

An Assay of RNA Synthesis in Hepatic Nuclei from Control and *Streptococcus pneumoniae*-Infected Rats^{1,2} (41597)

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Abstract. Hepatic nuclei were isolated, purified, and partially characterized from control and *Streptococcus pneumoniae*-infected rats. Biochemical and morphologic examination showed little contamination by other cell organelles. An *in vitro* system for the incorporation of 2-[¹⁴C]uridine-5'-triphosphate into ribonucleic acid (RNA) was developed and characterized. Although the stimulatory effects of cytosol on incorporation of labeled precursors into RNA have been previously reported, nuclei isolated from the livers of *S. pneumoniae*-infected rats were stimulated to a significantly greater extent than were nuclei isolated from the livers of control rats. In the presence of cytosol prepared from either control or infected rats, the increased incorporation of labeled precursor into RNA by nuclei isolated from infected rats was observed over broad pH and temperature ranges. The increased activity of infected nuclei was eliminated when albumin was substituted for cytosol, and could not be accounted for by differences in endogenous precursor pool size. These results are consistent with other infection-induced alterations in hepatic RNA and protein synthesis.

It is well documented that host metabolism involving the liver is markedly altered during *Streptococcus pneumoniae* infection (1, 2). There is an increased uptake of zinc (3, 4) and amino acids (4-6) by the liver, and altered carbohydrate (7), lipid (8-10), protein (6, 11), and nucleic acid metabolism (11, 12). There is also an increase in the concentration of certain plasma proteins referred to as acute-phase proteins (e.g., α_1 -glycoprotein, ceruloplasmin, and haptoglobin) (13) during *S. pneumoniae* infection in the rat. There is evidence to suggest that the acute-phase response may represent a nonspecific host adaptation, since it occurs in bacterial, viral, and rickettsial infections and a number of other

clinical states such as myocardial infarction, neoplasia, burns, and sterile inflammatory lesions [for review see (14)]. A significant increase occurs in hepatic ribonucleic acid (RNA) production (12) prior to the increased plasma protein synthesis. Previous reports have shown that transcription rates may be altered during infection (12) as a result of an early and continuing derepression of hepatic chromatin template activity (15).

These experiments were designed to determine the effects of *S. pneumoniae* infection on RNA synthesis in hepatic nuclei. It was first necessary to isolate, purify, and characterize nuclei from both control and infected rats.

Materials and Methods. *Chemicals.* Sucrose (density gradient ultrapure) was obtained from Schwarz/Mann, Orangeburg, New York. Spermidine was purchased from Calbiochem, Inc., Gaithersburg, Maryland. Hydrofluor was obtained from National Diagnostics, Parsippany, New Jersey. Scintisol Complete was obtained from Isolab, Inc. Akron, Ohio. Nucleotides, cholesterol, calf thymus deoxyribonucleic acid (DNA), phosphoenol pyruvate, pyruvate kinase, yeast RNA, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, Missouri. Tetramethyl-ammonium

¹ In conducting the research described in this report, the investigators 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.

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hydroxide was purchased from J. T. Baker Chemical Co., Phillipsburg, New Jersey. Dithiothreitol was obtained from Eastman Kodak Co., Rochester, New York. 2-[¹⁴C]Uridine-5'-triphosphate ([¹⁴C]UTP) (40–60 mCi/mmol) was obtained from New England Nuclear, Boston, Massachusetts. All other chemicals were of analytical grade and obtained from commercial sources.

Animals. Male albino rats (200–250 g) of the Sprague-Dawley strain (CrI:COBSR-(SD)BR) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.) and maintained on commercial laboratory feed and tap water *ad libitum*. All rats were housed in temperature- and light-controlled rooms (12 hr day-night cycle) and acclimated for 14 days prior to experimentation to standardize circadian variations. Rats were inoculated subcutaneously with 3×10^5 to 6×10^5 heat-killed (control) or virulent (infected) colony-forming units of *S. pneumoniae*, serotype I, A5 (U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Md.). The organisms were grown to the early stationary phase in 3.7% Brain Heart Infusion (Difco) supplemented with 10% normal rabbit serum and 4% sterile defibrinated sheep blood. Prior to inoculation the organisms were diluted to $1-2 \times 10^6$ CFU/ml with sterile tryptose saline. For all experiments food was removed after inoculation, since infected rats were anorectic.

Isolation of nuclei. Rats were killed 22 or 46 hr after inoculation, at 8:00 A.M., a time corresponding to the midpoint of the night cycle. Rats were stunned by a blow to the head and killed by exsanguination. Nuclei were isolated by a modification of the method of Blobel and Potter (16). The liver was excised, freed of connective tissue, and weighed. All subsequent operations were done at 4°C. For both control and infected rats, a 5-g portion of liver was minced with scissors, mixed with 10 ml of ice-cold 0.25 M sucrose containing 50 mM Tris(hydroxymethyl)aminomethane (Tris)-chloride (pH 7.5), 25 mM KCl, and 5 mM MgCl₂ (0.25 M STKM), and homogenized (15 strokes) in a glass homogenizer fitted with a Teflon pestle (Arthur H. Thomas, Inc., Philadelphia, Pa.) with a TRI-R STIRR model K43 mechanical homogenizer (TRI-R Instruments, Inc., Rockville Center, N.Y.) at

a setting of 1100 rpm. The homogenate was then filtered through four layers of gauze. Nine milliliters of filtered homogenate were then mixed with 18 ml of 2.3 M STKM buffer and gently layered over 10 ml of 2.3 M STKM buffer contained in a 30-ml capacity cellulose nitrate tube. Nuclei were pelleted by centrifugation at 105,000g for 40 min in an SW 27 rotor with a Beckman model L2-65B ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). All material above the 2.3 M STKM buffer was aspirated, and the tube walls washed twice with 10 ml of 0.25 M STKM to remove contaminating cellular components. The 2.3 M STKM buffer was then aspirated and the nuclear pellet suspended in 0.125 M STKM to approximately 10 mg protein/ml.

Isolation of microsomes. Microsomes were isolated as previously described (17).

Preparation of cytosol. Several livers from control or infected rats were pooled, homogenized in 0.25 M sucrose (1:2 v/v) and centrifuged at 10,000g for 15 min in a Sorval RC2-B refrigerated centrifuge (Ivan Sorval, Inc., Norwalk, Conn.). The supernatant was centrifuged at 105,000g for 2 hr in a Ti-60 rotor with a Beckman model L2-65B ultracentrifuge. The supernatant from this step was filtered through four layers of gauze and centrifuged as above at 105,000g for 1 hr. This supernatant was designated as cytosol.

Analytical methods. Protein was assayed by the method of Lowry *et al.* (18) using BSA as a standard. RNA was assayed by the orcinol method (19) using yeast RNA as a standard. DNA was assayed by the method of Burton (20) using calf thymus DNA as a standard. Inorganic phosphate was determined by the method of Chen *et al.* (21).

Microscopy. Nuclei to be examined by electron microscopy were fixed for 3.5 hr in ice-cold phosphate-buffered Formalin [10% formaldehyde (v/v) in 0.1 M phosphate buffer, pH 7.6]. Following fixation the nuclear pellet was rinsed in the same buffer, postfixated for 2 hr in 2% OsO₄ (v/v) in 0.1 M phosphate buffer (pH 7.6), dehydrated in a series of graded ethanol and propylene oxide, and embedded in Epon-Araldite. Sections prepared with a diamond knife on a Reichert ultramicrotome were stained with uranyl acetate and lead citrate (22) and examined at 75 kV with a Hi-

tachi HU-2 electron microscope (Perkin Elmer Corp., Mountain View, Calif.).

Biochemical enzyme assays. Glucose-6-phosphatase (E.C. 3.1.3.9), 5'-nucleotidase (E.C. 3.1.3.5), acid phosphatase (E.C. 3.1.3.2), alkaline phosphatase (E.C. 3.1.3.1), malate dehydrogenase (E.C. 1.1.1.37), and glutamate dehydrogenase (E.C. 1.4.1.2) were assayed as described previously (23). NADH cytochrome C reductase (E.C. 1.6.99.3) was measured at room temperature by following the reduction of cytochrome C at 550 nm by the method of Beaufoy *et al.* (24). Choline phosphotransferase (E.C. 2.7.8.2) was assayed by the method of McMurray (25).

Incorporation of 2-[¹⁴C]UTP into RNA by nuclei isolated from control and infected rats. Incorporation of [¹⁴C]UTP into RNA by isolated nuclei was assayed by a modification of the method of Bastian (26) as modified by McNamara *et al.* (27). Nuclei (about 0.25 mg protein), isolated as described above from the livers of control or infected rats, were incubated in a shaking water bath at 35°C in a 0.5-ml system containing 50 mM Tris-chloride (pH 7.5), 2.5 mM MgCl₂, 2.0 mM dithiothreitol, 0.5 mM CaCl₂, 0.3 mM MnCl₂, 5.0 mM NaCl, 2.5 mM Na₂HPO₄, 5.0 mM spermidine, 2.5 mM adenosine triphosphate (ATP), 2.5 mM phosphoenol pyruvate, 2 mg/ml yeast RNA, 18 units pyruvate kinase, 1 mM cytidine triphosphate (CTP), 1 mM guanosine triphosphate (GTP), 0.25 nCi [¹⁴C]UTP, and with or without cytosol (7 mg protein/ml). At selected times, 50-μl samples were precipitated in 300 μl ice-cold 10% trichloroacetic acid (TCA) (w/v) contained in 400-μl microfuge tubes and centrifuged at 10,000g for 2 min in a Beckman model 152 microfuge. The supernatant was removed by aspiration and the nuclear pellet washed four times in ice-cold 10% TCA (w/v) to remove unincorporated radioactivity. After aspirating the fourth wash, the microfuge tube tips containing the nuclear pellets were cut off, placed in 20-ml scintillation vials, dissolved at 70°C in 0.4 ml of 25% tetramethylammonium hydroxide in methanol, acidified with 0.4 ml concentrated HCl, and counted in 14 ml of Hydrofluor in a Searle Mark III 6880 Liquid Scintillation System (Searle Analytic, Inc., Des Plaines, Ill.). Counting efficiency was approximately 91%. Quenching was monitored using

an internal standard; differential quench from vial to vial was not encountered.

Measurement of radioactivity extracted from labeled nuclei. This procedure was employed to determine the percentage of label incorporated into RNA. Nuclei isolated as described above from the livers of control and infected rats were incubated in Corex test tubes in the [¹⁴C]UTP incorporation system (1.0 ml total volume) and precipitated by the addition of 1.0 ml of ice-cold 20% TCA (w/v). Following centrifugation at 15,000g for 10 min in a Sorval refrigerated centrifuge, the supernatant was discarded and the pellet was washed twice by suspension and recentrifugation in 3.0 ml of ice-cold 10% TCA (w/v). The pellet was then extracted twice by suspension in 5 ml of 95% ethanol and centrifugation at 15,000g for 10 min. The ethanol-extracted pellet was suspended in 2.0 ml of 1.0 N KOH and incubated for 20 hr at 37° to hydrolyze RNA (28). Following this incubation, a 0.2-ml sample was counted in 20 ml Scintisol to determine total counts. The volume was adjusted to 2.0 ml with 1.0 N KOH, and neutralized with 0.4 ml of 6 N HCl. Following the addition of 2.0 ml of ice-cold 10% TCA (w/v), protein and DNA were pelleted by centrifugation at 15,000g for 10 min. A 0.2-ml sample of the supernatant containing hydrolyzed RNA was counted in Scintisol. The pellet was solubilized by incubation at 45°C for 30 min in 1.0 ml of 0.1 N NaOH in 0.1% (w/v) sodium dodecylsulfate. A 0.2-ml sample of the solubilized pellet was also counted in Scintisol. The percentage of total counts in the pellet and supernatant was determined.

Statistical analysis. Statistical significance was determined by analysis of variance (29) at $P \leq 0.05$.

Results. Table I shows the biochemical assessment of contamination of isolated nuclei by hepatic subcellular organelles. The specific activities of marker enzymes were determined in organelles where these enzymes occur as markers and also in the purified nuclei. The ratio of the specific activity of the enzyme in the nuclei to the specific activity of the enzyme in the organelles where it occurs as a marker was determined in order to assess nuclear purity. Alkaline phosphatase and 5'-nucleotidase were used as plasma membrane markers, glutamate and malate dehydroge-

TABLE I. BIOCHEMICAL ASSESSMENT OF CONTAMINATION OF ISOLATED NUCLEI BY OTHER ORGANELLES (46 hr FASTED/INFECTED)

	Specific activity (\pm SEM, $N = 6$) of													
	<i>S</i> -Nucleotidase ^a		Alkaline phosphatase ^b		Glutamate dehydrogenase ^c		Malate dehydrogenase ^d		NADH cytochrome C reductase ^e		Choline phospho-transferase ^f		Acid phosphatase ^g	
	C ^g	I ^h	C	I	C	I	C	I	C	I	C	I	C	I
Nuclei	0.332 \pm 0.003	0.239 \pm 0.011	0.219 \pm 0.024	1.036 \pm 0.151	N.D. ^h	N.D.	0.038 \pm 0.001	0.063 \pm 0.002	30.3 \pm 1.6	38.8 \pm 2.9	291.2 \pm 15.8	240.0 \pm 16.8	0.051 \pm 0.005	0.064 \pm 0.011
Plasma membrane	30.3 \pm 1.5	24.8 \pm 1.7	23.9 \pm 2.3	51.6 \pm 10.0										
Mitochondria					0.032 \pm 0.003	0.031 \pm 0.002	2.20 \pm 0.14	1.97 \pm 0.05						
Microsomes									341 \pm 38	564 \pm 38	2385 \pm 55	2084 \pm 15		
Lysosomes													11.1	N.Det. ⁱ
Ratio: nuclei/fraction %	1.10	0.95	0.92	2.01			1.72	3.18	8.9	6.9	12.0	11.5	0.46	

^a Micromoles or phosphate formed per 20 min per milligram protein.^b Nanomoles of *p*-nitrophenol formed per minute per milligram protein.^c Micromoles of nicotinamide adenine dinucleotide oxidized per minute per milligram of protein.^d Micromoles of nicotinamide adenine dinucleotide reduced per minute per milligram protein.^e Micromoles of cytochrome C reduced per minute per milligram protein.^f DPM/mg protein.^g Cell fraction. C = control; I = infected.^h Not detectable.ⁱ Not determined.

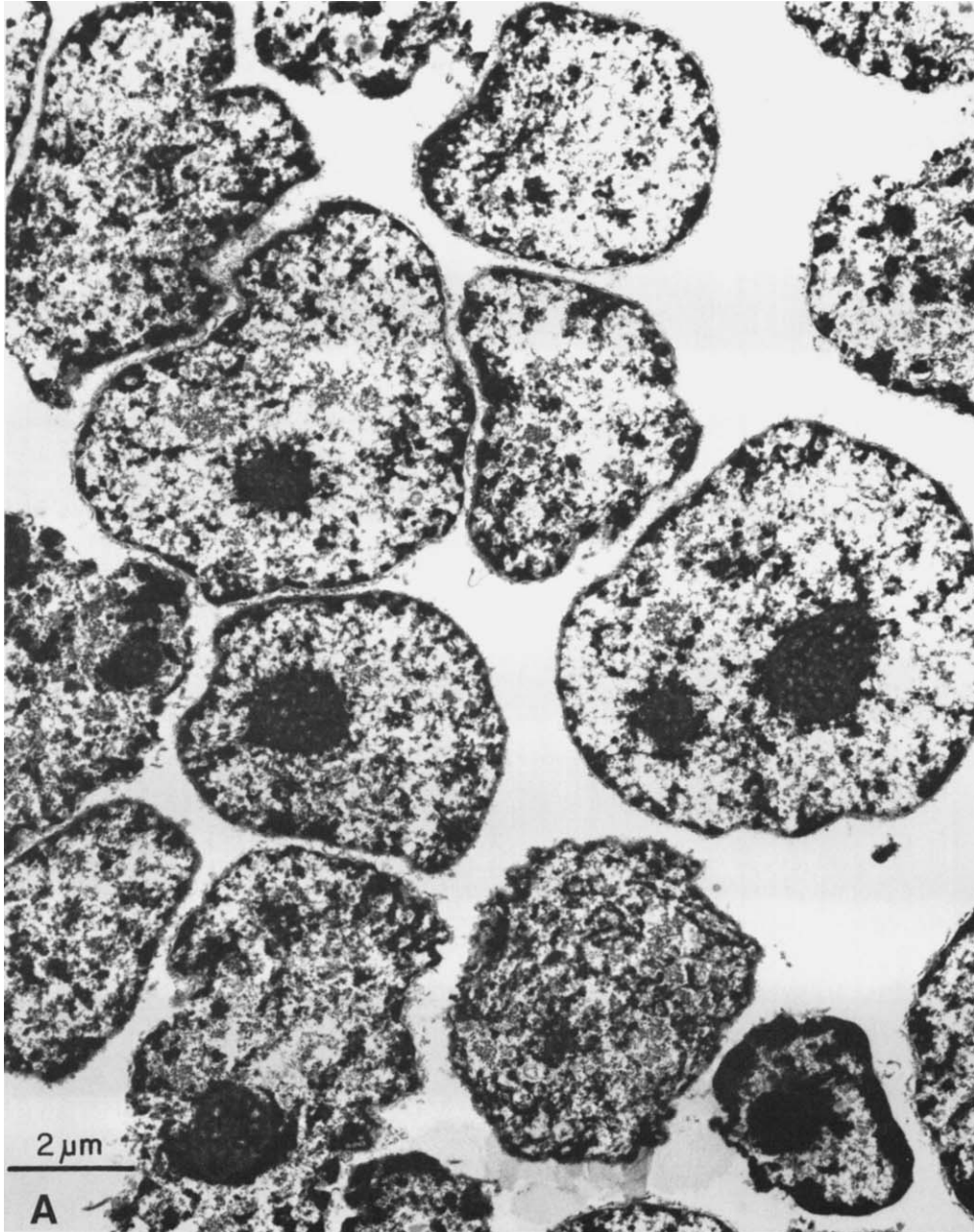


FIG. 1. Low-power electron micrograph of nuclei isolated from the livers of control (A) and infected rats (B). Both control and infected rats were fasted for 46 hr after inoculation and before isolation of nuclei. $\times 12,000$.

nase as mitochondrial markers, NADH cytochrome C reductase and choline phosphotransferase as endoplasmic reticulum markers, and acid phosphatase as a lysosomal marker. Table I shows that contamination of

nuclei by plasma membranes, mitochondria, lysosomes, and endoplasmic reticulum was less than 12%. The significant increase in the specific activity of alkaline phosphatase in the homogenate and purified plasma membranes

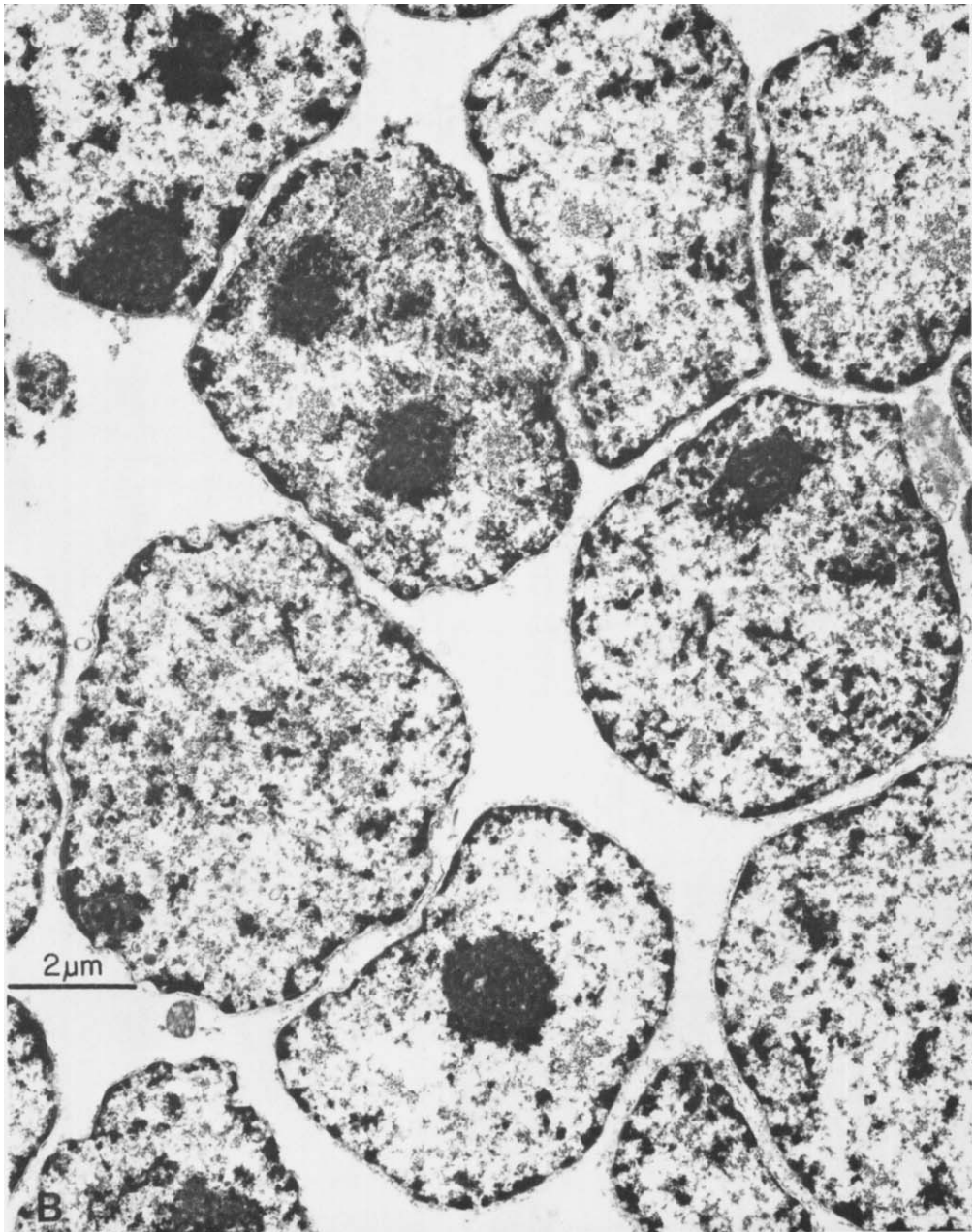


FIG. 1—*Continued.*

during infection (Table I) has been described elsewhere (23). Recovery (DNA in isolated nuclei/DNA in homogenate) was typically 55–75% for nuclei isolated from either control or infected rats.

Figure 1A and 1B show low-power electron micrographs of nuclei isolated from the livers

of control and infected rats; both preparations appear similar. It appeared that contamination by mitochondria, lysosomes, plasma membranes, and microsomes was minimal, thus confirming the biochemical results.

An assay for incorporating labeled UTP into RNA by isolated nuclei was developed. This

assay was optimized with respect to pH, temperature, yeast RNA, and nucleotide concentrations. Incorporation of label into RNA at several different pH values was examined. At all pH values tested (pH 6.5, 7.0, 7.5, 8.0, and 8.5) nuclei isolated from the livers of infected rats incorporated more label into RNA than did nuclei isolated from control rats. Although pH 8.5 appeared to result in maximum incorporation, nuclei aggregated at this pH. At pH 8.0 nuclei incorporated slightly more than at pH 7.5, whereas at pH 6.5, incorporation was approximately half maximal. pH 7.5 was chosen for the assay because it was more nearly physiological, and did not cause nuclear aggregation. The effect of incubation temperature on the incorporation of [¹⁴C]UTP into RNA by isolated nuclei was also examined. At all temperatures tested (25, 30, 35, and 40°C) nuclei isolated from infected rats incorporated more label into RNA than did nuclei isolated from control rats. This experiment showed an optimum temperature of 35°C. In an attempt to eliminate possible ribonuclease degradation of newly formed RNA and to improve linearity of UTP incorporation with time, several concentrations of yeast RNA was added to the incorporation system. A fourfold increase in yeast RNA [2 mg/ml, compared to 0.5 mg/ml (26)] resulted in maximum linearity. The effect of increasing concentrations of unlabeled nucleotides on the incorporation of labeled UTP into RNA was also examined. The optimum concentrations of ATP, CTP, and GTP were found to be 2.5, 1.0, and 1.0 mM, respectively, which was in agreement with previously published results. The incorporation of label into RNA by nuclei isolated from control or infected rats was linear through 2 mg/ml DNA. The incorporation was also linear through 6 min (Fig. 2). All subsequent experiments were done at pH 7.5, for 5 min at 35°C, and contained 2 mg/ml yeast RNA and nuclei containing approximately 0.8 mg/ml DNA.

To ensure that [¹⁴C]UTP was being incorporated into RNA, RNA was extracted from the nuclei after incubation in the incorporation system, and the percentage of radioactivity determined. In both control and infected nuclei, label was only incorporated into RNA.

The existence of a significant endogenous

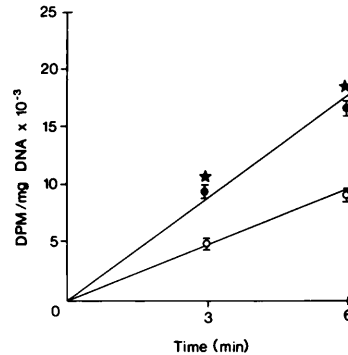


FIG. 2. Effect of infection on incorporation of [¹⁴C]UTP into RNA by nuclei isolated from the livers of control (○) or infected (●) rats. Nuclei were incubated as described in the presence of homologous cytosol. Values are mean ± SE of six determinations. Both control and infected rats were fasted for 46 hr after inoculation and before isolation of nuclei. (*) Indicates $P \leq 0.05$.

pool of nucleotides in the isolated nuclei that would affect the incorporation of [¹⁴C]UTP into RNA was also examined. Nuclei isolated from the livers of control and infected rats were incubated in the [¹⁴C]UTP incorporation system in the presence or absence of added ATP, CTP, and GTP. As shown in Fig. 3, in the absence of exogenous cold nucleotides, very little label was incorporated into RNA by nuclei isolated from either control or infected rats, thus suggesting small nuclear pools of ATP, GPT, or CTP in control or infected rats. Furthermore, in the presence of added unlabeled ATP (absence of unlabeled CTP, GTP, and cytosol), the incorporation of labeled UTP into RNA was reduced to 5.10% ± 1.16 (mean ± SE) for control and 4.97% ± 0.98 for infected.

Nuclei isolated 22 hr after infection incorporated approximately two-thirds as much label into RNA as did nuclei isolated 46 hr after infection, whereas nuclei isolated from 22- and 46-hr control rats incorporated approximately the same amount of labeled UTP into RNA.

The effect of cytosol on the incorporation of [¹⁴C]UTP into RNA was next determined (Table II). In the absence of cytosol, nuclei isolated from infected rats incorporated slightly but not significantly more [¹⁴C]UTP into RNA than did nuclei isolated from control rats. In the presence of homologous cytosol, nuclei isolated from the livers of control rats incorporated significantly more [¹⁴C]UTP

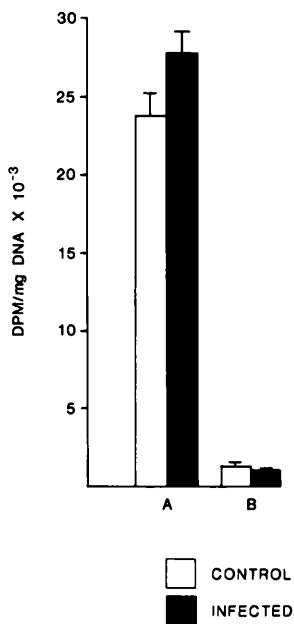


FIG. 3. Effect of ATP, CTP, and GTP on the incorporation of [¹⁴C]UTP into RNA by nuclei isolated from the livers of control (open) and infected (solid) rats. Nuclei were incubated as described in the [¹⁴C]UTP incorporation system in the absence of cytosol and the presence (A) or absence (B) of unlabeled nucleotides. At selected times, samples were removed and the amount of label incorporated into RNA determined. Values are mean \pm SE of six determinations. Both control and infected rats were fasted for 46 hr after inoculation and before isolation of nuclei.

into RNA than was observed in the absence of cytosol. Heterologous cytosol (from infected rats) did not stimulate control nuclei. In contrast, both homologous cytosol (from infected rats) and heterologous cytosol (from control rats) stimulated nuclei isolated from infected rats to incorporate [¹⁴C]UTP into RNA. More importantly, however, in the presence of either homologous or heterologous cytosol, nuclei isolated from infected rats incorporated significantly more [¹⁴C]UTP into RNA than did control nuclei. Table II also shows that when cytosol was replaced with albumin, there was no significant stimulation of label into RNA by either control or infected nuclei.

Discussion. Although *in vitro* transcriptional studies have shown early release of labeled RNA from the MPC-11 cell line (30), rat mammary tumor (30), chick embryo (30), Morris hepatoma 9121 (26), and regenerating

rat liver (26), this work represents the first isolation and characterization of hepatic nuclei from *S. pneumoniae*-infected rats and the employment of those nuclei in an *in vitro* RNA incorporation assay.

To examine the effect of infection on the incorporation of labeled precursor into RNA by isolated hepatic nuclei, it was first necessary to isolate, purify, and characterize nuclei from the livers of control or *S. pneumoniae*-infected rats. This infection, even at 46 hr after inoculation, did not alter the properties of the nuclei to preclude their isolation by conventional techniques. Characterization by both biochemical and morphological procedures showed nuclei isolated from control and infected rats to be relatively free of contamination by other cellular organelles.

The *in vitro* incorporation assay employed here was modified from existing procedures. A prime consideration during the development of this assay was the need to permit the determination of possible differences in the rate of incorporation of label into RNA by nuclei isolated from the livers of control and infected rats. Therefore, the assay conditions were optimized with respect to time, temperature, pH, yeast RNA, and nucleotide concentrations. All modifications were necessary to achieve linearity or stability of the reaction product. Through all modifications, hepatic nuclei isolated from infected rats incorporated more label into RNA than did hepatic nuclei isolated from control rats. It was necessary to ensure that the factor(s) enhancing incorporation of labeled UTP into RNA by nuclei isolated from infected rats was not spe-

TABLE II. EFFECT OF CYTOSOL ON THE INCORPORATION OF [¹⁴C]UTP INTO RNA BY NUCLEI ISOLATED FROM THE LIVERS OF CONTROL AND INFECTED RATS

	DPM/mg DNA \pm SEM (<i>N</i> = 6)	
	Control	Infected
No cytosol	18614 \pm 1227	21697 \pm 1209
Homologous cytosol	24654 \pm 604 ^b	30300 \pm 1326 ^{a,b}
Heterologous cytosol	19686 \pm 794	35532 \pm 1908 ^{a,b}
Albumin	14657 \pm 760	22491 \pm 1644 ^a

^a Infected value is significantly different than control value at *P* \leq 0.05.

^b Value within the column is significantly different than the no cytosol value in the same column at *P* \leq 0.05.

cific to cytosol prepared from the livers of infected rats. Therefore, crossover experiments were done where nuclei were incubated in the [¹⁴C]UTP incorporation assay in the presence of the opposite (heterologous) cytosol. Table II shows that in the presence of either homologous or heterologous cytosol, nuclei isolated from infected rats incorporated significantly more [¹⁴C]UTP into RNA than did control nuclei. Control cytosol appeared to stimulate infected nuclei to a significantly greater extent ($P \leq 0.05$) than did infected cytosol. At present this observation is not accounted for. These specific effects of cytosol were not mimicked by incubation of nuclei in the presence of albumin. A lack of incorporation of label into RNA in the absence of cytosol and unlabeled precursors indicated that nuclei contained a negligible pool of endogenous RNA precursors. Therefore, endogenous nuclear pools could not explain the observed results. This lack of incorporation was also observed in the presence of unlabeled ATP and the absence of cytosol, indicating that the decreased incorporation was not due to an energy deficiency, since an energy-dependent pool would be expected to permit incorporation of label into RNA.

If the initial rate of incorporation reflects RNA chain completion as proposed as McNamara *et al.* (27), these data suggest that the nuclei from infected rats were from liver cells actively engaged in an increased synthesis of RNA at the time of isolation, as compared to nuclei isolated from control rats. Although the precise role of the host transcriptional response to infection remains to be determined, this increased incorporation of label into nuclei isolated from the livers of *S. pneumoniae*-infected rats is consistent with infection-induced increased chromatin template activity (15); increased RNA synthesis during infection shown by *in vivo* experiments (12); increased synthesis, transport, and secretion of plasma proteins (16); and increased synthesis of α -1, α -2, and β globulins (6, 13).

Finally, these experiments indicate: (i) the presence of a factor or factors in the cytosol which significantly increases the [¹⁴C]UTP incorporation into RNA by nuclei isolated from the livers of infected rats, and (ii) that there is something unique about nuclei isolated from infected rats which permits a greater stimu-

lation by cytosol to synthesize RNA. Experiments are in progress to characterize both the cytosol factor(s) and the infected nuclei.

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