated from control (□) or infected (■) rat liver homogenates. At 40 h after inoculation with $3 \cdot 10^5$ heat-killed (control) or live (infected) *S. pneumoniae*, rats were injected intravenously with 100 μCi of [^{3}H]leucine and killed at the indicated times. Plasma membranes were prepared and the specific activity of the isotope determined. Each value represents the mean of 6 rats ± S.E.

The septic process in rats is also characterized by a marked incorporation of [^{3}H]leucine into hepatic plasma membranes (Fig. 6). It appears that this increased incorporation of labeled leucine represents enhanced de novo synthesis of hepatic plasma membranes and does not result from altered leucine pool size (Wannemacher et al., 1971, 1974), increased turnover of plasma membrane proteins and reutilization of label, or contamination from labeled secretory proteins. This view is supported by the observation that significantly more plasma membrane can be isolated from the infected livers, reflecting the significant increase in weight of this organ during infection (Little, 1978; Wannemacher et al., 1979).

The reason for the increased synthesis of hepatic plasma membranes and their altered enzymatic complement during infection is not clear. The increase in synthesis and secretion of plasma proteins during infection (Little, 1978) may require increased biogenesis of intracellular organelles involved in protein synthesis and fusion of secretory vesicles with plasma membranes (Dallner et al., 1966; Palade, 1975). This process probably contributes to the altered character of the plasma membrane.

IV. EFFECTS OF INFECTION ON HEPATIC METABOLISM

A. *Protein metabolism*

Bacterial (Lust, 1966; Powanda et al., 1975; Little, 1978), rickettsial (Mallavia and Paretsky, 1967; Stueckmann and Paretsky, 1971; Thompson and Paretsky, 1973) and viral infections (Squibb, 1964) are consistently associated with a marked increase in total liver protein synthesis. This increase is due principally to an enhanced rate of synthesis of a class of serum glycoproteins collectively termed 'acute-phase reactants.' The synthesis and secretion of these glycoproteins by the liver (haptoglobin, α_1-antitrypsin, α_1-acid glycoprotein, orosomucoid, ceruloplasmin, C-reactive protein and others) are associated not only with infection, but also with a number of other clinical states, such as myocardial infarction, neoplasia, burns and sterile inflammatory lesions (Owen, 1967; Koj, 1974). These proteins are thought to function in a compromised host to restrict tissue damage by inhibiting proteases released at inflammatory sites, aid in wound healing, modulate clotting, activate phagocytes, promote phagocytosis of microorganisms and necrotic tissue, and modulate the immune response (Powanda, 1977).

Another serum protein also synthesized by the liver which does not respond as an acute-phase protein is albumin. In contrast to the acute-phase glycoproteins, the plasma concentration of albumin decreases during infection (Jencks et al., 1956; Graham et al., 1958). It is uncertain whether this is due to decreased synthesis, increased degradation, or simply a redistribution of albumin to various extracellular compartments.

During infection, the initial step leading to enhanced serum glycoprotein synthesis by the liver is an increased flux of amino acids from skeletal muscle to the liver (Lust, 1966; Wannemacher et al., 1971, 1974; Little, 1978; Neufeld et al., 1978). Amino acids from skeletal muscle catabolism provide the nitrogen source for the increased hepatic synthesis of these proteins during infection (Wannemacher et al., 1974). This increase in liver protein synthesis represents a fundamental aspect of the host response to infection in that it occurs even in rats fed a protein-deficient diet (Powanda et al., 1972) or starved for 10 days prior to infection (Cockerell, 1973).

Williams et al. (1965) reported that staphylococcal infection in mice is associated with an increase in the synthesis of α- and β-globulins. Similarly, the synthesis of α_1-, α_2- and β-glycoglobulins is reported to increase in *S. pneumoniae*-infected rats (Powanda et al., 1975). In the study of Williams et al. (1965), there is a 5-fold increase in the incorporation of ^{14}C-labeled amino acids into serum proteins, a 10-fold increase in the rate of labeling of the globulin fraction and a 50% elevation in serum protein concentration. The isotopic labeling of the albumin fraction, on the other hand, decreases by 50% (Williams et

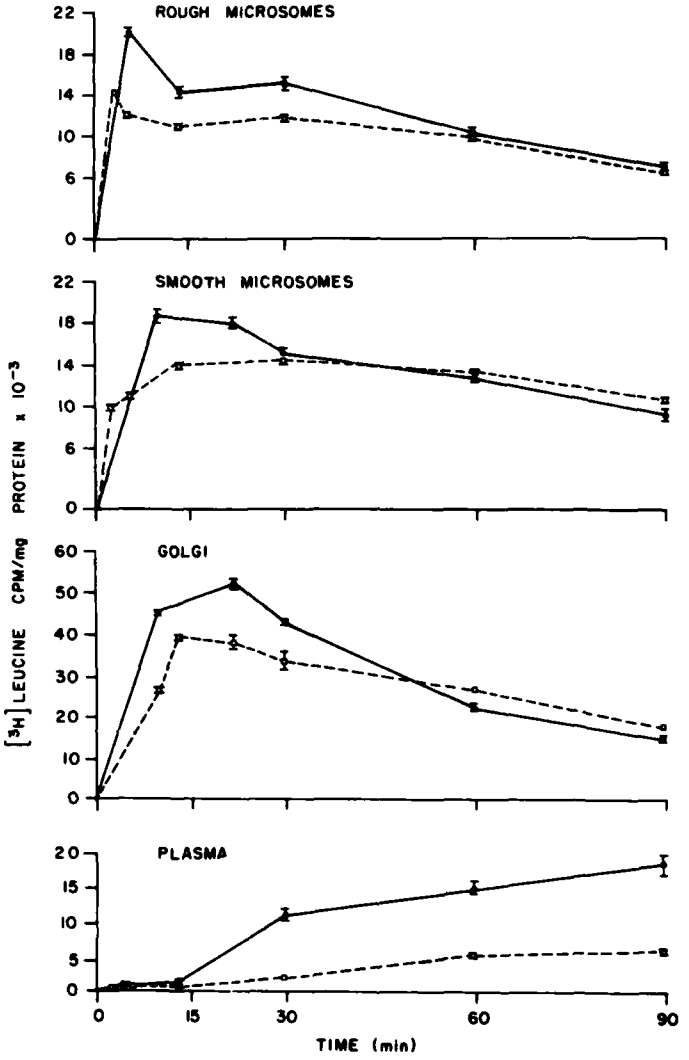


Fig. 7. Incorporation of [^{3}H]leucine into plasma and cell fractions isolated from control (□) or infected (●) rat liver homogenates. Methods for inoculation of rats and injection of isotope were as described in the legend to Fig. 6, except plasma and cell fractions were prepared and the specific activity of the isotope in these was determined. Each value represents the mean of 6 rats ± S.E. Data of Little, 1978.

al., 1965). Microsomes isolated from the livers of mice with staphylococcal infection have an increased capacity to synthesize proteins in vitro (Williams et al., 1965), suggesting that the protein synthetic machinery itself may be augmented during infection and that increased protein synthesis does not occur solely because of a greater availability of precursors and substrates. It has also been shown that bacterial infection does not bring about any major dysfunction

in liver cell organelles involved in the synthesis, transport, packaging, or secretion of proteins (Little, 1978). As in controls, protein in infected rats is synthesized on the rough endoplasmic reticulum, transported through the smooth endoplasmic reticulum to the Golgi, then makes its way to the plasma membrane and is finally secreted into the circulation. Although the amount of protein synthesized and secreted during sepsis is significantly increased (Fig. 7), the time from the initial synthesis of the protein until its release into the circulation is not affected by sepsis (Little, 1978). The mechanism by which the increased protein synthesis occurs during infection is only partly understood. It appears that there is an increase in the biogenesis of liver cell organelles involved in the secretory process, because the incorporation of the labeled amino acid into proteins of infected liver remains markedly higher than in uninfected controls even at 90 min (Fig. 8), a time point when most secretory protein should be in the circulation. This observation suggests that during infection there is an increase in the synthesis of some nonsecretory as well as secretory protein.

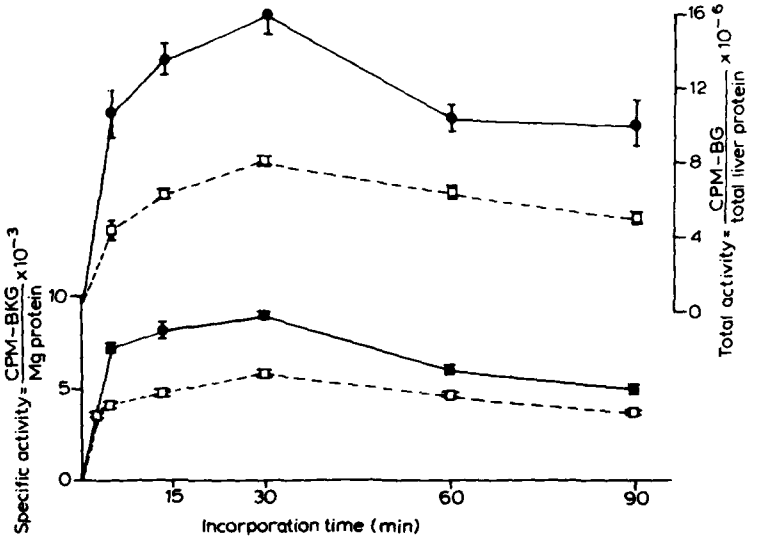


Fig. 8. Incorporation of [^{3}H]leucine into liver protein of control (□) or infected (●) rats. Methods were as described in the legend to Fig. 6, except at the time points indicated the rats were killed and the specific activity or total activity of isotope in liver protein was determined. Data of Little, 1978.

B. *Carbohydrate metabolism*

In acute infections, complex metabolic adaptations in the host result in sufficiently increased hepatic gluconeogenesis so as to maintain near normal levels of plasma glucose in spite of increased utilization of glucose by the body

(Beisel, 1975; Guckian, 1973). In contrast, severe infections are characterized by the development of hypoglycemia during the agonal stages of the disease process as a result of an impaired capacity of the liver to synthesize glucose (LaNoue et al., 1968b; Yeung, 1970; McCallum and Berry, 1973).

Wannemacher et al. (1980) assessed the gluconeogenic capacity of rat livers perfused with various substrates during the acute and agonal stages of pneumococcal infection. In the absence of any added gluconeogenic substrate, glucose release in the perfusate is small and not statistically different between livers taken from control and infected groups. The addition of alanine to the perfusate causes a significant stimulation of glucose formation in livers of acutely ill rats. In contrast, livers from the agonal stages of infection fail to show an increase in glucose formation when alanine is added to the perfusate. Rates of gluconeogenesis for pyruvate or lactate do not differ significantly between livers from control rats or those obtained during the acute stage of the infection. However, glucose formation in livers from rats during the agonal stages of the infection is severely depressed.

The search for specific biochemical lesions has yielded a number of proposals to account for the breakdown in glucose synthesis. Perfusion studies on isolated livers from *S. pneumoniae*-infected rats led Curnow et al. (1974) to conclude that a lack of reducing equivalents (H^+) was the specific point of biochemical failure. An inhibition of the induction of phosphoenolpyruvate carboxykinase (PEPCK) also has been proposed to account for the decline of blood glucose levels in mice infected with *S. typhimurium* (Moore et al., 1977).

Single cell suspension of isolated hepatocytes permits studies on regulatory mechanisms in a controlled environment removed from humoral factors. As with perfused livers, the capacity of hepatocytes isolated from agonal rats to synthesize glucose from pyruvate and lactate decreases markedly when compared to control cells (Table 5). Increasing the level of reducing equivalents through oxidation of ethanol added to hepatocytes fails to correct the impairment in glucose production. On the other hand, enzymatic measurements show significantly reduced levels of PEPCK in livers of agonal rats (Fig. 9). The modulation of PEPCK activity of agonal rats as compared to fasted controls could account for the impairment of gluconeogenesis during severe infections.

The cause of the depressed PEPCK activity, however, is unclear. PEPCK is one of a number of hepatic enzymes whose synthesis is enhanced by glucocorticoids (Exton, 1972). In acute infections, both increased secretion and a loss of the diurnal fall in plasma corticosteroids serves to elevate the concentration of plasma glucocorticoids (Beisel and Rapoport, 1969a, b; Szafarczyk et al., 1974). The rise in circulating glucocorticoids helps to promote a general anabolic response in the liver (Beisel and Rapoport, 1969a, b), increasing the activity of various hepatic enzymes and accelerating the rate of synthesis and secretion of acute-phase serum proteins. However, as an infectious illness persists or worsens, the liver appears to become refractory to the stimulatory effects of glucocorticoids. The activity of glucocorticoid-regulated enzymes, such as trypto-

TABLE 5

GLUCONEOGENESIS FROM PYRUVATE OR LACTATE IN ISOLATED SUSPENSIONS OF HEPATOCYTES OBTAINED FROM CONTROL AND *S. PNEUMONIAE*-INFECTED RATS AFTER 40 h OF FASTING

Substrate	Mean μg glucose formed/45 min per 10^7 cells ±S.E. (*n*)		
		Infected	
	Control	20 h	40 h
Pyruvate (10 mM)	284 ±40 (5)	254 ±22 (4)	164 ±22 (6)
Pyruvate + EtOH (2 mM)	528 ±45 (5)	373 ±28 (4)	237 ±32 (6)
Lactate[a] (10 mM)	264 ±12 (8)	267 ±13 (3)	171 ±14 (5)
Lactate[a] + EtOH (2 mM)	182 ±18 (6)	170 ±22 (3)	73 ±19 (5)

[a] 2 mM NH_4Cl added.

phan pyrrolase (Beisel and Rapoport, 1969a, b), tyrosine transaminase (Shambaugh and Beisel, 1968), glucose-6-phosphatase (Canonico et al., 1977a, b) and PEPCK decreases despite increasing plasma glucocorticoid concentrations (Beisel and Rapoport, 1969a, b).

Factors responsible for the liver's refractoriness to the effect of glucocorticoids are not well defined. Based on studies with endotoxin and other agents active on the reticuloendothelial system, Agarwal (1972) advanced the concept that hepatic refractoriness of tryptophan pyrrolase and tyrosine transaminase to glucocorticoid induction is mediated by factor(s) released from reticuloendothelial cells in response to injection of microorganisms or noxious agents.

C. *Lipid metabolism*

Perhaps the greatest alteration to occur during infection is in the metabolism of lipids. During acute or prolonged fasting the body normally increases its concentration of circulating blood ketone bodies. Ketones represent the major source of energy used by tissues such as skeletal muscle and brain (Cahill et al., 1971). This ketogenic adaptation to starvation reduces the need for energy derived from glucose, which in turn spares protein stores by decreasing the rate of amino acid usage for gluconeogenesis. During sepsis, however, the normal rise in hepatic and blood ketone concentration caused by fasting is abolished. The failure of the ketonemic adaptation to occur in anorectic-infected hosts is thought to result from a reduced ketogenic capacity of the liver as well as from a possible decrease in the supply of fatty acids to the liver (Wannemacher et al., 1979).

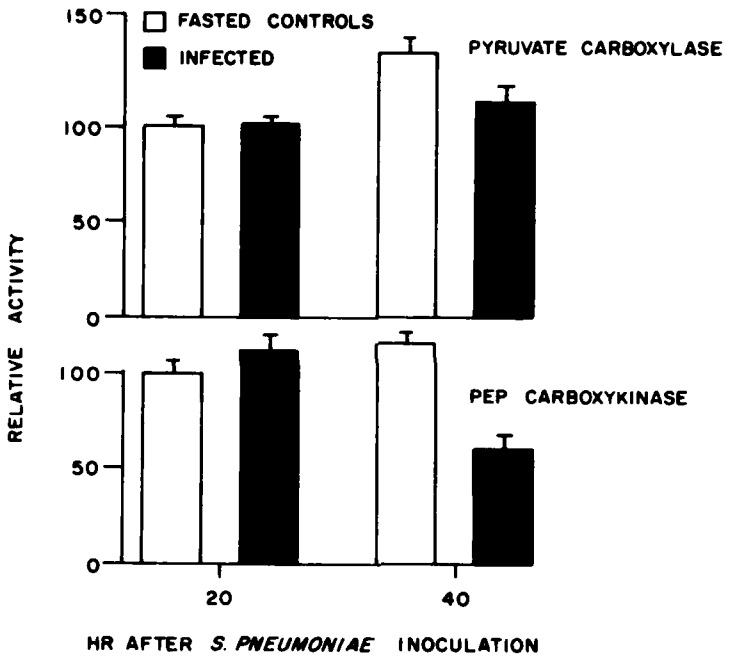


Fig. 9. Relative specific activity of liver pyruvate carboxylase and phosphoenolpyruvate (PEP) carboxykinase of *S. pneumoniae*-infected and pair-fasted control rats. Values shown are the mean of 6 livers per group. Bars indicate the S.E. of the mean.

The cellular basis for the reduced hepatic ketogenic capacity during infection has been intensively studied using a liver perfusion model. In this system, the rate of ketone body production from long-chain fatty acids such as oleic acid is reduced in *S. pneumoniae* infection, but no significant difference is found in the rate of ketogenesis from infusion of short-chain fatty acids such as octanoic acid (Wannemacher et al., 1980). The reduced rate of ketogenesis from oleic acid is not related to altered uptake of long-chain fatty acids by the perfused liver.

Mitochondria isolated from infected rat liver produce $^{14}CO_2$ and ketone bodies from [^{14}C]oleic acid at rates equivalent to those of control mitochondria. It appears, therefore, that the transfer of long-chain fatty acids into the mitochondria is not rate limiting in infected rat livers. Furthermore, this information indicates the presence of intact β-oxidation and Krebs cycle pathways (Wannemacher et al., 1979). The apparent lack of correlation in the rate of ketogenesis between perfused liver and isolated mitochondria promoted the as yet uneventful search for possible cytoplasmic regulatory factors.

The discovery by Lazarow and De Duve (1976) that hepatic peroxisomes contain enzymes necessary for β-oxidation of palmitoyl-CoA led to the postulation that peroxisomes may be a major site for β-oxidation of long-chain fatty acids. The depletion of hepatic peroxisomes and decrease in activity of some peroxisomal enzymes during infection led to the proposal that reduction in the

rate of fatty acid oxidation in bacterial infection and inflammation might result from a decrease in the activity of the peroxisomal fatty acetyl-CoA oxidizing system. However, the total capacity of hepatic peroxisomes to activate palmitic acid, measured by palmitate-CoA ligase activity, and to oxidize palmitoyl-CoA is not altered by infection (Foulke et al., 1979).

Another major consequence of acute generalized infections is an increase in hepatic lipogenesis, fatty acids, triglycerides and intracellular lipid droplet formation. The incorporation of [^{14}C]octanoic or [^{14}C]oleic acid into liver lipid during in vitro perfusion has been studied by Wannemacher et al. (1979) in infected rats (Table 6). These studies show that livers from infected rats

TABLE 6

EFFECT OF FASTING AND *S. PNEUMONIAE* INFECTION ON LIVER WEIGHT, LIPID CONTENT, AND INCORPORATION OF [^{14}C]OCTANOIC OR [^{14}C]OLEIC ACID INTO LIVER LIPID DURING IN VITRO PERFUSION STUDIES IN RATS

Treatment	Liver weight (g ±S.E.)	Liver lipid (±S.E.)		
		mg/liver	dpm/total liver lipid ($\times 10^6$)	
			[^{14}C]Octanoic acid	[^{14}C]Oleic acid
Fed	8.16 ±0.21	552 ±29	1.33 ±0.17	5.84 ±0.21
Fasted				
Heat-killed				
24 h	6.52 ±0.11	326 ±26	0.35 ±0.02	2.71 ±0.20
48 h	6.20 ±0.19	300 ±13	0.24 ±0.03	2.34 ±0.17
Infected				
24 h	7.13 ±0.20[a]	374 ±20	0.68 ±0.07[a]	3.62 ±0.17[a]
48 h	7.89 ±0.15	464 ±21[b]	1.30 ±0.12[b]	5.27 ±0.14[b]

[a] $P < 0.01$ vs. 24-h, fasted, heat-killed.
[b] $P < 0.01$ vs. 48-h, fasted, heat-killed.
Data of Wannemacher et al., 1979. $n = 6$.

increase in both weight and lipid content compared to pair-fed controls and incorporate significantly more labeled octanoic or oleic acid into the total lipid fraction. Incorporation of [^{14}C]acetate into lipid fractions of isolated hepatic parenchymal cells shows that cells from infected rats incorporate significantly more acetate into all lipid fractions than control cells. This includes an 18-fold increase in cholesterol and diglycerides, a 5-fold increase in triglycerides and a 6-fold increase in fatty acids (Table 7).

The increased lipogenesis in hepatic cells of infected rats implies that substantial modifications in the enzyme complement of rat liver endoplasmic reticulum occur during sepsis. In fact, marked changes in the specific activity of a number of endoplasmic reticulum enzymes accompany the infectious process

TABLE 7

INCORPORATION OF [1-^{14}C]ACETATE INTO LIPID FRACTIONS BY HEPATOCYTES FROM NORMAL OR INFECTED RATS

Lipid fraction	dpm/10^7 hepatocytes ($\times 10^{-2}$) ±S.E.		
	Normal (n=6)	Infected (n=6)	$P<$
Phospholipids and monoglycerides	5 ± 1	12 ± 2	0.05
Cholesterol and diglycerides	40 ±15	713 ±60	0.001
Fatty acids	25 ± 5	157 ±26	0.001
Triglycerides	53 ± 9	265 ±10	0.001
Cholesterol esters	6 ± 1	40 ±10	0.01

Data of Canonico et al., 1977a.

(Table 8). One enzyme whose activity is markedly altered is hydroxymethylglutaryl (HMG)-CoA reductase. The activity of this enzyme is about 6-times greater in infected rats. The higher levels of HMG-CoA reductase activity are of special interest because it is a key enzyme in cholesterol synthesis. This enzyme catalyzes the formation of mevalonic acid from HMG-CoA and in its absence cytoplasmic HMG-CoA is converted to acetoacetate (Bucher et al., 1960). Assessment of the effects of elevated HMG-CoA reductase levels on cholesterol synthesis in isolated suspensions of hepatocytes shows that liver cells from infected rats incorporate 10-times more [^{14}C]acetate into cholesterol than do cells from fasted control rats (Fig. 10).

It appears that during acute infection, the biochemical machinery of the liver is directed away from oxidation of fatty acids and toward the synthesis of lipid moieties. The exact nature of the controls involved in this process is unclear. An endocrine basis for the depression of ketone bodies during infection or inflammatory states has been proposed (Neufeld et al., 1977, 1980). The apparently inappropriate increase in insulin in the absence of a decrease in glucagon levels has been implicated in the regulatory processes. However, the failure to demonstrate a direct effect of insulin in vitro on the hepatic ketogenic capacity suggests that other indirect, more complex relationships may be operative.

The observed adaptation in lipid metabolism and in particular the elevated activity of HMG-CoA reductase are also of interest in relation to the synthesis of glycoproteins. The present concept of glycoprotein synthesis postulates that the carbohydrate moieties are preassembled on a lipid carrier, dolichol pyrophosphate, and then transferred to a protein acceptor. Low amounts of dolichol phosphate appear to be rate limiting in glycosylation reactions. The biosynthesis of dolichol phosphate is dependent upon the synthesis of mevalonate from HMG-CoA by reductase. Hence, the increased activity of this enzyme during infection may be appropriate in order to assure a sufficient supply of

TABLE 8

SPECIFIC ACTIVITY OF LIVER MICROSOMAL ENZYMES IN INFECTED OR CONTROL RATS

Enzyme	Specific activity[a]					
	24 h			48 h		
	Controls	Infected	$P <$	Controls	Infected	$P <$
Alkaline phosphatase[b]	10.3 ± 0.9	9.1 ± 0.3	n.s.	9.5 ± 0.1	9.0 ± 1.0	n.s.
Inosine 5′-diphosphatase[c]	8.3 ± 0.7	10.8 ± 0.8	n.s.	3.9 ± 0.3	4.9 ± 0.4	n.s.
Glucose-6-phosphatase[c]	7.6 ± 0.4	6.3 ± 0.4	0.05	6.0 ± 0.2	2.8 ± 0.3	0.001
5′-Nucleotidase[c]	2.4 ± 0.1	1.6 ± 0.1	0.01	1.8 ± 0.1	0.8 ± 0.1	0.001
Esterase[d]	5.4 ± 0.5	5.2 ± 0.2	n.s.	5.1 ± 0.3	3.4 ± 0.4	0.01
NADPH-cytochrome *c* reductase[e]	212 ± 5	222 ± 9	n.s.	207 ± 8	170 ± 6	0.01
NADH-cytochrome *c* reductase[e]	328 ±19	479 ±53	0.05	341 ±38	564 ±38	0.001
HMG-CoA reductase[f]	17.3 ± 3.3	19.2 ± 5.0	n.s.	3.6 ± 0.3	18.3 ± 1.4	0.001

[a]Mean of 6 rats ± S.E. n.s., not significant.
[b]nmol formed/min per mg of microsomal protein.
[c]P_i released (μmol/20 min per mg of microsomal protein).
[d]μmol formed/min per *mg of microsomal protein.*
[e] nmol of substrate converted/min per mg of microsomal protein.
[f] nmol formed/h per mg of microsomal protein.
Data of Canonico et al., 1977a.

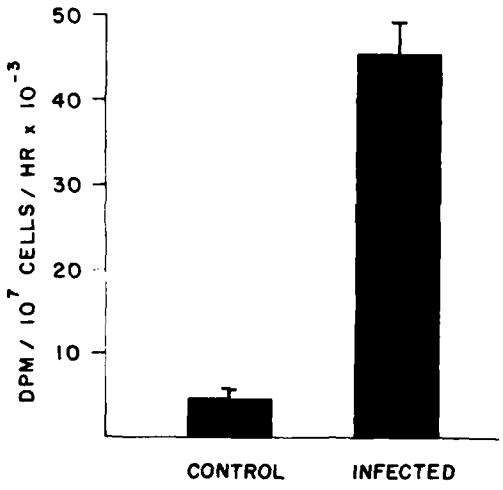


Fig. 10. [1-^{14}C]Acetate conversion to cholesterol by single cell suspension of hepatocytes isolated from infected and control rats. Experimental rats were inoculated with $3 \cdot 10^5$ *S. pneumoniae* and fasted for 40 h, and hepatocytes were isolated. Control hepatocytes were isolated from rats fasted for 40 h. All rats were killed at a time corresponding to the midpoint of the night cycle. Data of Canonico et al., 1977a.

dolichol phosphate required to meet the increased demand for synthesis and transport of serum glycoproteins.

D. *Nucleic acid metabolism*

The increased production by the liver of serum glycoproteins in response to acute injury or infection is preceded by an increase in hepatic RNA synthesis (Neuhaus et al., 1966; Thompson et al., 1976). In both injury and infection, actinomycin D inhibits the acute-phase response, suggesting that in both instances the response is dependent on the synthesis of hepatic RNA specific for the increased production of serum glycoprotein. It appears that this increase in RNA synthesis requires the presence of glucocorticoid hormones, since it does not occur in adrenalectomized rats following the injection of leukocytic endogenous mediator (LEM) (Lust and Beisel, 1967; Thompson et al., 1976). In this case, the response to LEM is restored by the administration of cortisol to adrenalectomized rats.

Elevated production of RNA by the liver has been observed during bacterial (Thompson and Wannemacher, 1973), viral (Budillon et al., 1967; Kehoe and Lust, 1969) and rickettsial (Thompson and Paretsky, 1973) infections. During *S. pneumoniae* infection in rats, for example, incorporation of radiolabeled orotate into hepatic RNA increases, with most of the label becoming associated with the bound ribosomal fraction (Thompson and Wannemacher, 1973). Mice infected with Venezuelan equine encephalomyelitis virus show an increased in-

TABLE 9

MODULATION OF HEPATIC AND BLOOD PARAMETERS IN RESPONSE TO TISSUE INJURY (INFECTION OR INFLAMMATION) OR TREATMENT WITH ANTIHYPERLIPIDEMIC DRUGS

Parameter	Response[a]	
	Tissue injury	Antihyperlipidemic drugs
Peroxisome density	−	+
Catalase synthesis	−	+
Carnitine acetyltransferase activity	−	+
Fatty acid oxidation	−	+
In vitro peroxisomal fatty acid oxidation	0	+
Blood ketone bodies	−	+
Cholesterol synthesis	+	−
Fatty acid esterification	+	−
Bound/free ribosome ratio	+	−
Synthesis of serum proteins	+	−
Susceptibility to *S. pneumoniae* infection	Uniformly lethal	Increased resistance

[a] −, decreased; +, increased, 0, no change.
Data of Canonico et al., 1977b.

corporation of orotate into both hepatic nuclear and ribosomal RNA (Kehoe and Lust, 1969). RNA synthesis is also increased in *Coxiella burnetii*-infected guinea-pigs (Thompson and Paretsky, 1973). In this case, there is not only an increased incorporation of orotate into hepatic RNA (Stueckmann and Paretsky, 1971), but a doubling of the number of liver ribosomes as well (Thompson and Paretsky, 1973).

In contrast, the synthesis of hepatic DNA does not appear to be affected by injury or infection (Squibb, 1964; Chandler and Neuhaus, 1968). Hepatic chromatin template activity, on the other hand, is reported to increase in both *S. pneumoniae*- and *S. typhimurium*-infected rats. The increase precedes the onset of fever and other nonspecific host responses to infection (Earp, 1975). Adrenalectomy prior to infection both increases the severity of the infection and markedly blunts the increase in template activity. These data suggest that systemic infection alters hepatic metabolism through transcriptional control and that a permissive or stimulatory action of glucocorticoids is required (Earp, 1975).

V. CONCLUDING REMARKS

We have documented numerous biochemical and physiological changes which are known to occur in the liver during the septic process. However, the

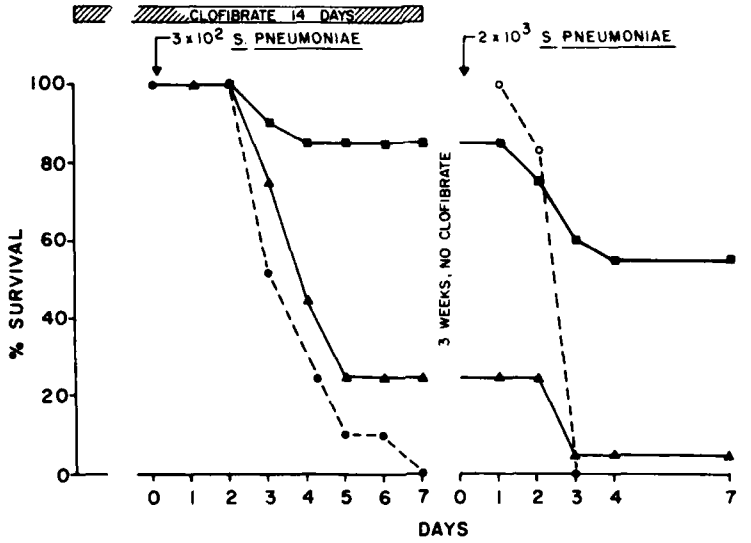


Fig. 11. Effect of dietary clofibrate on survival of rats inoculated with *S. pneumoniae*. Data of Powanda and Canonico, 1976. ●, controls ($n = 20$); ▲, 0.25% ($n = 20$); ■, 1.25% ($n = 20$); ○, rechallenge controls ($n = 12$).

relative importance of each specific adaptation to survival of the host is not certain in all instances. It would appear that the increased rate of synthesis of acute-phase serum globulins is an appropriate response designed to assure an adequate supply of proteins which, in part, assists the host in limiting inflammatory damage, aids in wound healing and promotes an immunological response. On the other hand, the impairment of ketogenesis may be conceived as an inappropriate response, requiring the host to mobilize its protein reserves in order to provide precursors for gluconeogenesis.

A list of changes in a number of metabolic parameters which accompany infection and inflammation is given in Table 9. These include a decrease in hepatic peroxisomes, catalase, carnitine acetyltransferase, fatty acid oxidation and an increased capacity to synthesize cholesterol and triglycerides. It is of interest to note that rats treated with antihyperlipidemic drugs, such as clofibrate, show changes in the above parameters which are inversely related to that produced by infection or inflammatory stress. Yet, rats treated with clofibrate are markedly resistant to *S. pneumoniae* infection (Fig. 11).

These observations indicate that certain host adaptations are, in fact, inappropriate and that their selective reversal may increase host resistance to infections. To achieve this goal, however, a more complete understanding of the cellular biochemical events which accompany infection is required. The knowledge to be gained from this area of research has great potential not only from its practical applications, but also for our more lucid understanding of the interrelationships between subcellular events and the well-being of the host which they ultimately control.

Note

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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