RESULTS
AND
DISCUSSION
5.0 RESULTS AND DISCUSSION

5.1 Temperature response

The mean rectal temperature of a number of guinea pigs was $38 \pm 0.5^\circ C$. The results are in agreement with those observed by Lennette and Schmidt (1979). The mean rectal temperature of mock infected guinea pigs and guinea pigs infected with *Q. burnetii* is presented in Fig. 9. Mock infected guinea pigs maintained the normal range of temperature ($38 \pm 0.5^\circ C$) throughout the period of observation. The guinea pigs infected with *Q. burnetii* exhibited pyrexia between 5th and 9th PI days. The mean rectal temperature during the febrile period was $39^\circ C$ on the 5th, $40^\circ C$ on the 6th, $41^\circ C$ on the 7th, $39.5^\circ C$ on the 8th and $39^\circ C$ on the 9th PI day. The temperature returned to normal on the 10th PI day ($38.5^\circ C$) and was maintained as such throughout the remaining period of observation (Fig. 9).

The mock infected guinea pigs were active and had a normal appetite. During the febrile period, the infected guinea pigs were lethargic, did not feed well and seemed to be in distress. The febrile response observed in the infected guinea pigs was generally
FIG. 9 RECTAL TEMPERATURE AND BODY WEIGHT OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF FIVE INDIVIDUAL DETERMINATIONS.
similar to the classical description of the infection of Q fever agent in guinea pigs (Derrick, 1937).

5.2 **Body weight**

The mock infected animals maintained their body weight and showed a slight, but progressive increase till the 28th PI day, when the experiments were terminated. The guinea pigs infected with *C. burnetii* showed a progressive loss in the body weight from the 3rd PI day onwards. The mean loss in the body weight (as calculated from the pre-inoculation body weight) was 2.28 per cent on the 3rd, 8.72 per cent on the 7th, 12.56 per cent on the 10th, 10.38 per cent on the 14th, 10.10 per cent on the 17th, 8.93 per cent on the 21st, 7.33 per cent on the 24th and 6.47 per cent on the 28th PI day (Fig.9).

Paretsky *et al.*, (1964) as well as Shukla and Negi (1977) reported the pyrexial response and loss in the body weight of guinea pigs experimentally infected with the Nine Mile strain of *C. burnetii* (Phase I). The pyrexial response in guinea pigs reported by Paretsky *et al.*, (1964) was from day 1 to day 7 post infection. The guinea pigs lost 6% of body weight on day 1. The loss was 28 per cent on day 9.
Shukla and Negi (1977) also observed pyrexial response in Q. burnetii infected guinea pigs between 3rd and 6th PI day and loss in the body weight of guinea pigs was 5.04 per cent on day 2 and 26.1 per cent on day 9. In the present study, the loss in body weight was not as profound as was observed by the above authors. One of the reasons for the difference could be the difference of the phase of the infecting organism. Other reasons could be the breed, the age, and the diet of the animals. However there was a general agreement with our findings that there was pyrexia and loss of body weight.

5.3 Rickettsemia

Study of rickettsemia in Q. burnetii infected guinea pigs revealed the presence of organisms in the yolk sac smears of all the eggs which were inoculated with the 3rd and 7th PI day blood clot suspensions (Fig.10). The blood clot suspensions of mock infected guinea pigs were negative. These findings indicated that during the febrile period there was a phase or rickettsemia in the guinea pigs infected with Q. burnetii.
Fig. 10 Demonstration of *C. burnetii* in the smear of yolk sac of chick embryo, inoculated with blood clot suspension of guinea pig infected with *C. burnetii*. (Magnification x 3500)
5.4 Complement levels and CF antibodies

The complement titration was performed on the sera of mock infected guinea pigs and guinea pigs infected with *Q. burnetii* on different days after infection. The level of complement ("C") remained unchanged in the sera of mock infected guinea pigs throughout the entire period of observation (titre 1:70). The pre-inoculation ("O" day) "C" titre in four of the five guinea pigs sera was 1:70, while one had a titre of 1:80. Following infection with *Q. burnetii*, it was observed that the "C" titre in only one of the guinea pigs (which had the pre-inoculation titre of 1:80), dropped from 1:80 to 1:70 on the 7th PI day. It remained unchanged (titre 1:70) in the sera of remaining infected animals. However, on the 10th and 14th PI day, all the guinea pigs infected with *Q. burnetii* exhibited a drop in "C" titre from 1:70 to 1:60. On the 21st PI day, the level of "C" returned to 1:70 and was maintained as such till the end of observation period (28th PI day).

Mosher et al., (1977) studied the coagulation and complement system during Rocky Mountain spotted Fever in *Macaca mulatta* experimentally infected with
R. rickettsii. With the onset of fever and rickettsemia, animals developed hyperfibrinogenemia, mild thrombocytopenia and prolonged prothrombin time. Decrease in the amount of hemolytic titres of the functional C2 and C3 components of the complement were found in only one monkey that developed peripheral gangrenous ecchymoses when rickettsemia and agglutinating antibody were present (5th day of fever).

In the present study, the drop in titre of 'C' observed in the sera of C. burnetii infected guinea pigs was only marginal. Therefore, no significance could be attached to the observed effect. The phase II CF antibodies to C. burnetii were detected in the convalescent guinea pigs sera (28th PI day) in a titre ranging from 1:128 to 1:512. The sera of mock infected guinea pigs were negative. The immunological response of guinea pigs to C. burnetii is in agreement with the findings reported by earlier workers. (Fiset, 1957; Kazar et al., 1977; Kishimoto et al., 1978).

5.5 Enzymological studies

5.5.1 Units of enzyme activity

The International Union of Biochemistry has recommended the use of International Units (IU) to
express the enzyme activity of serum or plasma. One IU of an enzyme equals to that amount which will catalyze the transformation of one micromole (μ mol.) of substrate per minute under defined conditions. The enzyme activity could be expressed as IU/ml or IU/litre. Since the activity expressed as IU/ml would be extremely small, it is also sometimes expressed in terms of one thousandth part of a IU/ml i.e. mIU/ml (Bush, 1975) e.g. 0.002 IU/ml = 2 mIU/ml).

The enzyme activities expressed in terms of IU are not necessarily comparable unless the reaction conditions are identical. The enzyme activity depends upon a number of factors such as temperature, pH, nature of substrate and the buffer employed. It was therefore essential to estimate the normal levels of various serum enzymes in guinea pigs in our laboratory. Activities of certain enzymes viz. Alkaline phosphotase, serum glutamate oxalacetate transminase and serum glutamate pyruvate transminase are still commonly being expressed in the units described by the original investigators. This system has also been followed in the current studies.
5.5.2 Normal levels

The normal levels of serum enzymes estimated in adult guinea pigs in the present study are depicted in Table 8. The normal levels of blood glucose, tissue glycogen and liver enzyme such as glycogen phosphorylase and glycogen synthetase estimated in adult guinea pigs are presented in Table 9.

Keller (1979) as well as Metzenauer and Lutz (1981) estimated the plasma levels of alkaline phosphatase (AP) serum glutamate oxalaceta transminase (SGOT), lactic dehydrogenase (LDH), creatine phosphokinase (CPK) in normal guinea pigs. The levels were AP 105 ± 25 IU/L, SGOT 47 ± 17 mU/ml, LDH 130 ± 30 mU/ml and CPK 95 ± 35 mU/ml. Singh et al. (1963, 1963a) estimated the levels of blood glucose, liver glycogen, and certain liver enzymes such as glycogen phosphorylase and glycogen synthetase in normal adult guinea pigs. The values reported by the authors were as follows: blood glucose 105 ± 1.8 mg/dl; liver glycogen 32 ± 3.8 mg/gm of fresh liver, liver phosphorylase 23.6 ± 2.2 units/gm of wet weight of tissue and glycogen synthetase 5.78 ± 0.5 units/gm of wet weight of tissue. The normal level of serum enzymes in guinea pigs estimated in the present study could
## TABLE 8

Normal levels of serum enzymes in adult guinea pigs

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Enzyme Code No.*</th>
<th>Activity Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase (AP)**</td>
<td>EC, 3.1.3.1</td>
<td>8.35 ± 3.37 K.A.† Units/dl.</td>
</tr>
<tr>
<td>Serum Glutamate Oxalacetate Transaminase (SGOT)**</td>
<td>EC, 2.6.1.1</td>
<td>38.21 ± 7.02 Units/ml.</td>
</tr>
<tr>
<td>Serum Glutamate Pyruvate Transaminase (SGPT)**</td>
<td>EC, 2.6.1.2</td>
<td>30.25 ± 5.79 Units/ml.</td>
</tr>
<tr>
<td>Lactic Dehydrogenase (LDH)***</td>
<td>EC, 1.1.1.27</td>
<td>221.83 ± 31.55 Units/ml.</td>
</tr>
<tr>
<td>Malic Dehydrogenase (MDH)***</td>
<td>EC, 1.1.1.37</td>
<td>446.06 ± 115.59 Sigma Units/ml.</td>
</tr>
<tr>
<td>Creatine Phosphokinase (CPK)**</td>
<td>EC, 2.7.3.2</td>
<td>21.67 ± 9.79 Sigma Units/ml.</td>
</tr>
</tbody>
</table>

* Enzyme identification number assigned by the Enzyme commission of the International Union of Biochemistry.

** Each assay is the mean of 15-20 individual determinations.

*** Each assay is the mean of 30 individual determinations.

† King Armstrong Units.
### TABLE 9

**Normal levels of blood glucose, tissue enzymes and tissue glycogen content in adult guinea pigs**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>Blood glucose*</td>
<td>95.32 ± 13.23 mg per cent</td>
</tr>
<tr>
<td>Glycogen phosphorylase (Liver)**</td>
<td>26.74 ± 2.23 Units/gm of wet Weight of tissue</td>
</tr>
<tr>
<td>EC, 2.4.1.1</td>
<td></td>
</tr>
<tr>
<td>Glycogen synthetase (Liver)**</td>
<td>5.24 ± 0.5 Units/gm of wet Weight of tissue</td>
</tr>
<tr>
<td>EC, 2.4.1.11</td>
<td></td>
</tr>
<tr>
<td>Liver glycogen**</td>
<td>26.18 ± 5.9 mg/gm of wet Weight of tissue</td>
</tr>
<tr>
<td>Muscle Glycogen**</td>
<td>3.35 ± 1.0 mg/gm of wet Weight of tissue</td>
</tr>
<tr>
<td>Heart glycogen**</td>
<td>1.54 ± 0.19 mg/gm of wet Weight of tissue</td>
</tr>
</tbody>
</table>

* Each assay is the mean of 20 individual determinations.

** Each assay is the mean of 8-10 individual determinations.
not be compared with those mentioned by the above authors due to differences in the expression of units and also for the reasons mentioned above. However, the levels of blood glucose, liver glycogen as well as liver phosphorylase and glycogen synthetase estimated in the present studies were in general agreement with those reported by Singh et al. (1963, 1963a).

5.5.3 **Enzymological response in infected guinea pigs**

The results of the serum enzyme assays carried out on mock infected and *Q. burnetii* infected guinea pigs have been depicted in Fig.11 to Fig.15. The levels of alkaline phosphatase (AP) remained within the normal range (AP = 8.35 ± 3.38, K.A. unit per dl. of serum), in the sera of infected guinea pigs during the course of infection (Fig.11). The AP activity may be increased in hyperparathyroidism, Paget's disease, rickets, osteoblastic sacroma, metastatic carcinoma and obstructive jaundice (Harper, 1975). In hepatocellular disease, such as infectious hepatitis, serum alkaline phosphatase may be slightly raised (Wooton, 1964). Raised levels of AP have also been reported in three cases of Q fever in the humans...
SERUM ALKALINE PHOSPHATASE LEVELS OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF EIGHT INDIVIDUAL DETERMINATIONS.
(Piccoli et al., 1960) but the rise was detected in the early convalescence (21st day of the onset of illness).

The results of serum glutamate oxalacetate transaminase (SGOT) (Fig. 12) and serum glutamate pyruvate transaminase (SGPT) (Fig. 13) assays in the infected guinea pigs indicated that the SGPT level in the infected guinea pigs remained within the normal range (30.25 ± 5.79 units per ml. of serum) during the course of infection. However, significant elevations in the SGOT levels were observed from 7th PI day (66 units per ml.) onwards and the enzyme levels remained elevated until the 28th PI day (Fig. 12). Both transaminases are widely distributed in the animal tissues. Heart and liver are particularly rich in GOT. Kidney and skeletal muscle are also active sources of this enzyme. Liver contains a fairly larger amount of GPT but other tissues such as kidney, heart and skeletal muscle also have relatively significant quantities.

SGOT rises rapidly after myocardial infarction, peak values which may be 2-20 times normal, are reached within 24-48 hours. The normal levels are regained within 3-5 days. SGPT is not elevated in myocardial
Fig. 12. Serum glutamate oxalacetic transaminase levels of guinea pigs infected with C. burnetii (Phase II). Each assay is the mean of ten individual determinations.
FIG 13 SERUM GLUTAMATE-PYRAVATE TRANSAMINASE LEVELS OF GUINEA PIGS.

EACH ASSAY IS THE MEAN OF TEN INDIVIDUAL DETERMINATIONS.
infarction unless the lesion is a large one or is associated with the liver damage (Wooton, 1964). Both transaminases are elevated in hepatocellular damage caused by the hepatotoxic drugs, infectious hepatitis and primary or secondary liver cancer. The rise in the level of SGPT is particularly marked in infectious hepatitis and the level of SGPT often exceeds that of SGOT (Beeson et al., 1975). Raised serum transaminases are also seen in Laennec's cirrhosis, biliary cirrhosis and obstructive jaundice (Wootton, 1964; Thompson and Wooton, 1970). SGOT has been reported to be elevated in the humans suffering from Q fever. Sheridan and associates (1974) reported two cases of Q fever (serologically diagnosed) with raised SGOT. Provvidenza and Ciarla (1971) reported a human case of Q fever (also diagnosed serologically) with raised serum transaminases. Raised serum transaminases in the humans suffering from Kyasanur Forest disease – a viral infection, has also been reported (Banerjee, 1978).

Myocarditis has been reported in guinea pigs experimentally infected with the Nine Mile and M-44 strains of C. burnetii (Rychlo and Pospisil, 1958;
Johnson et al., 1977). The myocardial lesions caused by the Nine Mile strain were severe on 6th day of infection and were characterized by focal areas of necrosis accompanied by mononuclear cell infiltrates beneath the epicardium and occasionally adjacent to the small blood vessels. Infection with M-44 vaccine strain in guinea pig resulted in a mild, multifocal, acute to subacute myocarditis, characterized by mononuclear cell infiltrates with an occasional neutrophil. Sheridan et al. (1974) reported two cases of Q fever (diagnosed serologically) in humans, in which elevated SGOT was a prominent feature. The authors estimated the enzymes by a micro method with a 'Calbiochem' Kit (the units were described as one thousandth part of IU/ml i.e. mU/ml). The SGOT levels in these two cases were as follows (case 1, SGOT 250 mU per ml., normal 10-50 mU per ml., case 2, SGOT 33 IU/L, normal 0-16 IU/L). It is peculiar that the authors have expressed the activity of SGOT in different units in two different cases. Provvidenza and Ciarla (1971) described a case of Q fever (also diagnosed serologically) with raised transaminases. Endocarditis caused by
Q. burnetii infection in humans has also been well documented. Palmer and Young (1982) recorded endocarditis in 92 (11 per cent) of the 839 confirmed cases of human Q fever in England and Wales. Similarly, Tobin et al. (1982) had also reported ten cases of endocarditis in Dublin, Ireland. Four of these cases had history of possible environmental exposure to C. burnetii. All the cases studied, showed valvular lesions, 6 cases were presented with febrile illness and 4 had congestive heart failure. Laboratory investigation in 7 out of 8 cases revealed thrombocytopenia. Haldane et al. (1983) and Elise et al. (1983) have also described cases of human Q fever in which myocarditis and endocarditis were the prominent features.

It appears therefore that the significant rise in the SGOT levels of infected guinea pigs observed in the present study could be due to myocarditis caused by C. burnetii. The levels of SGPT and AP were within normal limits.

Alkaline phosphatase is secreted by the liver cells and excreted through the bile. Obstruction in the passage of the enzyme in the bile produces high serum levels in cases of obstructive jaundice and in
cases of hepatitis with the swelling of liver cells. It appears from the AP data that there is no obstruction in the biliary tract anywhere.

SGPT is a cytosol enzyme and is present in greater proportion in liver as compared with heart or skeletal muscle (Sherlock, 1983). Increase of SGPT level is therefore specific of liver damage. In the present studies, the SGPT level of C. burnetii infected guinea pigs was maintained within the normal limits throughout the period of observation. This indicates that the parenchymatous cells of liver have not been damaged to the extent that SGPT should be elevated due to C. burnetii infection.

Handley et al. (1967) reported the electron microscopic observations of the Nine Mile strain of C. burnetii (phase I) in the guinea pig. Examination of the infected guinea pig liver at 84 hours post infection revealed numerous large clusters of C. burnetii in the cytoplasm surrounded by the limiting membrane, which was sometimes multilayered. Many organisms were also found free in the cytoplasm and sinusoids. The organisms were found to be enclosed in a limiting membrane within the cytoplasm of the parenchymal cells of liver. Various rickettsial
forms such as short bacillary and ovoid types were found within the vacuoles. Despite the multiplication of *C. burnetii* in the liver tissue, only a little cytopathological change was observed. The cytoplasm and the nuclei of the infected cells were apparently normal. The only difference noted by the authors was the highly vacuolated appearance of the cytoplasm of the infected liver cells as compared with the uninfected controls. This seems to be the probable reason for the maintenance of SGPT level within the normal limits.

The study of total serum or plasma LDH activity and its isozyme pattern serves as a useful indicator in detecting the organs involved in the pathogenesis. When tissue damage takes place, particular isozyme leaks out from the damaged cells (Boyd, 1983). The LDH isozymes are present in varying concentrations in the body tissues. Heart, kidney, brain and erythrocytes are rich in LDH-1, while liver and skeletal muscles are rich in LDH-5 (Plagemann *et al.*, 1960; Galen, 1975). The LDH-2, LDH-3 and LDH-4 are mostly present in thyroid, pancreas and spleen (Wilkinson, 1970).
The results of serum LDH activity in mock infected and *C. burnetii* infected guinea pigs are presented in Fig.14. Rise in the serum LDH activity of *C. burnetii* infected guinea pigs was noted on 7th PI day onwards, with a peak on the 14th PI day (334 units per ml). The enzyme levels gradually declined from 21st PI day (259 units per ml) and reached normal levels (235 units per ml) on the 28th PI day (Fig.14).

Heat inactivation studies were carried out on guinea pig sera to estimate the thermostable moiety of LDH. The results revealed the absence of thermostable fraction of LDH in the sera of mock infected and *C. burnetii* infected guinea pigs on the 3rd PI day. However, a sharp rise in the thermostable LDH activity was observed in the sera of infected guinea pigs on the 7th and 10th PI day, with a peak on the 10th PI day (Fig.15). These findings were suggestive of some degree of damage to the cardiac musculature because the LDH fraction of the cardiac origin is known to be heat resistant (Wroblewski and Gregory, 1961).

The plasma LDH isozyme pattern of mock infected and *C. burnetii* infected guinea pigs on different PI
FIG 14 SERUM LACTIC DEHYDROGENASE LEVELS OF GUINEA PIGS.

EACH ASSAY IS THE MEAN OF 10 INDIVIDUAL DETERMINATIONS.
FIG 15 THERMOSTABLE SERUM LACTIC DEHYDROGENASE LEVELS OF GUINEA PIGS.

EACH ASSAY IS THE MEAN OF 5 INDIVIDUAL DETERMINATIONS.
days is presented in Fig.16 to Fig.25. The per cent
distribution of LDH isozymes is presented in Table 10.
The LDH isozyme pattern of infected guinea pig plasma
revealed an increase in the LDH-5 isozyme (Cathodal -
slow moving) on the 3rd PI day (mock infected - 21.1
per cent), C. burnetii infected - 31.5 per cent). On
the 7th PI day the LDH-2 and LDH-3 were elevated in
the infected animals, viz. 33.1 per cent and 31.2 per
cent respectively. The mock infected controls on the
other hand showed LDH-2, 26.2 per cent and LDH-3, 21.1
per cent. The LDH-1 (anodal - fast moving) was found
to be elevated in C. burnetii infected guinea pig
plasma on the 10th (44.2 per cent) 14th (38.4 per cent)
and 21st (36 per cent) PI day. The control animals
had the constant levels of 20 per cent of LDH-1.
LDH-4 remained unaltered during the course of infection.
Heat inactivation of mock infected and C. burnetii
infected guinea pig plasma collected from 7th PI day
onwards, revealed the presence of thermostable LDH-1
only. (Fig.18). However, on the 10th PI day, the
proportion of thermostable LDH-1 in C. burnetii
infected guinea pig plasma was 2½ times more in
comparison with the mock infected control (Fig.21).
Plasma LDH Isozyme pattern of guinea pigs infected with *C. burnetii*

3rd Post Inoculation Day (PID)

*a* = Mock infected control

*b* = Infected with *C. burnetii*, Nine Mile strain (Phase II).
Plasma LDH Isozyme pattern of guinea pigs infected with *C. burnetii*

7th Post Inoculation Day (PID)

a = Mock infected control

b = Infected with *C. burnetii*, Nine Mile strain (Phase II).
Fig. 18
Heat inactivated plasma LDH isozyme pattern of guinea pigs infected with C. burnetii

7th Post Inoculation Day (PID)

a = Mock infected control
b = Infected with C. burnetii, Nine Mile strain (Phase II).
Plasma LDH Isozyme pattern of guinea pigs infected with \textit{C. burnetii}

10th Post Inoculation Day (PID)

\textbf{a = Mock infected control}

\textbf{b = Infected with \textit{C. burnetii}, Nine Mile strain (Phase II).}
Fig. 20

Heat inactivated plasma LDH isozyme pattern of guinea pigs infected with *C. burnetii*

10th Post Inoculation Day (PID)

*a* = Mock infected control

*b* = Infected with *C. burnetii*, Nine Mile strain (Phase II).
Plasma LDH Isozyme pattern of guinea pigs infected with *C. burnetii*

14th Post Inoculation Day (PID)

- *a* = Mock infected control
- *b* = Infected with *C. burnetii*, Nine Mile strain (Phase II).
Plasma LDH Isozyme pattern of guinea pigs infected with C. burnetii

14th Post Inoculation Day (PID)
Fig. 23

Heat inactivated plasma LDH isozyme pattern of guinea pigs infected with *C. burnetii*

14th Post Inoculation Day (PID)

- **a** = Mock infected control
- **b** = Infected with *C. burnetii*, Nine Mile strain (Phase II)
Fig. 24

Plasma LDH Isozyme pattern of guinea pigs infected with C. burnetii

21st Post Inoculation Day (PID)

a = Mock infected control

b = Infected with C. burnetii, Nine Mile strain (Phase II).
Fig. 24 a

Heat inactivated plasma LDH isozyme pattern of guinea pigs infected with C. burnetii

21st Post Inoculation Day (PID)

a = Mock infected control

b = Infected with C. burnetii, Nine Mile strain (Phase II).
Fig. 25

Plasma LDH Isozyme pattern of guinea pigs
infected with *C. burnetii*

28th Post Inoculation Day (PID)

\[ a = \text{Mock infected control} \]
\[ b = \text{Infected with } C. \text{ burnetii,}
\text{Nine Mile strain (Phase II).} \]
Fig. 25 a

Heat inactivated plasma LDH isozyme pattern of guinea pigs infected with \textit{C. burnetii}.

28th Post Inoculation Day (PID)

\textbf{a} = Mock infected control

\textbf{b} = Infected with \textit{C. burnetii}, Nine Mile strain (Phase II).
TABLE 10

Percent distribution of LDH isozymes in guinea pig plasma

<table>
<thead>
<tr>
<th>PI day</th>
<th>Status</th>
<th>LDH-5</th>
<th>LDH-4</th>
<th>LDH-3</th>
<th>LDH-2</th>
<th>LDH-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Control*</td>
<td>21.1</td>
<td>15.1</td>
<td>21.4</td>
<td>23.4</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>Infected**</td>
<td>31.5</td>
<td>15.1</td>
<td>13.8</td>
<td>10.4</td>
<td>24.1</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>18.3</td>
<td>14.4</td>
<td>21.1</td>
<td>26.2</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>12.1</td>
<td>14.8</td>
<td>31.2</td>
<td>33.1</td>
<td>13.6</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>19.7</td>
<td>17.2</td>
<td>17.6</td>
<td>25.5</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>10.5</td>
<td>20.1</td>
<td>18.8</td>
<td>10.5</td>
<td>44.2</td>
</tr>
<tr>
<td>14</td>
<td>Control</td>
<td>19.2</td>
<td>16.4</td>
<td>18.4</td>
<td>26.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>15.3</td>
<td>17.7</td>
<td>17.2</td>
<td>11.2</td>
<td>38.4</td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
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<td>25.9</td>
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</tr>
<tr>
<td></td>
<td>Infected</td>
<td>17.5</td>
<td>19.0</td>
<td>18.7</td>
<td>8.9</td>
<td>36.0</td>
</tr>
<tr>
<td>28</td>
<td>Control</td>
<td>20.7</td>
<td>17.9</td>
<td>18.4</td>
<td>23.6</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>18.5</td>
<td>17.4</td>
<td>18.7</td>
<td>22.4</td>
<td>24.0</td>
</tr>
</tbody>
</table>

© Each value represents the mean of 5 separate determinations.

* Mock infected control.

** Infected with Q. burnetii (phase II)
It gradually declined on the 14th PI day (Fig. 23a) but persisted at low level till the end of observation period (Fig. 25a).

Total serum LDH activity and its isozymes have been extensively studied in many infectious diseases. Al-Saffar and Al-Mudhaffer (1979) reported elevated serum LDH activity in 49 human patients suffering from Kala-azar. The activity of serum LDH in the cases of Kala-azar was in the range of 416-3850 IU/L (Normal control - 152-250 IU/L). Satpathy et al. (1982) investigated the LDH activity and its isozymes in serum and cerebro-spinal fluid (CSF) of 30 cases (22 inflammatory and 8 non-inflammatory) of neurological disorders of the central nervous system (CNS). The cases of inflammatory disorders included pyogenic meningitis, tuberculous meningitis and viral meningo-encephalitis; while the cases of epilepsy, cardiovascular stroke, Pott’s paraplegia, infective polyneuritis, hemiplegia and hypertensive encephalopathy belonged to the category of non-inflammatory disorders. The authors observed highest total CSF-LDH activity in pyogenic meningitis (162 units per ml) followed by tuberculous meningitis (109 units per ml). In viral meningo-encephalitis (specific virus infection not
mentioned), and non-inflammatory CNS disorders, the total CSF-LDH activity was within normal limits (48 and 25 units per ml respectively, controls 4-40 units per ml). A characteristic dominance of LDH-4 in tuberculous meningitis and of LDH-3 in pyogenic meningitis was observed in serum and CSF respectively. However, in viral meningo-encephalitis and non-inflammatory CNS disorders, the isozyme pattern did not deviate significantly from the normal pattern. Sarojini and Ramakrishnan (1983) observed a significant increase in the LDH activity of the ascitic fluid in cases of malignant ascites (171 ± 16.4 IU/L and tuberculous pleural effusion (191 ± 24.4 IU/L). The total LDH activity in the ascitic fluid was 33 per cent more and that in the pleural fluid was 25 per cent more than that observed in the respective serum samples. All the five isozymes of LDH were prominent in malignant ascites, while LDH-1, LDH-2 and LDH-3 were prominent in the pleural fluid with a greater rise in LDH-2.

Among the rickettsial diseases, the serum LDH activity was investigated in the humans suffering from boutonneuse fever caused by Rickettsia conori (Mansueto et al., 1983). The authors noted increased LDH activity in 86 per cent of adults and 89 per cent of children in
the first week of illness. The mean LDH activity in adults and children was 419 and 423 units per litre respectively. In the second week, it was found to be increased in 53 per cent of adults (252 units per litre) and 66 per cent of the children (291 units per litre, normal 240 units per litre). The cases of Rocky Mountain Spotted fever (RMSF) caused by *Rickettsia rickettsii* were investigated by Walker *et al.* (1982) for the serum LDH activity. They found that 80 per cent of the cases had highly elevated LDH activity. The authors correlated the elevated activity with the other markers of severity of infection including the number of organs involved and the level of thrombocytopenia. The serum LDH concentration was higher on each successive day of illness up to day 7 post infection. Sheridan *et al.* (1974) reported a case of human Q fever (serologically diagnosed) with elevated serum LDH activity (430 mU per ml, normal 30–85 mU per ml).

Besides these, elevated serum LDH and its isozyme activity has also been reported in cardiovascular diseases (Cohen *et al.*, 1964); renal infarction (London *et al.*, 1968); muscular dystrophy (*Wieme*
and Herpol, 1962); hematological disorders (Palaheimo and Ikkala, 1965; McCarthy et al., 1966) and in certain malignancies (Frank et al., 1982; Bhatnagar et al., 1983).

The significant increase in the serum LDH activity of guinea pigs infected with C. burnetii observed in the present study could be related to the pathophysiology of Q fever. C. burnetii causes injury to the reticuloendothelial cells (Mansueto et al., 1983), leading to inflammation and subsequent damage to different tissues in varying degrees.

The damage to the tissues results in the leakage of the enzyme into the circulation, resulting in the rise in the serum enzyme concentration (Boyd, 1983). Since LDH is predominantly present in kidney, heart, liver and skeletal muscles (Oser, 1965), it could be possible that these organs could have undergone some degree of damage during the acute phase of the disease. It is a well established fact that, heart, kidney, brain and erythrocytes are rich in LDH-1 (anodal - fast moving). LDH-2, LDH-3 and LDH-4 are mostly present in the thyroid, pancreas and spleen (Wilkinson, 1970). The liver and the skeletal muscle
are the sources of LDH-5 (cathodal - slow moving).

The present study of plasma LDH isozymes in *C. burnetii* infected guinea pigs showed an increase in the LDH-5 noted on the 3rd PI day, indicating the involvement of liver or skeletal muscle. The increase in the relative proportion of LDH-2 and LDH-3 suggested the involvement of spleen, while the increased LDH-1 activity on the 10th, 14th and 21st PI day were indicative of pathological changes in the heart and kidney.

The results of the CPK assays performed on the serum samples of guinea pigs on different PI days are presented in Fig.26. The serum CPK levels in *C. burnetii* infected guinea pigs increased from 7th PI day (68 Sigma units/ml, control 28 Sigma units/ml). Significant elevations in the CPK level of infected guinea pigs were observed between 7th and 14th PI days with a peak on the 10th PI day (Fig.26). The enzyme level returned almost to the level of mock infected control guinea pigs on the 21st PI day and reached normal level by 28th PI day.

The rise in CPK was 2–2½ times of controls, between 7th and 14th PI days. This could be correlated
FIG. 26 SERUM CREATINE PHOSPHOKINASE LEVELS OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF 5 INDIVIDUAL DETERMINATIONS.
with the loss in body weight of infected guinea pigs (Fig. 9). It was interesting to note that the highest CPK activity (84 Sigma units/ml) observed on the 10th PI day coincided with the maximum loss in the body weight (12.56 per cent) which was also recorded at the same time. This was suggestive of some degree of damage to the skeletal muscle as well as myocardium during the acute phase of infection. The skeletal muscle and myocardium are rich in CPK (Eschar and Zimmerman, 1967). The damage to the myocardium by \textit{C. burnetii} has been discussed earlier. It is therefore possible that the heart also contributes to the increase of serum CPK activity.

MDH is one of the important enzymes of the Tricarboxylic acid (TCA) cycle (Lehninger, 1978). An outline of the TCA cycle is depicted in Fig. 27. MDH exists in two different forms viz. the cytoplasmic fraction and the mitochondrial fraction. In the mammalian plasma both the forms of MDH are present but the elevation of plasma MDH activity is contributed mainly by the cytoplasmic form of the MDH (Delbruck \textit{et al}., 1959).

In the present study, the cytoplasmic form of MDH was assayed in the plasma of guinea pigs. The
FIG. 27

AN OUTLINE OF TRICARBOXYLIC ACID CYCLE

Pyruvate
\[ \rightarrow \text{Acetyl Coenzyme A} \]

\[ \text{Oxalacetate} \]

\[ \uparrow \text{Malic Dehydrogenase (MD)} \]
\[ \text{Malate} \] \[ \uparrow \text{Fumarase} \]
\[ \text{Fumarate} \]

\[ \uparrow \text{Succinic Dehydrogenase} \]
\[ \text{Succinate} \] \[ \leftarrow \text{Alpha-ketoglutaric Oxidase} \]

\[ \downarrow \text{Condensing Enzyme} \]
\[ \text{Citrate} \]

\[ \downarrow \text{Aconitase} \]
\[ \text{Cis-aconitate} \] \[ \downarrow \text{Aconitase} \]
\[ \text{Isocitrate} \]

\[ \downarrow \text{Isocitric Dehydrogenase (ICD)} \]
\[ \text{Alpha-ketoglutarate} \]
results of plasma MDH assays performed in guinea pigs are presented in Fig. 28. It was observed that the plasma MDH activity in *C. burnetii* infected guinea pigs was significantly elevated on the 10th (925 Sigma units/ml, control 540 Sigma units/ml) and 14th (797 Sigma units/ml, control 476 Sigma units/ml) PI days. The enzyme level returned to normal on the 21st PI day and was maintained as such till the end of observation period (Fig. 28).

The increase in the serum MDH activity has been reported in muscular dystrophy (Chowdhury et al., 1962). According to these authors, the elevated serum MDH activity was due to the continuous leakage of the enzyme from the muscle into the circulation. The leakage of the enzyme was probably due to the increased permeability of the cell membrane. In the present studies, elevated level of plasma MDH in infected guinea pigs could probably be due to this phenomenon.

Thus, it appears from the enzymological response of guinea pigs infected with *C. burnetii* (Phase II) that SGOT, LDH, CPK and MDH enzymes are elevated in the acute stage of infection.

The LDH enzyme is widely distributed in kidney, heart, spleen and skeletal muscle (Oser, 1965). Rise in serum LDH activity observed in *C. burnetii* infected
FIG 28 SERUM MALIC DEHYDROGENASE LEVELS OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF 8 INDIVIDUAL DETERMINATIONS.
guinea pigs between 7th and 14th PI days could probably be due to some degree of cellular damage in these tissues. Rise in CPK levels between 7th and 14th PI days were indicative of damage to the skeletal muscle and myocardium.

Histopathological studies on various organs in guinea pigs following experimental infection with the virulent Nine Mile and Henzerling strains in Phase I as well as M-44 attenuated vaccine strain of *C. burnetii* has been dealt with in detail in section 2.8.3. Severe lesions were produced at the peak of infection by the virulent Henzerling and the Nine Mile strains. The salient features were diffuse interstitial inflammation of heart, lungs, liver, spleen, adrenals and lymph nodes. A characteristic feature was the presence of large vacuolated reticuloendothelial cells containing intracellular colonies of *C. burnetii*. On the other hand, the lesions produced by the M-44 attenuated vaccine strain were milder in nature and characterized by mild, multifocal myocarditis and multifocal splenitis accompanied by small necrotic foci in the liver of infected guinea pigs.

In the present studies, the Nine Mile strain of *C. burnetii* in phase II was employed. The histopatho-
logical findings on guinea pig tissues following experimental infection with \textit{C. burnetii} in phase II have not been reported. Organisms in phase II tend to become less virulent during the process of egg adaptation (Kazar \textit{et al.}, 1974; Johnson \textit{et al.}, 1977). The strain employed in the present study had undergone 150 egg passages. It is therefore expected that the lesions produced by \textit{C. burnetii} in phase II would be milder in nature. Therefore, the damage to the liver seems to be mild. From the degree of liver damage produced by phase I Nine Mile strain (Rychlo and Pospisil, 1960 and 1962) one would expect high rise in SGPT levels. However, histopathology of various organs in guinea pigs after infection with \textit{C. burnetii} in phase II needs investigation.

5.6 Studies on glucose and glycogen metabolism

Disturbance in the carbohydrate metabolism such as progressive depletion of hepatic glycogen, diminution of glycogen synthetase and elevation of liver phosphorylase enzymes have been encountered in the liver of guinea pigs during experimental infection with \textit{C. burnetii} (phase I). (Paretsky \textit{et al.}, 1964; Tsung and Paretsky, 1968; Paretsky and
Therefore, certain parameters of glucose metabolism were investigated in guinea pigs infected with *C. burnetii* in phase II. The parameters studied included the monitoring of blood glucose, the glucose tolerance test, effect of insulin on the blood glucose level, estimation of tissue glycogen and assay of liver enzymes viz. glycogen phosphorylase and glycogen synthetase.

5.6.1. **Blood glucose levels**

The blood glucose level of mock infected and *C. burnetii* infected guinea pigs was monitored during the course of infection (Fig. 29). The blood glucose level of *C. burnetii* infected guinea pigs was significantly elevated on the 3rd (149 mg/dl $p < 0.001$), 7th (146 mg/dl $p < 0.001$) and 10th (135 mg/dl $p < 0.01$). It gradually returned to normal level from 14th PI day (113 mg/dl) and was maintained within the normal limits till the end of observation period. The mock infected guinea pigs maintained a normal blood glucose level throughout the period of observation. The hyperglycemia observed in *C. burnetii* infected guinea pigs during the acute phase of infection (i.e. 3rd, 7th and 10th PI days) could be
FIG. 29 BLOOD GLUCOSE LEVELS OF GUINEA PIGS.

EACH ASSAY IS THE MEAN OF 5 INDIVIDUAL DETERMINATIONS
due to augmentation of glycogenolysis, since the infected guinea pigs showed progressive depletion of hepatic glycogen accompanied by increase in the phosphorylase and diminution of glycogen synthetase activity (vide infra).

5.6.2 Glucose tolerance test

The findings of the glucose tolerance test (GTT) performed on mock infected and C. burnetii infected guinea pigs (8th PI day) are presented in Fig.30. The highest blood glucose level during the GTT in mock infected (221.87 mg/dl) and C. burnetii infected guinea pigs (282.5 mg/dl) was observed at 60 and 90 minutes respectively. In comparison with the blood glucose levels of mock infected guinea pigs during the entire course of the GTT (30, 60, 90 and 120 minutes), the corresponding blood glucose levels of C. burnetii infected guinea pigs were markedly elevated. It remained elevated (247 mg/dl) even at the end of 120 minutes.

5.6.3 Effect of insulin on blood glucose

Sub-cutaneous administration of 1-2 units of insulin in infected guinea pigs before the performance of GTT had only a mild effect in lowering the blood
Fig 30. Glucose Tolerance Test in Guinea Pigs.

Each assay is the mean of 5 individual determinations.
glucose level. Guinea pigs with one and two units of
insulin had 220 and 213 mg/dl of blood glucose respec-
tively at 120 minutes (Fig. 31). Thus, it can be concluded
that the infected guinea pigs could not utilise the
exogenous glucose for glycogenesis in liver.

5.7 **Tissue glycogen levels**

The hepatic glycogen estimations were performed
on mock infected and *Q. burnetii* infected guinea pigs
on 3rd, 5th, 7th, 10th, 14th and 28th PI days. The
results are presented in Table 11. *Q. burnetii* infected
guinea pigs showed a drastic depletion of hepatic
glycogen from 3rd PI day (1.17 mg per gm. of wet weight
of tissue). The lowest hepatic glycogen in *Q. burnetii*
infected guinea pigs was recorded on the 5th PI day
(0.355 mg per gm of wet weight of tissue). The
depletion of hepatic glycogen was observed from 3rd
to 10th PI day. The hepatic glycogen content of
infected guinea pigs increased from 14th PI day and
was restored to normal levels by 28th PI day, indicating
recovery of liver. The mock infected guinea pigs
maintained a normal hepatic glycogen throughout the
period of observation (26.7 ± 2.2 mg. per gm of wet
FIG. 31—EFFECT OF PRIOR PRIMING WITH INSULIN ON BLOOD GLUCOSE LEVELS DURING GLUCOSE TOLERANCE TEST IN GUINEA PIGS INFECTED WITH C. burnetii (Phase II)
TABLE II

Liver glycogen* of guinea pigs infected with the
Nine Mile strain of C. burnetii (Phase II)

<table>
<thead>
<tr>
<th>Status of guinea pig</th>
<th>Post inoculation days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Mock infected**</td>
<td>26.1</td>
</tr>
<tr>
<td>Infected with**</td>
<td>1.172</td>
</tr>
</tbody>
</table>

*C Glycogen mg per gm of wet weight of tissue

** Each assay is the mean of 4 to 6 individual determinations
The muscle and the heart glycogen estimations performed in control and infected guinea pigs at the peak of infection (7th PI day) showed no appreciable change in the muscles and heart glycogen content (muscle - normal 2.35 - 3.35; infected 2.39 mg. per gm. of wet weight of muscle, heart - normal 1.3 to 1.7; infected 1.4 mg. per gm. of wet weight of tissue).

5.8 Liver enzymes

Results of glycogen phosphorylase and glycogen synthetase estimations performed on the liver tissues of guinea pigs are presented in Fig. 32 and Fig. 33 respectively. Liver phosphorylase activity in C. burnetii infected guinea pigs was significantly elevated on the 3rd PI day (44 units per gm. of wet weight of tissue). The enzyme activity declined on the 5th (21 units) and 7th (17 units) PI day. It was restored to almost normal level (23 units) on the 14th PI day (Fig. 32). The mock infected guinea pigs maintained a normal level of phosphorylase activity throughout the period of observation (24-28 units per gm. of wet weight of tissue).

The glycogen synthetase activity in the liver tissue of C. burnetii infected guinea pigs was signifi-
FIG. 32 LIVER GLYCOGEN PHOSPHORYLASE LEVELS IN GUINEA PIGS.

EACH ASSAY IS THE MEAN OF 4 INDIVIDUAL DETERMINATIONS PERFORMED IN QUADRUPLE.
FIG. 33 LIVER GLYCOGEN SYNTHETASE LEVELS IN GUINEA PIGS.

EACH ASSAY IS THE MEAN OF 4 INDIVIDUAL DETERMINATIONS PERFORMED IN QUADRUPLE.
cantly decreased on the 3rd, 5th and 7th PI days (Fig. 33). The enzyme activity gradually increased from 10th PI day. The mock infected guinea pigs displayed glycogen synthetase activity within the normal limits ($5.25 \pm 0.5$ units per gm. of fresh liver).

The depletion of hepatic glycogen observed in the guinea pig infected with C. burnetii occurs as well in a broad range of bacterial (Berry and Smythe, 1960; Klein et al., 1966; Lindell et al., 1964; Singh et al., 1963; Wilder and Sword, 1967), and protozoan infections (Mercado and Von Brand, 1954, 1960; Von Brand and Mercado, 1956). Glycogen depletion has also been reported during starvation in rats (Tata, 1964), chemical poisoning and tumour transplants (Nigam, 1962, Sie and Hablanian, 1965; Tata, 1964).

The drastic depletion of hepatic glycogen observed in the guinea pigs infected with C. burnetii was also accompanied by increase in the glycogen phosphorylase and diminution of glycogen synthetase activities. The findings of the present study indicated that during the course of experimental Q fever in guinea pigs, there were alterations in the activities of these enzymes involved in the glycogenesis and glycogenolysis. The depletion of hepatic glycogen could be due to the
enhanced phosphorylase activity (Fig. 32) leading to glycogenolysis and also due to the suppressed glycogen synthetase activity (Fig. 33). This was actually observed during the peak of infection (3rd to 7th PI day). A parallel observation of glycogenolysis accompanied by increased phosphorylase and decreased glycogen synthetase activities was reported by Belcopitow (1961) in epinephrine mediated glycogenolysis in rats. The increase in the phosphorylase activity observed in C. burnetii infected guinea pigs could be the result of series of reactions and might be hormone linked. The stress due to the infection in guinea pigs could stimulate epinephrine secretion leading to increased phosphorylase activity (Fig. 34) (Cory and Illingworth, 1956; Sutherland and Rall, 1958). Marecki et al. (1978) reported changes in the liver plasma membranes of guinea pigs infected with C. burnetii. The infected guinea pig liver had much greater levels of cyclic 3', 5'-adenosine monophosphate (cAMP). cAMP is known to inactivate glycogen synthetase (Fig. 35) increase liver glycogenolysis, increase synthesis of liver protein and increase lipolysis during Q fever (Bernier et al., 1974). This could be the probable reason for
FIG. 34

AMPLIFICATION CASCADE IN THE STIMULATION OF GLYCOGENOLYSIS BY EPINEPHRINE IN THE LIVER CELL

Stimulus \rightarrow \text{Adrenal medulla} \\
\text{Epinephrine} \\
\text{Epinephrine receptor} \rightarrow \text{blood} \\
\text{Cell membrane} \\
\begin{align*}
\text{ATP} & \rightarrow \text{cAMP + PP}_1 \\
\text{Protein kinase} & \rightarrow \text{Protein kinase + cAMP - R} \\
& \text{(inactive)} \\
& \text{(active)} \\
\text{ATP} + \text{Dophospho-phosphorylase kinase} & \rightarrow \text{Phospho-phosphorylase kinase + ADP} \\
& \text{(inactive)} \\
& \text{(active)} \\
\text{ATP} + \text{phosphorylase b} & \rightarrow \text{Phosphorylase a + ADP} \\
& \text{(inactive)} \\
& \text{(active)} \\
\text{Glycogen} + \text{P}_i & \rightarrow \text{Glucose 1-phosphate} \\
\text{Glucose 6-phosphate} \\
\text{Glucose + P}_i \\
\end{align*}

Blood glucose
FIG. 35

INHIBITION OF GLYCOGEN SYNTHETASE
IN LIVER BY EPINEPHRINE

Stimulus → Adrenal medulla
↓
Epinephrine
Epinephrine receptor

Membrane
Liver cell

ATP → cAMP + PP
Protein kinase
CR (inactive)
→ Protein kinase + cAMP
C (active)

ATP + Dephospho-glycogen synthase
(active)
→ Phospho-glycogen synthase
+ ADP
(inactive)
diminution of glycogen synthetase activity and depletion of hepatic glycogen in C. burnetii infected guinea pigs as observed in the present studies.

The Embden-Meyerhof pathway of glycolysis and the pathway of glycogenesis as well as glycogenolysis in the liver has been depicted in Fig. 36 and Fig. 37 respectively. It appears from the study of certain parameters of glucose metabolism in C. burnetii (Phase II) infected guinea pigs that, during the acute stage of infection (3rd to 7th PI day), the glucose and glycogen metabolism have been affected as evidenced by hyperglycemia, depletion of hepatic glycogen and alterations in certain liver enzymes. The hyperglycemia observed during the acute stage is the apparent result of augmentation of glycogenolysis. Another interesting observation is that the exogenous glucose administered during the GTT did not enter the pathway of glycogenesis. In fact higher levels of blood glucose were observed in C. burnetii infected guinea pigs even at 120 minutes. This could probably be due to certain changes in the membrane permeability of liver cells. The glucose apparently could not enter the liver cells.
FIG. 36

EMBDEñ-MEYERHOP PATHWAY OF GLYCOLYSIS

Glycogen → Glucose → Hexokinase → Glucose-6-PO₄ → Phospho-hexose isomerase → Fructose-6-PO₄

Fructose-1, 6-Di-PO₄ → Phosphoglycerate kinase → Phosphoglycerate

Phosphoglycerate → Glyceraldehyde-3-PO₄ → Dehydrogenase → NADH + H⁺ → Oxidation TC cycle → Lactic dehydrogenase → Lactate

Glyceraldehyde-3-PO₄ → Phosphotriose isomerase → 2 Phosphoglycerate → Enolase → Phosphoenolpyruvate

Phosphoenolpyruvate → Pyruvate kinase → Pyruvate → Lactic dehydrogenase → Lactate

Leaks out
FIG. 37

PATHWAY OF GLYCOGENESIS AND GLYCOGENOLYSIS IN THE LIVER

GLYCOGEN [(1,4 and 1,6 Glucosyl)\textsubscript{x} units

Branching enzyme

(1,4 Glucosyl units)\textsubscript{x} Glucose

\text{Glycogen synthetase}

Glucose

\text{Glucose-1-phosphate} \leftarrow \text{Phosphoglucose mutase}

\text{Glucose-6-phosphate} \leftarrow \text{Phosphoglucomutase}

\text{To direct oxidative pathway}

\text{To uronic acid Pathway}

UDPG

\text{UDPG pyrophosphorylase}

Insulin

Glucagon

\text{Cyclic AMP}

\text{Debranching enzyme}

\text{Phosphorylase and Debranching enzyme}

Enhances

Stimulation

Inhibition
It has been shown that increased phosphorylase and diminution of glycogen synthetase activities occurred in guinea pigs infected with \textit{C. burnetii} in phase I (Paretsky \textit{et al.}, 1964) during the acute phase of infection.

Studies on the pathway of glycogenesis in guinea pigs infected with \textit{C. burnetii} (phase I) revealed that the inhibition in hepatic glycogenesis occurred at the level of glycogen synthetase (Paretsky \textit{et al.}, 1964). This was attributed to the release of an inhibitory factor by \textit{C. burnetii} (Steuckemann and Paretsky, 1971) similar to the bacterial endotoxin (Berry \textit{et al.}, 1966).

The presence of an endotoxic lipopolysaccharide (LPS) has been demonstrated in the cell wall of \textit{C. burnetii} in phase I and phase II (Baca and Paretsky, 1974; Schramek and Brezina, 1979; Baca \textit{et al.}, 1980). (See section 2.7.2). Therefore, it could be surmised at present that the enzymological response, the progressive depletion of hepatic glycogen and the alterations in the phosphorylase and glycogen synthetase enzyme activities in the infected guinea pigs could be due to the release of an endotoxic LPS from the cell wall of \textit{C. burnetii} into the host system.
during the febrile period of infection.

The concentration of an enzyme in serum depends upon the relative rates of enzyme flow into the circulation. An increase in the serum enzyme could be due to either greater enzyme flow into the circulation or slower enzyme clearance (Posen, 1970; Friedel et al., 1979). The increased enzyme flow into the plasma could be either due to increased leakage of the enzyme from the damaged cells or increased leakage of the enzyme from cells which are over producing the enzyme. The rate of enzyme leakage from the damaged tissue into the plasma is influenced by a) The extent, rapidity and severity of the damage and b) The concentration, rate of synthesis, subcellular localization and diffusibility of the enzyme (Boyd, 1983).

Thompson and Paretsky (1973) studied the RNA and protein syntheses in guinea pig liver during infection with C. burnetii (phase I). Protein and RNA synthesis increased progressively during 96 hours post infection coincident with increased hepatic cortisol concentrations. Enhanced synthesis of 28S, 18S and 4S RNA species was parallel with the increased number of hepatic ribosomes.
It appears therefore, that rise in the serum enzymes of guinea pigs observed in the present study could partially be due to the increased synthesis of protein (enzyme) and partially due to the leakage of the enzyme from the damaged cells.

Further, Baca and Paretsky (1974) observed that when LPS isolated from C. burnetii (phase I) was inoculated IP in guinea pigs, the animal responded with an elevated plasma and liver cortisol, increased RNA, increased plasma and liver protein synthesis and leucocytosis. The responses of guinea pig to LPS were comparable to those observed in active Q fever infection in guinea pig. These observations strongly suggested the role of LPS in causing biochemical changes in C. burnetii infected guinea pigs.

5.9 Response of guinea pigs to LPS of C. burnetii

To elucidate the role of an endotoxic LPS of C. burnetii in producing the pathological and biochemical changes in C. burnetii infected guinea pigs, further studies on the isolation of LPS and the response of guinea pigs to LPS were carried out. The amount of extractable LPS in phase II C. burnetii is 1/10th of the amount of extractable LPS found in phase I.
Therefore, LPS of Co. burnetii in phase I was isolated and employed for further studies.

The pathophysiological and biochemical (enzymological) parameters were investigated in guinea pigs inoculated with the LPS isolated from Co. burnetii in phase I. The LPS inoculated guinea pigs exhibited hyperthermia on the 1st and 2nd PI day (Fig. 38) accompanied by progressive loss in the body weight (Fig. 38). The loss in the body weight (as calculated from the pre-inoculation body weight) was 3.59 per cent on the 1st, 7.36 per cent on the 3rd, 5.63 per cent on the 5th and 3.93 per cent on the 7th PI day. The maximum loss in the body weight (7.36 per cent) of guinea pigs inoculated with the LPS, was recorded on the 3rd PI day. The mock infected guinea pigs maintained their body weight and showed a slight but progressive increase in the body weight till the end of observation period. The changes in certain serum enzymes were accompanied by depletion of hepatic glycogen. Although the AP and SGPT remained within the normal limit (Fig. 39 and Fig. 41) the SGOT remained persistently elevated (Fig. 40) from 1st PI day to 7th PI day with a peak on the 3rd PI day (74 units per ml) (Fig. 40). The LDH enzyme was elevated on the 3rd PI day (LDH 351 units per ml)
Fig. 38 Rectal temperature and body weight of guinea pigs. Each observation is the mean of 6 individual determinations.
FIG. 39  SERUM ALKALINE PHOSPHATASE
LEVELS OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF SIX INDIVIDUAL DETERMINATIONS.
FIG. 40  SERUM GLUTAMATE OXALACETATE TRANSAMINASE LEVELS IN GUINEA PIGS.

EACH ASSAY IS THE MEAN OF 6 INDIVIDUAL DETERMINATIONS.
FIG 41 SERUM GLUTAMATE PYRUVATE TRANSAMINASE LEVELS IN GUINEA PIGS.

EACH ASSAY IS THE MEAN OF 6 INDIVIDUAL DETERMINATIONS.
(Fig. 42). The level of MDH was elevated on the 2nd and 3rd PI day (930 and 1250 Sigma units per ml respectively) (Fig. 43) and was subsequently returned to normal levels. The serum enzyme levels in mock infected guinea pigs remained within the normal limits.

Monitoring the blood glucose level of LPS inoculated guinea pigs it was observed that the blood glucose level of LPS inoculated guinea pigs was elevated (135 mg/dl) on the 3rd PI day (Fig. 44) and subsequently returned to normal level on the 5th PI day and was maintained within normal limits till the end of observation period. The mock infected guinea pigs maintained a normal blood glucose level throughout the entire period of observation. LPS infected guinea pigs showed all the enzymological and biochemical changes as were observed in Coxiella burnetii infected guinea pigs. However, the response was of accelerated type and of a shorter duration.

The transient hyperglycemia observed in the LPS inoculated guinea pigs could be due to enhanced glycogenolysis, since the glycogen phosphorylase of LPS inoculated guinea pigs displayed enhanced activity (45 units per gm of tissue) on the 3rd PI day accompanied by depletion of hepatic glycogen (5.89 mg per gm of tissue). The observed effect could be due to the increased level of cAMP, since it has been reported that
FIG. 42  SERUM LACTIC DEHYDROGENASE LEVELS OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF SIX INDIVIDUAL DETERMINATIONS.
FIG. 43  SERUM MALIC DEHYDROGENASE LEVELS OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF SIX INDIVIDUAL DETERMINATIONS.
FIG. 44 BLOOD GLUCOSE LEVELS OF GUINEA PIGS.
EACH ASSAY IS THE MEAN OF SIX INDIVIDUAL DETERMINATIONS.
cAMP levels were increased in the guinea pig liver 84 hours after infecting the animals with the LPS of \textit{C. burnetii} in phase I (Baca and Paretsky, 1974).

Anti-LPS antibodies were detected in the convalescent guinea pig sera on 28th PI day by CF test in a titre ranging from 1:16 to 1:32. Investigating the pathophysiological changes in guinea pigs induced by the LPS of \textit{C. burnetii} in phase I, Baca and Paretsky (1974) observed hyperthermia, loss in body weight, hepatomegaly and leucocytosis. Our findings are in agreement with Baca and Paretsky (1974).

The physiological and biochemical response of the guinea pigs to infection with \textit{C. burnetii} and to the LPS extract were comparable in many ways. There was hyperthermia, loss in body weight accompanied by changes in certain serum enzymes, a rise in blood glucose level, a lowered glucose tolerance and enhanced glycogen phosphorylase and diminished glycogen synthetase activity accompanied by depletion of hepatic glycogen during the acute stage of infection.

Thus, in conclusion, it could be stated that many of the biochemical changes observed in guinea pigs infected with \textit{C. burnetii} could be due to the release of a toxic LPS from the cell envelope of \textit{C. burnetii} into the host system. The causal relation-
ship between the LPS of C. burnetii and the pathophysiological and biochemical changes that occur during Q fever could be established.

5.10 Mechanism of action of lipopolysaccharide

Lipopolysaccharide (LPS) (synonyms "endotoxin" or pyrogen) are the constituents of the cell walls of gram-negative bacteria forming the outer membrane of the cell wall. They were detected in cell free filtrates of autolyzed gram negative cultures of bacteria indicating that some cells release these substances spontaneously into the medium (Nowotny, 1969). Some cells release endotoxin readily under the effect of mild treatments or due to the specific nutritional environments (Work et al., 1966).

The two major constituents of the bacterial endotoxins were discovered by Bovin et al. (1933) who described these materials as glycolipids. Mild acidic hydrolysis precipitated a lipid and left a degraded polysaccharide in the supernatant fluid. The polysaccharide consists in most cases, of a large number of different carbohydrates, the most common being glucose, galactose and mannose. In addition, pentoses, hexosamines, heptoses octonic acid derivatives and different deoxysugars are frequently present,
in similar endotoxin preparations. The carboxylic acids of the lipid moiety are the usual even numbered saturated and unsaturated fatty acids (Davies, 1960; Luderitz et al., 1966). The chemical composition of the LPS of C. burnetii has been dealt with in detail under section 2.7.2 which appears to be similar to the LPS of gram negative bacteria.

Although the effects of LPS preparation of C. burnetii (phase I) on the susceptible hosts such as guinea pigs, mice and chick embryo have been reported (Baca and Paretsky, 1974; Schramek and Brezina, 1976) its precise underlying mechanism of action in host system has not yet been resolved. However, there are several reports on the mechanism of endotoxic reaction of LPS derived from gram negative bacteria on various host systems concerning pyrogenicity (Atkins, 1960; Bennett, 1961; Dinarello and Wolff, 1982), leucocytosis (Des Prez, 1964; Des Prez and Bryant, 1966), effects on reticuloendothelial system (Heilman, 1964; Heilman, 1965), and effects on blood vascular system (Zweifach, 1964).

Inflammation is the summation of action taken by the defence system of the host after infection.
Enhanced phagocytosis, fibrin formation and activation of metabolic enzymes like phosphorylase kinase and phosphorylase are the characteristic features of endotoxin reactions (Homosh and Shapiro, 1960; Des Prez, 1964). Enhanced capillary permeability facilitates the exit of phagocytic cells and plasma constituents from the blood vessels and makes it possible to reach the site of invasion (Nowotny, 1969).

The investigation of the mode of endotoxic reaction was facilitated by studying the fate of injected endotoxin. According to number of investigators, the bacterial endotoxin seems to accumulate in the cells of reticulo-endothelial system. The spleen and liver appear to be primarily involved (Levy et al., 1967). Other organs where accumulation could be observed were endothelial cells lining the blood vessels (Rubenstein et al., 1962) and the lung alveoli (Barnes et al., 1952). Whether these organs are the direct or indirect targets of endotoxic reaction is still not known. However, certain evidence from the recent reports indicates that the primary targets may be platelets or the leucocytes and the damage caused by the endotoxin results in the release of leucocytic pyrogen and blood clotting factors which elicit a chain
reaction acting on the central nervous system as well as on the vascular system.

The most important and the extensively studied property of an endotoxin is the pyrogenicity. It is a well established fact that the pyrogenic effect produced by the endotoxin on the host system is mediated by the leucocytic pyrogen. The role of leucocytic pyrogen is well documented (Dinarello and Wolff, 1978; Bernheim et al., 1979). The current hypothesis of fever is that regardless of a stimulus, whether infectious or non-infectious, the fever is mediated by a substance of leucocyte origin. Further, once released from leucocytes into the blood stream, the leucocytic pyrogen acts on the thermoregulatory centre in the hypothalamus, which results in upward resetting from normal body temperature levels to those observed during the febrile state (Fujiwara et al., 1984).

A number of agents have been shown to induce production of leucocytic pyrogen. Circulating leucocytic pyrogen can be detected in rabbits following the injection of influenza virus (Atkins and Huang, 1958) or Coxsackie viruses (King, 1964). The ability of viruses to induce the leucocytic pyrogen has been shown to be associated with the presence of viral
hemagglutinins (Kanoh and Kawasaki, 1966). Gram positive organisms such as *Staphylococcus albus*, *pneumococci*, *Bacillus subtilis* or *Listeria monocytogenes* have been known to produce fever in rabbits and circulating leucocytic pyrogen has been demonstrated in the febrile plasma (Atkins and Freedman, 1963). The pyrogenicity of gram negative bacteria is due to the LPS (endotoxin). It has been demonstrated that most of the biological and toxic properties including pyrogenicity are clearly due to lipid A moiety of LPS (Wolff, 1973). Besides these, live or killed yeast cells, spirochetes and a number of non-organic substances have been shown to produce fever (Briggs and Atkins, 1966; Butler et al., 1981; Petersdorf et al., 1957).

Rosendorff (1976) postulated that leucocytic pyrogen circulates to the hypothalamus, where it induces production of a metabolite of arachionic acid. This substance in turn, increases the synthesis of norepinephrine that in turn increases the production of cAMP. This then, directly causes alterations in the activity of temperature sensitive neurons that bring about increase in heat conservation or heat production or both.
Recent reviews on the pathogenesis of fever (Bernheim et al., 1979; Dinarello and Wolff, 1982) have described the ability of leucocytic pyrogen to alter the hypothalamic thermoregulatory centre by increasing the arachidonic acid metabolite levels, which seems to be the most likely mechanism of pathogenesis of fever. Recently it has been shown that leucocytic pyrogen is probably identical to interleukin 1 (Rosen Wasser et al., 1979; Murphy et al., 1980). Duff and Darum (1982) tested the effect of increased temperature on T cell proliferation in vitro stimulated by Interleukin 1 and 2 (IL-1 and IL-2). They found that T cell proliferation in response to IL-1 and IL-2 in the presence of Concanavalin A (Con A) was greatly increased at 39°C as compared with 37°C. While B cell proliferation stimulated by LPS was not increased. These findings suggested a potentially important immunoregulatory function of the fever response.

From the above discussion it seems reasonable to hypothesise at this juncture that the mechanism of action of LPS of *C. burnetii* on the susceptible host system appears to be similar to that of LPS derived
from gram negative bacteria; since the chemical composition of LPS derived from *C. burnetii* and that of gram negative bacteria have qualitative similarities.