CULTURAL STUDIES ON LEPTOSPIRAE

I. ON THE GROWTH OF LEPTOSPIRAE IN LIQUID MEDIUM INACTIVATED AT 100°C AND 121°C

Ryo YANAGAWA, Takashi HIRAMUNE, Takeshi SHIMIZU
and Susumu ISHII

National Institute of Animal Health, Tokyo

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Inactivation of the liquid medium of leptospira has been performed at 56°C, or 60°C after the addition of rabbit serum, but almost no particular studies have been published on the inactivation at 100°C and 121°C.

Rabbit serum has been accepted as the best serum for the growth of leptospira, and is substituted by horse serum by some workers^{1,4)}, although a poor growth of leptospira was reported with the use of horse serum by others³⁾. On the other hand, cattle serum has been considered unfavourable for the growth of leptospira.

Attempts have been made to inactivate the medium of leptospira at 100°C and 121°C after the addition of serum. The sera examined, besides the rabbit, were those of horse, cattle and sheep. The data concerning the experiments are described in this paper.

MATERIALS AND METHODS

Medium: 4.5 ml of Cox's liquid medium²⁾ was poured into each tube. Sera from 20 rabbits, 5 cattle, 3 horses and 3 sheep were used. The medium was inactivated for sixty minutes at 56°C, thirty minutes at 100°C, and fifteen minutes at 121°C, respectively.

Strain: Strain TR-1 (*L. icterohaemorrhagiae*) which was isolated from the rat in 1956, by Dr. YAMAMOTO, was used in this experiment. Each tube was inoculated with 0.5 ml of the seed culture. Media to which were added horse, cattle and sheep sera were inoculated with a strain of the 6th passage through the homologous medium in order to avoid the influence of rabbit serum.

Incubation: 28±1°C for three weeks.

Counting of leptospira: Number of leptospira per ml of the medium was counted on the 3, 7, 10, 14 and 21st days of incubation by means of the counting chamber previously reported⁵⁾. The average number in each of the two tubes was plotted and the growth curve of leptospira was thus figured.

RESULTS

Sera from 10 rabbits were pooled and added to the liquid medium. Contents of tubes were then inactivated at 56, 100 and 121°C, respectively. More abundant and rapid growths of leptospira were observed in the media which were inactivated at 100°C and 121°C than in those inactivated at 56°C (Fig. 1). Almost the same growth was observed in the media to which were added the individual rabbit sera. Poor growth or inhibition of leptospira was obtained in the media to which were added the sera of 10 other rabbits by the inactivation at 121°C, but not at 100°C.

The effect of various degrees of inactivation on the growth of leptospira in the medium

Fig. 1. Effect of Various Degrees of Inactivation of Media added with Rabbit Serum on the Growth of Leptospira

Fig. 2. Effect of Various Degrees of Inactivation of Media added with Horse Serum on the Growth of Leptospira

Fig. 3. Effect of Various Degrees of Inactivation of Media added with Cattle Serum on the Growth of Leptospira

to which was added pooled horse serum, was examined (Fig. 2). The number of leptospira per ml reached 8.8×10^7 in the medium inactivated at 56°C or not inactivated. While over 10^8 of leptospira per ml was obtained in the medium inactivated at both 100°C and 121°C. Almost the same effect of various degrees of inactivation on the growth of leptospira was observed in the media to which the individual horse sera were added.

A marked difference in the growth of leptospira was demonstrated in the media to which were added sera from 3 calves of Japanese native stock (Fig. 3). Poor growth was observed in the medium which was inactivated at 56°C or not inactivated. On the contrary, good growth was obtained in the medium inactivated at 100°C and 121°C. The same difference in the growth was observed in the medium to which was added the individual cattle serum.

On the other hand, in the media to which were added sera from 2 Holstein cows, the effect of various degrees of inactivation on the growth of leptospira was similar to that of the media to which horse sera were added.

In the media to which sheep sera were added, almost the same growth of leptospira was observed. The number of leptospira per ml, however, hardly reached to 5×10^7 .

In the experiments above described, the media were preliminarily inactivated at 56°C prior to the 100°C and 121°C inactivations. Almost the same growth of leptospira was obtained without the preliminary 56°C inactivation. But, in the media inactivated at 121°C, a poor growth or the inhibition of leptospira was occasionally observed, which was frequently avoided by a gradual inactivation up to 121°C.

Decrease in the number of leptospira on the 3rd day of incubation was frequently observed in the media to which were added horse, cattle and sheep sera. This decrease disappeared when the medium was inactivated at 100°C.

Agglutinability and infectivity of the strain were examined before and after the 11th passage through the medium inactivated at 100°C. No changes in the agglutinability for homologous immune rabbit serum and the infectivity for guinea pigs were involved by the passage.

DISCUSSION

A more abundant and rapid growth of leptospira was observed in the liquid medium inactivated at 100°C or 121°C than in those inactivated at 56°C after the addition of rabbit serum. The same phenomenon was also observed in the media to which were added horse, cattle and sheep

sera. These results may give some suggestions for the investigation of the role of serum as a medium for the growth of leptospira.

Media, to which the sera from the calves of Japanese native cattle were added were demonstrated to be suitable for the growth of leptospira after the inactivation at 100°C, but not at 56°C. On the other hand, media to which the sera of Holstein cows were added, were suitable for the growth of leptospira after inactivation either at 100°C, or 56°C. The difference between these sera will come into question from the view point of both the species and the age of the animals.

In the media to which were added cattle and horse sera the number of leptospira per ml reached 8×10^7 , which is considered to be of use for the antigen of agglutination-lysis test. The growth of leptospira in the medium to which sheep sera were added, however, was considered to be inadequate for the antigen owing to the relative small number of leptospira cells. The temporary decrease in the number of leptospira per ml on the 3rd day of incubation, which was observed in the media inactivated at 56°C, frequently disappeared after the inactivation at 100°C.

Almost the same growth of leptospira was observed in the media inactivated at 100°C and 121°C. By inactivation at 121°C, however, occasional poor growth, or inhibition of leptospira, was observed. This was considered to be due to the rapid heating. By heating gradually this effect could be frequently avoided.

No changes in the agglutinability of the strain for homologous immune rabbit serum or the infectivity for guinea pigs were involved by the passage through the medium inactivated at 100°C. Facilities in sterilization of the medium and the use of horse and cattle sera by the inactivation at 100°C and 121°C may be useful in practice.

SUMMARY

Attempts were made to inactivate the liquid medium of leptospira at high temperatures, such as 100°C and 121°C, after the addition of rabbit serum. The growth of leptospira was generally more increased in the media which were inactivated at 100°C or 121°C than in those which were inactivated at 56°C or not inactivated. The same phenomenon was observed in the medium to which was added horse, cattle and sheep serum, respectively. In the medium inactivated at 121°C, however, occasional poor growth or inhibition of leptospira was demonstrated.

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レプトスピラの培養に関する研究

I. 100°Cおよび121°Cで非働化された血清加液体培地
におけるレプトスピラの発育について

梁川 良・平棟孝志・清水 健・石井 進

農林省家畜衛生試験場

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Coxの液体培地(家兎血清を10%の割合に含む)を100°Cまたは121°Cで非働化して、レプトスピラを移植したところ、56°Cで非働化された同じ培地におけるよりも、レプトスピラは優勢に且つ速かに発育、増殖する傾向が認められた。同じような現象は馬、牛および緬羊血清をそれぞれ加えた培地

でも認められた。したがって、56°C非働化ではレプトスピラの発育に好適でない培地が100°Cまたは121°C非働化ではじめてレプトスピラの発育に好適となる例があった。但し121°Cで非働化された培地では、時にレプトスピラの発育が著るしく不良となる場合が認められた。