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

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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 12 h and was maximal by 16–20 h 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., 1971a). When the serum proteins were fractionated by electrophoresis, the major infection-related increase in synthesis was observed in the $\alpha_1$, $\alpha_2$ and $\beta$ 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., 1971a), 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, 1971b). 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-14C]Leucine (250 mCi/mmol), [methoxy-3H]-puromycin dihydrochloride (1.1 mCi/mmol) and [9-14C]orotate hydrate (55 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass.,
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., 1971a). The infectious process was initiated by the subcutaneous injection of 0.1 ml of 0.9% NaCl-diluted culture containing 10⁷ 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 10⁷ 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., 1971b) and utilized for the incorporation in vitro of amino acid ([14C]-leucine) by procedures of Cooper et al. (1968). The number of active ribosomes was measured by the [3H]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 [6-14C]-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., 1971b). 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., 1971a), 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 [14C]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 10⁷ *D. pneumoniae* or with 10⁷ heat-killed (56°C, 20 min) organisms and killed either 12 or 28 h later. Hepatic microsomal fraction was prepared by the procedures of Lust (1966). The protein-synthetic system in vitro contained in 1 ml: 1 mg of microsomal protein, 800 μg of cell-sap protein, 250 μmol of sucrose, 65 μmol of KCl, 10 μmol of MgCl₂, 50 μmol of Tris–HCl buffer, pH 7.6 at 25°C, 5 μmol of ATP, 0.1 μmol of GTP, 19 naturally occurring L-amino acids (minus leucine) in the concentrations found in the liver cell (Wannemacher et al., 1968) and 0.8 nmol of L-[U-¹⁴C]leucine (0.2 μCi). Samples were incubated for 15 min and protein was extracted by the procedure of Cooper et al. (1968). Results are recorded as the means ± S.E.M. for six rats.

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

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

Ribosomes were prepared by centrifuging the postmitochondrial supernatant through 1 M sucrose as described by Wannemacher et al. (1971b). The protein-synthetic system in vitro contained in 1 ml: 40 μg of rRNA, 800 μg of cell-sap protein, 250 μmol of sucrose, 65 μmol of KCl, 10 μmol of MgCl₂, 50 μmol of Tris–HCl buffer, pH 7.6 at 25°C, 5 μmol of ATP, 0.1 μmol of GTP, 19 naturally occurring L-amino acids (minus leucine) in the concentrations found in liver cells (Wannemacher et al., 1968) and 0.8 nmol of L-[U-¹⁴C]leucine (0.2 μCi). For the determination of active ribosomes the reaction mixture contained in 1 ml: 50 μmol of Tris–HCl buffer, pH 7.8, 80 μmol of KCl, 12.5 μmol of MgCl₂, 10 μmol of 2-mercaptoethanol, 5 μmol of ATP, 0.05 μmol of GTP, 1 μmol of phosphoenolpyruvate, 10 μg of pyruvate kinase, 90 pmol of [¹⁴H]puromycin (1 μCi) and 150 μg of rRNA. The mixture was incubated at 37°C for 30 min. The [¹⁴H]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.

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

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 24 h after inoculation with *D. pneumoniae*, a significant increase was observed in the number of hepatic ribosomes that were actively synthesizing proteins, whereas at 24 h 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
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 10 µCi of [6-14C]orotate/100 g body wt. in rats 16 h after a subcutaneous injection of 107 D. pneumoniae (●) or in pair-fed controls (▲). In Figs. 1(c) and 1(d) the specific radioactivity of total liver RNA after a 4 h pulse dose (10 µCi/100 g body wt.) of [6-14C]orotate is plotted at various times after subcutaneous inoculation with 107 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. (1971b). 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.

The rate of incorporation of a 4 h pulse dose of [14C]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. 1c and 1d). By 12 h 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 20 h after inoculation a maximum increase was noted in the 6% and 18% casein-fed infected rats respectively. By 28 h, no difference was observed in the rate of RNA synthesis in infected or control rats fed on either diet.

At 16 h 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. 2b), whereas the percentage of radioactivity in the nuclear fraction was decreased in the

RNA synthesis in various fractions of liver from infected animals

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

incorporation of [14C]leucine into nascent protein in vitro. Similar infection-related changes were observed in rats fed on the low-protein diet.
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-\textsuperscript{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. (1971b). The livers are from rats fed on an 18% casein diet and injected subcutaneously with either 10\textsuperscript{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. 2a). At the same time-period, a significant increase was observed in specific radioactivity (Fig. 2b) and percentage of total radioactivity (Fig. 2c) 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. 2f) 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. 2e). 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 [\textsuperscript{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
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. 4a and 4b). 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. 4c and 4d). 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., 1971a; 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 incorporation *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
EFFECTS OF *D. PNEUMONIAE* INFECTION ON HEPATIC RNA SYNTHESIS

(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 28 h 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. (1971b). Results are expressed as means ±S.E.M. for six rats.

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 28 h 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. (1971b). 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 4 h 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
Changes in liver cyclo[1-14C]leucine (-----) and serum amino acids concentrations (---) from Wannemacher et al. (1971b); rate of incorporation of [6-14C]orotate into hepatic bound rRNA (-----), present paper; rate of incorporation of [5,6-3H]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., 1971a; 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., 1971a), 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, 1971b). 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


