SEASONAL VARIATIONS IN ENDOTOXIN SUSCEPTIBILITY OF RABBITS

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Endotoxins from Gram-negative bacteria have been known to induce intoxications in numerous animal species, with considerable variations of toxic symptoms and species susceptibility. Rabbits are known to be the most susceptible among laboratory animals and as the toxic manifestations, dyspnea, diarrhea, hypothermia and late prostration appear. The dyspneic response, the most characteristic one in this animal species has been shown to be mediated by an epinephrine discharge which is prone to occur in this animal species (FUKUDA). During the past two years' experiences in our laboratory it has been noticed that there exists a marked seasonal variation in the endotoxin susceptibility in this animal, being increased in winter and the least in summer. The present report concerned with these.

METHODS

Rabbits weighing about 2.5 kg were used. They were maintained with "Okara" (soybean curds waste 300 g, salt 0.5 g) throughout the year. The environmental temperature in which they were kept varied considerably, reaching over 30°C in hot summer and below 10°C in cold winter with considerable diurnal variations. The toxic manifestations and the lethality for the first time inoculation of endotoxin (Salmonella typhosa, Difco) in dose of 300 µg/kg were determined in each season in an experimental environmental temperature ranging from 20°C to 24°C. In hot summer and cold winter the test animals were accustomed to these temperatures at least for a few hours. In preliminary experiments of artificial cooling or heating of the experimental animals it was found that the induction of an increased susceptibility in cold environment (below 15°C) or a decreased susceptibility in hot environments (over 28°C) required at least several days. Therefore, warming or cooling to the indifferent temperatures for short time periods did not seem to affect the endotoxin susceptibility and the results of the observations could be interpreted as a real seasonal variation.

All the observations were made in a free state without any restraintment. The animals once inoculated with endotoxin were never used, because of the long continuance of the tolerance (unpublished data). The techniques for the determinations of the blood sugar and the liver glycogen were the same as reported previously. For thyroid feeding a desiccated thyroid preparation (U.S.P) was administered 16 mg/kg per day for 5 days.

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RESULTS

Seasonal variations in the lethality and the toxic manifestations As is shown in Table 1 the susceptibility to endotoxin was increased in winter and decreased in hot summer season. The dyspnea, the most characteristic toxic symptom in this animal species was marked in winter and did not appear in summer. The diarrheic tendency, although lessened in summer, was observed throughout the year. But an intense watery diarrhea appeared only in spring and autumn. In winter, although the diarrheic response occurred most frequently, its intensity was not so severe. These seasonal variations seemed to have close correlation with the environmental temperature of the breeding room. The winter type of heightened susceptibility appeared when the daily maximum temperature was reduced below 18°C, while the summer type of non-susceptibility appeared when it was elevated over 28°C. As has been noticed above, an artificial cooling in summer or warming in winter could convert the response type readily, but it required at least several days.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Seasonal Variations in Toxic Symptoms and Lethality in Endotoxin Intoxication (S. typhosa, 300 μg/kg i.v.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total Cases</td>
</tr>
<tr>
<td>Winter</td>
<td>15</td>
</tr>
<tr>
<td>Spring</td>
<td>15</td>
</tr>
<tr>
<td>Summer</td>
<td>15</td>
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<td>Autumn</td>
<td>15</td>
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Relation of the thyroid gland activity to the toxic manifestations As the dyspneic response has been shown to be induced by massive epinephrine discharge in endotoxin intoxication (FUKUDA), it might be expected that the marked tendency to dyspnea in winter is the result of intense epinephrine secretion and/or increased epinephrine susceptibility. As the cause of these the activation of thyroid glands in winter was taken into consideration and the effects of thyroidectomy and thyroid medication were examined in autumn. The results were given in Table 2. One week after thyroidectomy the dyspneic response could no more be evoked. However, the diarrheic tendency was intensified and the depletion of the liver glycogen below 1000 mg% occurred as usual. On the other hand the thyroid medication markedly intensified the hyperglycemic response and the dyspnea. Thus it could be inferred that the marked dyspneic response in winter might be the result of activation of thyroid glands. However, the non-susceptibility in summer did not seem to be due solely to a hypothyroid state, since the diarrheic tendency and the depletion
of liver glycogen, as mentioned below, were also markedly reduced in this season. The above mentioned lessening of the severity of diarrheic response in winter which has been shown to be of vagal origin\(^3\) seemed to be due to the antagonistic effect of intense epinephrine secretion.

*The summer non-susceptibility* In summer not only the toxic symptoms but also the characteristic depletion of liver glycogen after endotoxin\(^2,3\) were markedly ameliorated. As is shown in Fig. 1 this non-susceptibility of the liver glycogen could also be observed in adrenalectomized animals. These animals which were extremely susceptible to the hypoglycemic deterioration due to endotoxic depletion of the liver glycogen\(^3\) could thus survive in summer the toxic dose here used. Thus it is sure that the enhanced utilization of the liver glycogen in response to endotoxin, being reduced in summer, contributes to the summer non-susceptibility. As is already noticed by Fuji\(^5\) the liver glycogen content itself undergoes an appreciable seasonal variation, high in winter and low in summer. This could be noticed also in Fig. 1 and might have an causal relation to the endotoxic reaction, as will be discussed later.

In connection with the non-susceptibility in summer, the marked seasonal variations in the blood picture\(^6\), especially the neutropenia and lymphocytosis in summer, should be also considered. This is because the immediate leucopenic response, especially the neutropenic one on administration of endotoxin has been discussed to have intimate relations to the febrile response\(^7\) or Shwartzman reaction\(^7\). Although it is not yet established whether the a-
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Liver Glycogen Content

Winter

Summer

FIG. 1. Effects of endotoxin upon liver glycogen content. 3 hours after intravenous administration of endotoxin (300 µg/kg). Glycogen contents in wet weight percentage.

Fig. 2. Relation between the initial counts of pseudoeosinophiles and the changes in rectal temperature 3 hours after intravenous administration of endotoxin (100 µg/kg). Experiments in late spring, 1962. No correlation could be seen.
mounts of leucocytic pyrogen (Wood) liberated from neutrophiles (here pseudo-
eosinophiles) might have a causal relation to the induction of intoxication, the
relation between the absolute counts of pseudoeosinophiles and the degree of
intoxication was examined by estimating the latter by the degree of the late
toxic hypothermia. Experiments were done in late spring selecting animals
with wide variations in the pseudoeosinophile counts. As depicted in Fig. 2
changes in the rectal temperature 3 hours after endotoxin inoculation (100 μg/kg)
showed no correlation with the initial level of pseudoeosinophile counts. Thus
it might be concluded that the neutropenic and lymphocytotic tendency in
summer does not have a causal relation to the non-susceptibility to endotoxin.

DISCUSSION

Concerning the heightened susceptibility to endotoxin in winter it was
shown that the activation of thyroid gland participated in enforcing the dysp-
neic response via epinephrine discharge, however, the depletion of the liver
glycogen was independent of the thyroid activity. The latter which most
probably induced by the disposal of endotoxin in the hepatic reticuloendothelial
cells, might perhaps be intensified by the heightened hepatic metabolism in
the second stage of cold adaptation, the maintenance of which seems to be
independent of both thyroid and adrenal glands. The reverse might be the
case in heat acclimatization. This state seemed to be characterized by the
lowered glycogen content of the liver. The supposition that the lessening of
the liver glycogen depletion in summer might be the result of the lessening
of the intoxication symptoms in this season would not be accepted, since as
has been reported previously (Fukuda), the liver glycogen depletion together
with the hypoglycemic deterioration are independent of the toxic manifestations,
such as dyspnea and diarrhea. The latter resulting from the central autonomic
disturbances in relation to the activation of the febrile mechanism could be
sedated by antipyretics, leaving the liver glycogen depletion uninfluenced.

Concerning the lessening of the toxic symptoms in summer, a central stabi-
ization similar to the effect of antipyretics (Fukuda) might be induced by
the continued activation of the heat dissipating mechanisms. The macromole-
cular hematic syndrome, such as leucocytic response was also induced in
summer as in winter, except that the magnitude was lessened by virtue of
the neutropenic tendency. However, this did not seem to be related with the
lessening of intoxication in summer and the full febrile response could also be
evoked by non-toxic doses of endotoxin. At any rate, it is difficult to suppose
that the trigger mechanisms for the induction of intoxication might be absent
or weakened in summer.
SUMMARY

The susceptibility to endotoxin intoxication in rabbits was found to undergo a marked seasonal variation, being increased in winter and the least in summer. The activation of thyroid gland in winter was found to be related to the intense dyspneic response due to epinephrine discharge in this season. However, the characteristic metabolic derangements, such as the depletion of the liver glycogen especially pronounced in winter, was shown to be independent from it and seemed to be related to the enhanced hepatic metabolism in cold adaptation. Concerning the summer non-susceptibility, besides the lessening of the above potentiating factors, an assumption of some central alterations in reducing the disturbances in the autonomic nervous system in relation to the activation of the febrile mechanism seemed to be necessary.

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