Biochem. J. (1973) **134**, 79–87
Printed in Great Britain

Effects of Infection with *Diplococcus pneumoniae* on Synthesis of Ribonucleic Acids in Rat Liver

By WILLIAM L. THOMPSON and ROBERT W. WANNEMACHER, JR.*
United States Army Medical Research Institute of Infectious Diseases, Frederick, Md. 21701, *U.S.A.*

(*Received* 7 *December* 1972)

Rats infected with virulent *Diplococcus pneumoniae* developed a significant increase in the rate of incorporation of labelled orotate into hepatic RNA when compared with pair-fed controls inoculated with heat-killed organisms. The finding was readily detected in rats raised on either a low-protein diet (6% casein) or a diet containing adequate amounts of protein (18% casein). The increase in hepatic RNA synthesis was observed by 12h and was maximal by 16–20h after inoculation with the *D. pneumoniae*. Most of the infection-related increase in RNA synthesis was associated with the bound ribosomal RNA fraction of the liver. A small but less significant increase was observed in the synthesis of free ribosomal RNA. The increased synthesis of RNA in the liver of infected rats resulted in a marked elevation of the liver RNA/DNA ratio, the major increase being observed in concentration of bound ribosomal RNA fraction. When followed sequentially, the infection-related increase in synthesis of hepatic RNA was preceded by a flux of amino acids into liver and was followed by elevated synthesis rates of serum globulin proteins. These findings suggest that the infectious process was able to regulate hepatic RNA synthesis by altering the rate of transcription of new RNA. This mechanism was stimulated even in rats that had been severely depleted of body protein and amino acids by feeding them on a low-protein diet. The infection-related stimulation of liver RNA and protein synthesis thus appeared to take place at the expense of other body proteins.

Recent observations in our laboratories indicated that the synthesis of certain serum proteins was markedly increased in rats infected with *Diplococcus pneumoniae* (Wannemacher *et al.*, 1971*a*). When the serum proteins were fractionated by electrophoresis, the major infection-related increase in synthesis was observed in the α_1, α_2 and β fractions with a concomitant decrease in the rate of synthesis of albumin (Powanda *et al.*, 1972). In contrast, the synthesis of total liver proteins was not significantly altered by the infectious process (Wannemacher *et al.*, 1971*a*), but increased activities of hepatic tyrosine transaminase and tryptophan oxygenase have been observed in rats or mice infected with *D. pneumoniae* (Rapoport *et al.*, 1968; Shambaugh & Beisel, 1968). Thus in rats infected with *D. pneumoniae* it would appear that the liver was stimulated to increase its synthesis of certain serum proteins and the activity of several hepatic enzymes. However, the pattern of synthesis was different from that observed in the non-infected, pair-fed controls. These observations thus raised the question as to the mechanism whereby the infectious process was able to regulate hepatic protein synthesis. Previous observations in our laboratory suggested that both the translational (synthesis of protein) and transcriptional (synthesis of template) processes were increased in the liver of mice infected with pneumococci (Lust, 1966; Kehoe & Lust, 1969). The present studies were designed to evaluate in greater detail the effects of infection on the synthesis and synthetic ability of hepatic ribosomes.

When weanling (23-day-old) rats were fed on a low-protein diet (6% casein) for 28 days, hepatic amino acid concentration and rRNA protein-synthetic ability and rate of synthesis were all markedly decreased compared with rats given an adequate amount of protein (Wannemacher *et al.*, 1968, 1971*b*). If the rats that were fed on the low-protein diet were infected with *D. pneumoniae*, serum protein synthesis was significantly increased compared with pair-fed non-infected controls (Powanda *et al.*, 1972). Thus another objective of the present study was to determine whether infection was able to stimulate similar responses in RNA metabolism in livers from protein-deprived rats.

Materials and Methods

Materials

L-[U-^{14}C]Leucine (250mCi/mmol), [*methoxy*-^{3}H]-puromycin dihydrochloride (1.1mCi/mmol) and [6-^{14}C]orotate hydrate (55mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass.,

* To whom reprint requests should be addressed.

U.S.A. ATP, GTP, L-amino acids, phosphoenolpyruvate, pyruvate kinase and antiserum to horse ferritin were obtained through Calbiochem, Los Angeles, Calif., U.S.A.

Animals

Male weanling (23-day-old) rats of the Fisher Dunning strain (Microbiological Associates, Walkersville, Md., U.S.A.) were placed in individual cages and fed on an agar-gel diet (Allison *et al.*, 1964) *ad libitum* for 28 days. One group was fed on a diet that contained 6% casein, which, in effect, represents a low-protein diet. The remaining rats were fed with an adequate amount of protein (18% casein). All rats were maintained on a 12h-light (06.00–18.00h)/12h-dark schedule and at a temperature of 25–26°C with 40% relative humidity. In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care' as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council. The facilities are accredited by the American Association of Accreditation of Laboratory Animal Care.

Micro-organism and resulting infection

Virulent *D. pneumoniae* type I strain A5 served as the infecting bacteria. Cultures were prepared for inoculation by previously described procedures (Wannemacher *et al.*, 1971*a*). The infectious process was initiated by the subcutaneous injection of 0.1ml of 0.9% NaCl-diluted culture containing 10^7 *D. pneumoniae*. Body-temperature changes were measured by rectal probe; bacteraemia was measured quantitatively by plate counting on blood–agar. Control pair-fed rats were injected with 10^7 heat-killed (56°C, 20min) organisms and were killed at 6, 12, 16 or 28h after inoculation.

Analysis of hepatic microsomal and ribosomal amino acid-incorporating ability, number of active ribosomes and polyribosomal pattern

A hepatic microsomal fraction was prepared by the procedure of Lust (1966). The system of Cooper *et al.* (1968) for amino acid incorporation *in vitro* was utilized in these experiments and the reaction was stopped after 15min incubation. The microsomal fractions were analysed for RNA and protein content by the procedure of Wannemacher *et al.* (1965). The results were expressed as amount of radioactivity per mg of protein or per μg of RNA.

A ribosomal fraction was prepared as previously described (Wannemacher *et al.*, 1971*b*) and utilized for the incorporation *in vitro* of amino acid ([^{14}C]-leucine) by procedures of Cooper *et al.* (1968). The number of active ribosomes was measured by the [^{3}H]peptidyl-puromycin technique of Wool & Kurihara (1967), and polyribosomal patterns were studied by methods of Drysdale & Munro (1967).

Incorporation of orotate into various hepatic cellular RNA fractions in vivo

Rats were injected intraperitoneally with [6-^{14}C]-orotate (10μg/100g body wt.) and were killed at ½, 1, 2 or 4h after injection. The radioactivity in the RNA of total liver homogenate, nuclear, free ribosomal, total ribosomal and non-sedimentable fractions was measured by previously described procedures (Wannemacher *et al.*, 1971*b*). Samples of liver homogenate and cellular fractions were analysed for RNA, DNA and protein by previously described procedures (Wannemacher *et al.*, 1968). To overcome the possible differences in size of the nucleotide pool, incorporation of orotate into the various RNA fractions was expressed as specific radioactivity (c.p.m./μg of RNA), percentage of total RNA radioactivity, and amount of RNA radioactivity per unit of DNA (specific radioactivity multiplied by the ratio μg of RNA/μg of DNA).

Statistics

Group mean values were compared by Student's *t* test and the difference between two means was considered significant at $P<0.01$ under the null hypothesis.

Results

Effect of infection on the protein-synthetic ability of microsomal RNA from liver cells

In both dietary groups the course of the infection was similar to that described previously (Wannemacher *et al.*, 1971*a*), except that the mean survival rate was 35.6±0.8h for the infected rats fed on 6% casein as opposed to 46.8±2.6h for the rats fed on the adequate protein diet (18% casein). Six infected and six control rats were killed at 12 or 28h after inoculation with *D. pneumoniae*. When the results were expressed as d.p.m. of [^{14}C]leucine incorporated into nascent protein/mg of microsomal proteins, a 54% increase in protein-synthetic ability was observed in the microsomal fraction from liver of the rats infected for 28h as compared with pair-fed controls (Table 1). These results were very similar to those reported by Lust (1966) in mice infected with *D. pneumoniae*. However, if the results were expressed as the amount of radioactivity incorporated into microsomal protein/μg of RNA, no significant difference was noted at either 12 or 28h after inoculation in the hepatic microsomal fractions from the infected rats as compared with pair-fed controls (Table 1). Thus the increase in

Table 1. *Effect of infection on protein-synthetic ability of liver microsomal preparations*

Livers were from 51-day-old rats that had been fed on an 18%-casein diet for 28 days. Rats were either infected with 10^7 *D. pneumoniae* or with 10^7 heat-killed (56°C, 20min) organisms and killed either 12 or 28h later. Hepatic microsomal fraction was prepared by the procedures of Lust (1966). The protein-synthetic system *in vitro* contained in 1ml: 1mg of microsomal protein, 800μg of cell-sap protein, 250μmol of sucrose, 65μmol of KCl, 10μmol of $MgCl_2$, 50μmol of Tris–HCl buffer, pH7.6 at 25°C, 5μmol of ATP, 0.1μmol of GTP, 19 naturally occurring L-amino acids (minus leucine) in the concentrations found in the liver cell (Wannemacher *et al.*, 1968) and 0.8nmol of L-[U-^{14}C]leucine (0.2μCi). Samples were incubated for 15min and protein was extracted by the procedure of Cooper *et al.* (1968). Results are recorded as the means ±S.E.M. for six rats.

Group	Time after inoculation (h)	Amino acid incorporation (d.p.m.) (per mg of protein)	(per μg of RNA)
Pair-fed controls	12	212±16	6.24±0.59
Infected	12	249±13	6.31±0.26
Pair-fed controls	28	258±11	8.36±0.45
Infected	28	385±44	9.74±0.67

Table 2. *Effect of infection on protein-synthetic activity and composition of liver ribosomes*

Ribosomes were prepared by centrifuging the postmitochondrial supernatant through 1M-sucrose as described by Wannemacher *et al.* (1971*b*). The protein-synthetic system *in vitro* contained in 1ml: 40μg of rRNA, 800μg of cell-sap protein, 250μmol of sucrose, 65μmol of KCl, 10μmol of $MgCl_2$, 50μmol of Tris–HCl buffer, pH7.6 at 25°C, 5μmol of ATP, 0.1μmol of GTP, 19 naturally occurring L-amino acids (minus leucine) in the concentrations found in liver cells (Wannemacher *et al.*, 1968) and 0.8nmol of L-[U-^{14}C]leucine (0.2μCi). For the determination of active ribosomes the reaction mixture contained in 1ml: 50μmol of Tris–HCl buffer, pH7.8, 80μmol of KCl, 12.5μmol of $MgCl_2$, 10μmol of 2-mercaptoethanol, 5μmol of ATP, 0.05μmol of GTP, 1μmol of phosphoenolpyruvate, 10μg of pyruvate kinase, 90pmol of [^{3}H]puromycin (1μCi) and 150μg of rRNA. The mixture was incubated at 37°C for 30min. The [^{3}H]peptidyl-puromycin was separated and its radioactivity counted by the Millipore-filter method of Wool & Kurihara (1967). Polyribosomes were separated on 10–40% sucrose gradients by the procedure of Drysdale & Munro (1967). Rats were treated as described in Table 1. Results are recorded as the means ±S.E.M. for six animals.

Group	Time after inoculation (h)	Amino acid incorporation (d.p.m./μg of RNA)	% of active ribosomes	% of polyribosomes
Pair-fed control	12	13.4±0.3	13.3±0.2	52.8±2.3
Infected	12	13.6±0.2	15.2±0.2	53.4±0.8
Pair-fed control	28	15.3±0.8	12.5±0.5	56.1±1.6
Infected	28	14.5±0.7	16.0±0.8	44.6±2.3

the protein-synthetic rate that Lust (1966) observed in microsomal fractions from liver of infected mice could be related to the amount of rRNA templates present in the microsomal fractions and did not represent an increased rate of utilization of the template for synthesis of nascent proteins in the livers of mice exposed to *D. pneumoniae*. Similar results were observed in microsomal fractions from livers of infected rats fed on a 6%-casein diet.

Effect of infection on hepatic ribosomal integrity

At both 12 and 24h after inoculation with *D. pneumoniae*, a significant increase was observed in the number of hepatic ribosomes that were actively synthesizing proteins, whereas at 24h there was a decrease in the percentage of polyribosomes in the livers from infected rats as compared with pair-fed controls (Table 2). However, these changes in hepatic ribosome patterns had no significant effect on the rate of

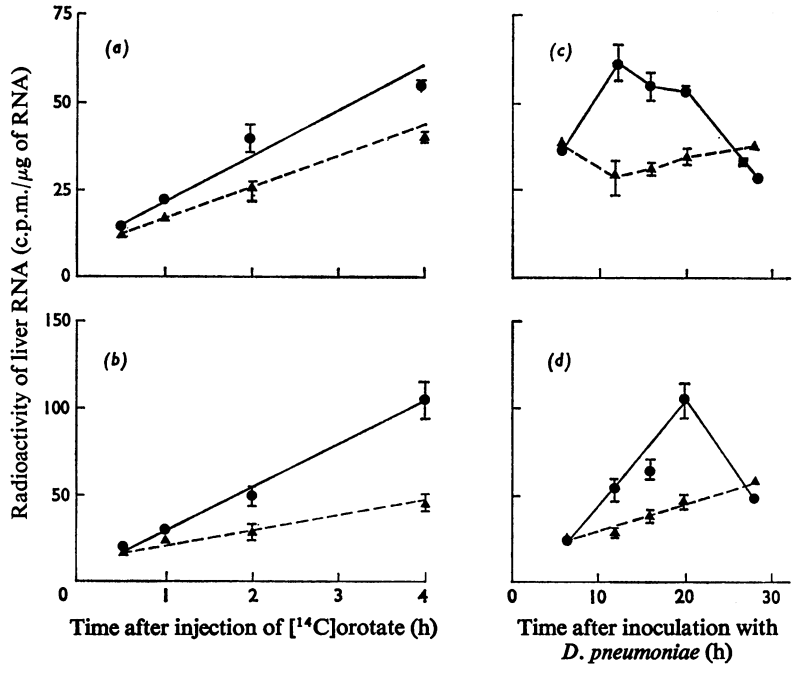

Fig. 1. *Effect of diet and infection on incorporation of orotate into hepatic RNA*

Livers were obtained from 51-day-old rats that had been fed on either a 6%-casein (*a* and *c*) or an 18%-casein (*b* and *d*) diet for 28 days. In Figs. 1 (*a*) and 1(*b*) the specific radioactivity of total liver RNA is plotted at various times after a single intraperitoneal injection of 10μCi of [6-^{14}C]orotate/100g body wt. in rats 16h after a subcutaneous injection of 10^7 *D. pneumoniae* (●) or in pair-fed controls (▲). In Figs. 1(*c*) and 1(*d*) the specific radioactivity of total liver RNA after a 4h pulse dose (10μCi/100g body wt.) of [6-^{14}C]orotate is plotted at various times after subcutaneous inoculation with 10^7 *D. pneumoniae* or in control pair-fed rats inoculated with heat-killed micro-organisms. Symbols are the same as in Figs. 1(*a*) and 1(*b*). RNA was extracted by the procedure of Wannemacher *et al.* (1971*b*). Each point is the mean for six rats. The bars represent ±S.E.M. and are plotted when the differences between groups have a probability (P)<0.01.

incorporation of [^{14}C]leucine into nascent protein *in vitro*. Similar infection-related changes were observed in rats fed on the low-protein diet.

RNA synthesis in various fractions of liver from infected animals

At 16h after inoculation with *D. pneumoniae* the specific radioactivity (c.p.m./μg of RNA) of total liver RNA after a pulse dose of [^{14}C]orotate was significantly increased in infected rats as compared with pair-fed controls (Figs. 1*a* and 1*b*). In both dietary groups the specific radioactivity of liver RNA increased in a linear fashion over the 4h time-period after injection of the [^{14}C]orotate. At 2 and 4h after injection of the pulse dose of the labelled pyrimidine the specific radioactivity of the liver from infected rats was significantly greater than that of the pair-fed controls.

The rate of incorporation of a 4h pulse dose of [^{14}C]orotate into hepatic RNA was compared at various times after inoculation of virulent or heat-killed *D. pneumoniae* in rats fed on either adequate or low-protein diets (Figs. 1*c* and 1*d*). By 12h after inoculation with the infectious organisms, a significant increase was observed in the specific radioactivity of liver RNA in both dietary groups as compared with pair-fed controls. At 12 and 20h after inoculation a maximum increase was noted in the 6%- and 18%-casein-fed infected rats respectively. By 28h, no difference was observed in the rate of RNA synthesis in infected or control rats fed on either diet.

At 16h after inoculation with *D. pneumoniae* in rats fed on the 18%-protein diet, the specific radioactivity of the RNA from the nuclear fraction of the liver of infected rats was similar to that observed in pair-fed controls (Fig. 2*b*), whereas the percentage of radioactivity in the nuclear fraction was decreased in the

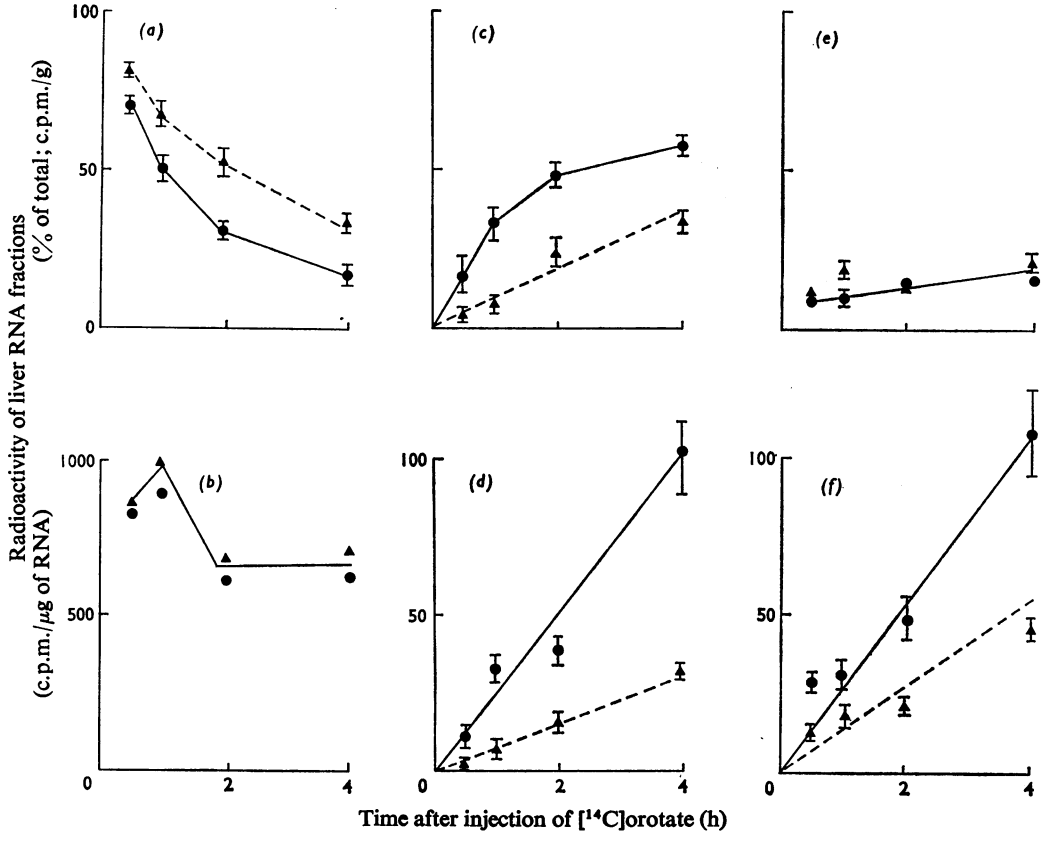

Fig. 2. *Specific radioactivity (c.p.m./μg of RNA) and percentage of total hepatic RNA radioactivity in the nuclear and bound or free ribosomal RNA fractions at various times after a single intraperitoneal injection of* [6-^{14}*C*]*orotate*

Nuclear RNA (*a,b*), bound rRNA (*c,d*) and free rRNA (*e,f*) were isolated by the procedures of Wannemacher *et al.* (1971*b*). The livers are from rats fed on an 18%-casein diet and injected subcutaneously with either 10^7 *D. pneumoniae* (●) or heat-killed (▲) organisms 16h before the pulse dose of orotate. Each point is the mean for six rats. The bars represent ±S.E.M. and are plotted when the difference between the means had a probability (*P*) of <0.01. The mean values for specific radioactivity of nuclear and percentage total radioactivity of the free rRNA fractions were essentially the same for both the infected and pair-fed controls rats; therefore a single line was plotted.

infected rats (Fig. 2*a*). At the same time-period, a significant increase was observed in specific radioactivity (Fig. 2*d*) and percentage of total radioactivity (Fig. 2*c*) in the bound ribosomal fraction (total minus free rRNA) of the infected rats as compared with the pair-fed controls. A significant increase was also observed in the specific radioactivity of the free rRNA fraction (Fig. 2*f*) in livers of infected rats as compared with the pair-fed controls, but when expressed as percentage of total radioactivity, no significant difference was observed (Fig. 2*e*). No significant differences were observed in specific radioactivity or percentage of total radioactivity in the non-sedimentable RNA fraction from livers of infected and pair-fed control rats. Similar results were obtained in the various hepatic RNA fractions from infected and non-infected rats fed on the 6%-casein diet.

In Fig. 3 the results for incorporation of a 4h pulse of [^{14}C]orotate into RNA from various cellular fractions of pair-fed controls and infected rats at 16h after inoculation are expressed as amount of radioactivity in these RNA fractions per μg of total cellular DNA. Since DNA per cell does not change appreciably over the 16h time-period, this is a means of expressing the results on a per-cell basis. The amount of radioactivity in the various cellular fractions of livers of rats fed on 6% casein was less than that in rats fed on the adequate-protein diet. When

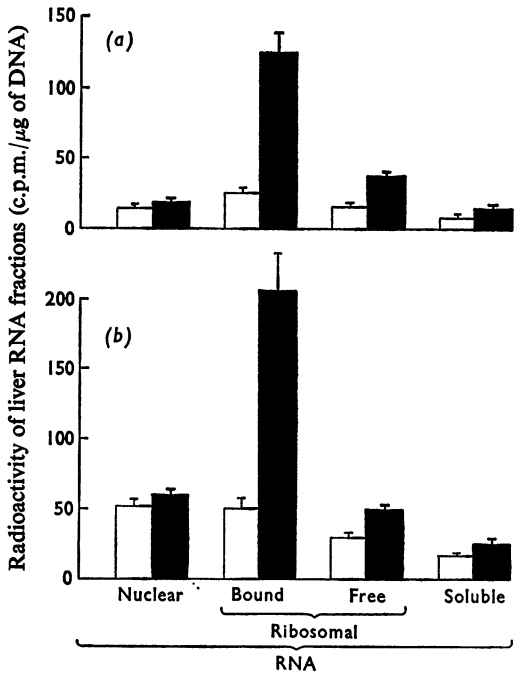

Fig. 3. *Cellular distribution of radioactivity in liver after a pulse dose of orotate in infected and protein-deprived rats*

Radioactivity of hepatic nuclear, bound and free ribosomal, and soluble RNA fractions on a cellular basis (c.p.m./μg of RNA × μg of RNA/μg of DNA) after a 4h pulse dose of [6-^{14}C]orotate in rats inoculated 16h previously with *D. pneumoniae* (■) or heat-killed organisms (□) and fed on either a 6%- (*a*) or 18%-(*b*) casein diet for 28 days. Results are expressed as means ±S.E.M. for six rats.

either dietary group was inoculated with *D. pneumoniae* the rate of incorporation of orotate into the bound rRNA fraction of the liver in rats fed on 18% and 6% casein was increased by 422 and 495% respectively. Similarly, the synthesis of free hepatic rRNA was increased by 142 and 250% respectively. No significant change was observed in the nuclear or soluble fractions of the liver from infected rats as compared with pair-fed controls.

The RNA/DNA ratio was significantly increased in the liver of rats infected with *D. pneumoniae* by 16h after inoculation and continued to increase linearly up to 28h (Figs. 4*a* and 4*b*). The infection-related increase in hepatic RNA was observed in rats fed on either the adequate-protein or low-protein diet. When the RNA/DNA ratios were compared in the various cellular fractions at 28h, a significant increase in the cellular concentration of bound rRNA was observed in the infected rats as compared with pair-fed controls (Figs. 4*c* and 4*d*). No significant difference was observed in the amount of RNA present in the free ribosomal or soluble RNA fractions.

Discussion

Infection with *D. pneumoniae* in rats was characterized by an early movement of amino acids from muscle to liver cells and a subsequent increase in the synthesis of serum globulin proteins (Wannemacher *et al.*, 1971*a*; Powanda *et al.*, 1972). Previous observations by Lust (1966) and Kehoe & Lust (1969) suggested that the infection-stimulated increases in protein synthesis in mice were regulated at both the translational and transcriptional sites within the liver cell. These earlier investigators utilized a microsomal fraction to measure rates of amino acid incorportation *in vitro* into proteins and expressed their results per unit of microsomal protein; similar results were derived from the present studies of pneumococcal infection in rats. However, if our present results were expressed on an RNA basis (Table 1) no difference was observed in the rates of protein synthesis *in vitro* in microsomal fractions from livers of infected or pair-fed control rats. Similarly, when the amino acid-incorporating ability of ribosomal fractions from livers of infected and control rats was compared, no differences were observed (Table 2). Although there was a small but significant increase in the number of ribosomes that were actively synthesizing protein in the fractions from the infected rats, the process did not produce an overall increase in the protein-synthetic activity of the total ribosomal fraction. Therefore it seemed evident that the major effects of infection in regulating hepatic RNA synthesis were not at the translational sites. These observations thus lead to further studies during infection as to the possible regulation of hepatic protein synthesis at the transcriptional sites.

By 12h after inoculation with *D. pneumoniae*, RNA synthesis was significantly increased in the liver of infected rats as compared with pair-fed controls. The increase in synthesis of hepatic RNA takes place at approx. 6–8h before the observed increments in serum protein synthesis (Powanda *et al.*, 1972). Thus the increase in the rate of serum protein synthesis would appear to be regulated at the transcriptional level and was preceded by a stimulation in the rate of new hepatic RNA production. Similar observations of an increase in hepatic RNA synthesis were reported by Kehoe & Lust (1969) in mice infected with *D. pneumoniae*.

The specific radioactivity in the nuclear RNA fractions in the liver of our infected rats was similar to that observed in the pair-fed controls, which would suggest that the changes in synthesis of other cellular RNA fractions do not represent an alteration in

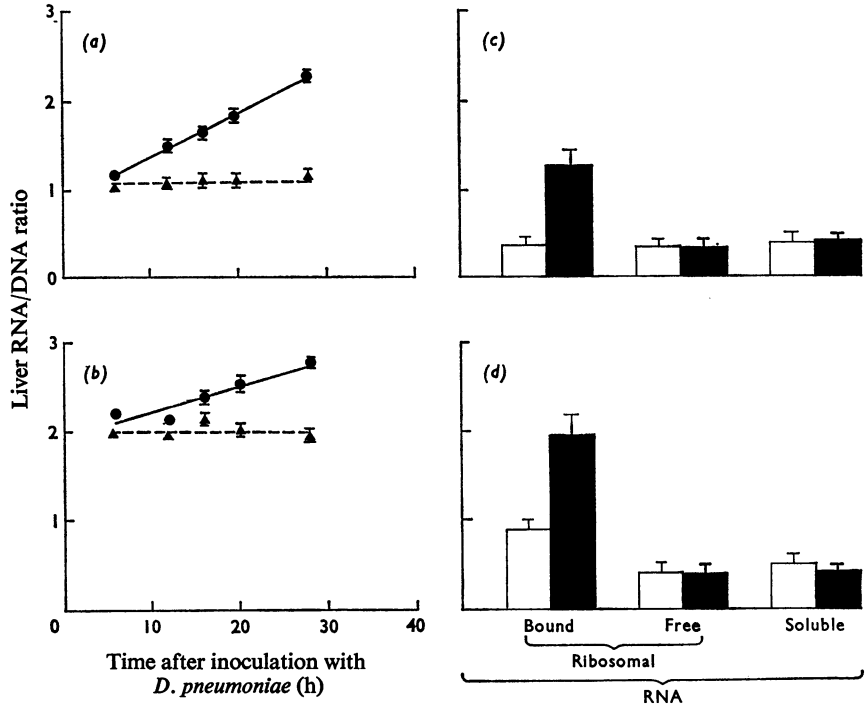

Fig. 4. *Liver RNA/DNA ratio in infected and protein-deprived rats*

(*a*) and (*b*) Total liver RNA/DNA ratio at various times after inoculation with *D. pneumoniae* (●) or heat-killed organisms (▲) in rats that were fed on either a 6%-(*a*) or 18%-(*b*) casein diet for 28 days. Each point is the mean for six rats. The bars represent ±S.E.M. and are plotted when the difference between the means has a probability (*P*) <0.01. (*c*) and (*d*) RNA/DNA ratios in bound and free ribosomal and soluble liver fractions at 28h after inoculation with *D. pneumoniae* (■) or heat-killed organisms (□) in rats fed on either a 6%-(*c*) or 18%-(*d*) casein diet. The livers were fractionated by the procedures of Wannemacher *et al.* (1971*b*). Results are expressed as means ±S.E.M. for six rats.

nucleotide pool size. Thus the increases in specific radioactivity in the bound and free rRNA fractions from the liver of infected rats represent elevations in the rates of synthesis of these ribosomal moieties. When expressed as a percentage of total hepatic radioactivity, the marked increases in RNA in the bound ribosomal fraction result in a decreased percentage in the nuclear and little change in the free ribosomal fractions. Therefore not only was there a marked increase in the rate of synthesis of bound ribosomes but also an elevated rate of passage of the newly synthesized RNA moieties from nucleus to cytoplasm. This difference was more evident when the radioactivity was expressed on a per-cell basis. In the rats receiving a 4h pulse of orotate there was nearly a fourfold increase in the amount of radioactivity in the bound rRNA fraction from the liver of infected rats as compared with the pair-fed controls. A similar but smaller increase was observed in the free rRNA fraction. The increase in RNA synthesis in the liver of infected rats resulted in a significant accumulation of cellular RNA as shown by the elevated RNA/DNA ratio; most of this increase was noted in the bound rRNA fraction.

Several investigators (Redman, 1969; Hicks *et al.*, 1969; Ganoza & Williams, 1969) suggested that free ribosomes synthesized mostly internal proteins of the hepatic cells, whereas bound ribosomes mainly synthesized proteins for export (serum proteins). Thus the observed infection-related increase in synthesis of serum globulin proteins would be in agreement with the hypothesis of an increase in RNA directed to the bound ribosomes, which are at the sites of synthesis of these serum proteins destined for export. Although little change was noted in the rate of synthesis of total liver proteins in the infected rat

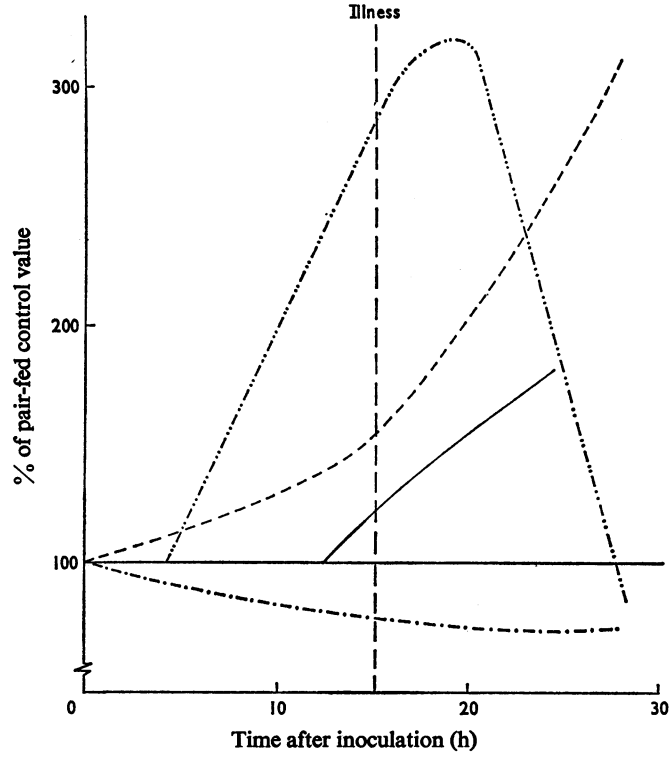

Fig. 5. *Schematic representation of sequential biochemical changes in liver of rat infected with D. pneumoniae*

Changes in liver cyclo[1-^{14}C]leucine (----) and serum amino acids concentrations (-·-·-) from Wannemacher *et al.* (1971*b*); rate of incorporation of [6-^{14}C]orotate into hepatic bound rRNA (-··-··-), present paper; rate of incorporation of [5,6-^{3}H]leucine into serum proteins (——) from Powanda *et al.* (1972). Rats were inoculated subcutaneously with 10^7 *D. pneumoniae*. The results are expressed as percentages of the values from pair-fed controls which were inoculated with the same number of heat-killed organisms. Illness (vertical broken line) is characterized by the onset of fever and bacteraemia.

(Wannemacher *et al.*, 1971*a*; Powanda *et al.*, 1972), certain specific intracellular proteins of the liver such as tyrosine transaminase (Shambaugh & Beisel, 1968) and tryptophan oxygenase (Rapoport *et al.*, 1968) have been reported to increase in rats or mice that were infected with *D. pneumoniae*. The small increase in free rRNA fraction in the liver of infected rats may be associated with the synthesis of such specific proteins, such as tyrosine transaminase and tryptophan oxygenase.

As illustrated in Fig. 5 the sequential changes in the liver of rats infected with *D. pneumoniae* can be characterized by a rapid increase in flux of amino acids into liver and decrease in serum amino acid concentrations by 4h (Wannemacher *et al.*, 1971*a*), an increase in hepatic bound rRNA synthesis by 10h, and subsequent elevation in the rate of formation of serum globulin proteins (Powanda *et al.*, 1972) by 16h after inoculation of the virulent pneumococci. This pattern of change emphasizes the relationship that has been reported between the movement in concentration of cellular free amino acids and regulation of RNA and protein synthesis within a given cell (Wannemacher, 1972).

When weanling rats were raised on a low-protein diet (6% casein), hepatic cells showed decreased amounts of free amino acids, bound rRNA and ability for synthesis of hepatic proteins or new rRNA *in vivo* or *in vitro* when compared with rats raised on an adequate protein diet (Wannemacher *et al.*, 1968, 1971*b*). However, if rats raised on this low-protein diet were infected with *D. pneumoniae*, a very marked increase was observed in the synthesis of bound rRNA. Again, this increase in bound rRNA was correlated with an influx of amino acids into liver and synthesis of serum globulin proteins in rats fed

on the low-protein diet and infected with *D. pneumoniae* (Powanda *et al.*, 1972). These observations would suggest that the infection-related increase in hepatic utilization of amino acid will take place at the expense of other tissue proteins, even in the protein-depleted host.

References

Allison, J. B., Wannemacher, R. W., Jr., Banks, W. L., Jr. & Wunner, W. H. (1964) *J. Nutr.* **84**, 383–388

Cooper, W. K., Muramatsu, K. & Wannemacher, R. W., Jr. (1968) *Biochim. Biophys. Acta* **169**, 269–271

Drysdale, J. W. & Munro, H. N. (1967) *Biochim. Biophys. Acta* **138**, 616–618

Ganoza, M. C. & Williams, C. A. (1969) *Proc. Nat. Acad. Sci. U.S.* **63**, 1370–1376

Hicks, J. J., Drysdale, J. W. & Munro, H. N. (1969) *Science* **164**, 584–585

Kehoe, J. M. & Lust, G. (1969) *J. Infec. Dis.* **120**, 411–418

Lust, G. (1966) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **25**, 1688–1694

Powanda, M. C., Wannemacher, R. W., Jr. & Cockerell, G. L. (1972) *Infec. Immunity* **6**, 266–271

Rapoport, M. I., Lust, G. & Beisel, W. R. (1968) *Arch. Intern. Med.* **121**, 11–16

Redman, C. M. (1969) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **28**, 726 (abstr.)

Shambaugh, G. E., III & Beisel, W. R. (1968) *Endocrinology* **83**, 965–974

Wannemacher, R. W., Jr. (1972) *Proc. Nutr. Soc.* **31**, 284–290

Wannemacher, R. W., Jr., Banks, W. L., Jr. & Wunner, W. H. (1965) *Anal. Biochem.* **11**, 320–326

Wannemacher, R. W., Jr., Cooper, W. K. & Yatvin, M. B. (1968) *Biochem. J.* **107**, 615–623

Wannemacher, R. W., Jr., Powanda, M. C., Pekarek, R. S. & Beisel, W. R. (1971*a*) *Infec. Immunity* **4**, 556–562

Wannemacher, R. W., Jr., Wannemacher, C. F. & Yatvin, M. B. (1971*b*) *Biochem. J.* **124**, 385–392

Wool, I. G. & Kurihara, K. (1967) *Proc. Nat. Acad. Sci. U.S.* **58**, 2401–2407