INTRODUCTION

In an attempt to improve the anti-rabies treatment efforts have hitherto been centered around the production of vaccine with high antigenic potency, removal of nervous tissues, introduction of chick embryo adapted rabies vaccine, etc.

A few workers have tried adjuvants, in order to increase the antigenic potency, but in this method they only observed a prolongation of time in reaching the height of immunity. Consequently, adjuvant method is unsuited for the primary purpose of anti-rabies treatment.

However, the striking acceleration of the antibody formation of typhoid vaccine by the simultaneous injection of purified vaccine lymph (PVL) has been found by Yaoi as early as 1939, which was confirmed later with many other vaccines.

In the present study animal experiments were carried out to confirm whether the said combined vaccination based on the simultaneous use of PVL may be applied effectively to the anti-rabies treatment, and at the same time the effect of cortisone was compared to that of PVL.

EXPERIMENTALS

Methods and materials:

For the strain of fixed rabies virus the Nishigahara-strain was used as usual, and for immunization of animals two lots of Merzonin-inactivated vaccine were prepared using a brain emulsion of guinea pigs. Namely, 0.2 cc of $10^{-2}$ dilution of the mouse brain infected with seed virus ($LDD_50=10^{-7.2}$) was intracerebrally injected into guinea pigs and the brain removed was ground in a mortar to make a 10% emulsion in sterilized distilled water adjusted to pH 7.2.

The emulsion was passed through a 100-150 mesh metal screen, added with Merzonin in 0.1% and incubated as usual at 37°C for 5 days. All through the incubation period the container was shaken by hand 4 or 5 times a day. The Merzonin-vaccine thus obtained was used for the immunization of rabbits.
However, in order to suit the mouse protection experiments, another Merzonin-vaccine of a lower antigenic potency was obtained by incubating the same emulsion at 37°C for 8 days and standing it at room temperature further for 3 months before use.

The PVL was obtained from the Institute for Infectious Diseases.

As for cortisone, cortisone acetate of Merck was used as suspension in normal saline.

The mouse experiment was largely depended upon the usual technique of mouse protection test.

Immunizing effect on rabbits was evaluated by the formation of neutralizing antibodies.

Results:
(1) Results of protection experiment on mice.

Experiment 1:

White mice weighing 10 to 12g were divided into 5 groups and, leaving one group of mice for the control, the remaining 4 groups of mice were immunized with intraperitoneal doses of 0.25 cc each of a low-antigenic Merzonin-vaccine diluted to 0.5%.

First group of mice was immunized with 3 doses on alternate days, and 0.1 cc of PVL was injected subcutaneously into the thigh with every dose of vaccine. Second group received only 3 doses of vaccine, and the 3rd group 6 doses of vaccine. Fourth group received 3 doses of vaccine, and at every dose 1 mg of cortisone acetate was injected into the thigh muscle. Fifth group served as control as stated above. The "3-dose" group was injected intracerebrally with the challenge virus 7 days after, and the "6-dose" group was challenged 14 days after the beginning of immunization. The challenge inoculation was made with 0.025 cc each of the tenfold dilutions of a 10% suspension in broth of pH 7.6 of the mouse brain infected with the Nishigahara strain.

Table 1 indicates the result of above experiments. As clearly indicated in the table, the 1st group which received the 3-dose-vaccine with PVL showed a protection index of as high as 50,000, whereas the indices of the 2nd (3-dose-
vaccine) and 3rd groups (6-dose-vaccine) were 5,000 and 16,000, respectively.

In contrast, the 4th group which received a 3-dose-vaccine with cortisone showed a very low MLD protective value as 500. The 5th group indicates the titre of challenge virus, that is, \(LD_{50} = 10^{-7.5}\). As mentioned above, as compared with the MLD protective value, the 1st group is greater by 10 times than the 2nd group and 3 times than the 3rd group, and the 4th group corresponds to 1/100th of the 1st group and 1/10th of the 2nd group.

**Experiment 2:**

Using the same less antigenic vaccine as used previously, the 1st group was immunized with 3 doses of vaccine, simultaneously with 0.1 cc of PVL. The 2nd group received 6 doses of vaccine. The 3rd group which had received 3 doses of vaccine was injected intramuscularly with 1 mg each of cortisone into the thigh muscle for 3 successive days prior to the challenge inoculation. The 4th group received simply 3 doses of cortisone like the former group. The 5th group served as control.

The above-mentioned 5 groups were challenged alike 14 days after the beginning of immunization and observed for 14 days as usual.

Table 2 indicates the results. The 1st group which received 3 simultaneous injections of vaccine and PVL showed a protection index of 8 times as large as the 2nd group which received 6 doses of vaccine: the disparity between the 2 groups is more marked as compared to the former experiment. It was thus proved again that, when PVL was associated, higher immunizing effect could be attained by the 3-dose-vaccine.

On the other hand, the 3rd group which received 3 doses of vaccine and cortisone separately indicated a protection index of barely 25. Compared with each other on the basis of the index, the 3rd group corresponds to 1/50th of the 1st group and to 1/6th of the 2nd group. Further, the 4th group which received only cortisone showed an increase of susceptibility to the virus by 16 times on the basis that \(LD_{50}\) of the 4th group and that of control group was \(10^{-7.6}\) and \(10^{-6.4}\), respectively.

From the above-mentioned two protection experiments, it is evident that

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunization</th>
<th>Dilutions of challenge virus</th>
<th>(LD_{50})</th>
<th>MLD Protect. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>“3-dose”-vaccine+PVL</td>
<td>7/7 7/7 5/7 0/7 0/7</td>
<td>(10^{-5.3})</td>
<td>1,300</td>
</tr>
<tr>
<td>II</td>
<td>“6-dose”-vaccine</td>
<td>7/7 7/7 7/7 3/7 2/7</td>
<td>(10^{-4.2})</td>
<td>160</td>
</tr>
<tr>
<td>III</td>
<td>“3-dose”-vaccine+Cortisone</td>
<td>7/7 7/7 7/7 6/7 4/7</td>
<td>(10^{-5.0})</td>
<td>25</td>
</tr>
<tr>
<td>IV</td>
<td>Cortisone</td>
<td>7/7 7/7 4/7 2/7 2/7</td>
<td>(10^{-7.6})</td>
<td>-16</td>
</tr>
<tr>
<td>V</td>
<td>Control</td>
<td>7/7 5/7 1/7</td>
<td>(10^{-8.4})</td>
<td></td>
</tr>
</tbody>
</table>
the combined vaccination with PVL gives rise to immunity of several times
greater than that obtainable by the redoubled dose of vaccine. On the con-
trary, the immunizing effect was remarkably reduced by the simultaneous use
of cortisone. Moreover, a considerable reduction of immunity seemed to have
been caused also by the administration of cortisone immediately before the
challenge inoculation.

(II) Neutralization test using sera from rabbits.

For the immunization of rabbits, the Merzonin-vaccine with ordinary
antigenic potency was used.

Rabbits weighing 2,500 to 2,900 g were divided into 3 groups of 3 rabbits
each. These 3 groups of rabbits were injected intradermally with 0.2 cc each
of the vaccine on their side for 7 successive days. The first group received
only the vaccine, while the 2nd group was immunized with 7 doses of vaccine
and 2 doses of PVL, which were given subcutaneously, 0.2 cc on the 1st day
and 0.3 cc on the 4th day of immunization. The 3rd group was also immunized
with 7 doses of vaccine but at the same time treated daily with intramuscular
injections of cortisone of 4 mg/kg of body weight for 20 days.

Bleeding was done by cardiac puncture at 10, 14 and 21 days and the
serum was separated by centrifugation and inactivated by heating at 56°C for
30 minutes in water-bath.

Then 0.3 cc each of the sera was distributed into small test tubes, added
with 0.3 cc each of the virus suspension of from 10^-1 to 10^-7 dilutions, thorou-
gly shaken, and incubated at 37°C for 2 hours, shaking from time to time.
After being placed in a refrigerator at 4°C till the next morning, 0.03 cc each
of the said mixtures was injected intracerebrally into mice of 10 to 12 g in
weight, and observed for 14 days.

The findings are graphically presented in (figure 1.). As shown in the
figure, the mean N.I. of the 1st group which received only vaccine is shown
to be 116 at 10 days, 853 at 14 days and 2,050 at 21 days. The N.I. of the
2nd group which received vaccine and PVL was proved to be 483 already at
10 days, which is 4 times as large as that of the 1st group, and it further
increased to show a value twice as much as that of the 1st group at 21 days.
On the contrary, antibody formation in the 3rd group which received
vaccine and cortisone was retarded and showed a low value like 770 even at
21 days, which corresponded to 1/3rd of the 1st group and 1/6th of the 2nd
group.

As mentioned above, the combined vaccination with PVL gave rise to a
rapid and abundant formation of antibodies in rabbits, while vaccination
combined with cortisone has caused not only a marked inhibition of antibody
formation, but also a certain diminution of body weight in animals.

DISCUSSION

In the recent outbreak of rabies in Japan, there have been observed rather
many cases which indicated that the Pasteurian anti-rabies treatment may not
be as efficacious as it had been believed to be. Moreover, frequent occurrence
of neuroparalytic accident have roused public anxiety and the value of the anti-rabies treatment was doubted by a number of workers.

We also reported some inquiries into the causes of the failures of the Pasteurian anti-rabies treatment. Merzonin-vaccine was inaugurated by us in order to make up for the defects of the said method. Merzonin-vaccine not only confers a strong protection as shown by the Habel test but also produces abundant rabicidal antibodies in man and animal. By this method, failure and accident were strikingly reduced as compared to the old method.

However, in reference to the problem of neuroparalytic accident, there remained one more investigation into the effective immunization with a smaller quantity as well as a fewer number of injections as compared to the previous 10-successive intradermal injections in human rabies.

Under such circumstances, the application of the combined vaccination with PVL, which was first reported by Yaoi as early as 1939, was attempted, the result of which being quite satisfactory: the combined vaccination conferred a stronger protection for mice and produced more rabicidal antibodies in rabbits.

On the other hand, the increase of susceptibility to bacteria and viruses by the administration of cortisone or ACTH, and the suppression of immunizing effect by the same agents have been reported by many workers. In this experiment the combined vaccination with cortisone also gave rise not only to an unexpectedly large decrease in immunizing effect, but also an increase of susceptibility to rabies virus and emaciation of the animals.

Further, as shown in the 2nd mouse protection experiment, cortisone given after the vaccination appeared to have lowered the immunizing effect. However, as it has been reported that cortisone has no effect upon the formed antibodies, it seems worthwhile to investigate the mechanism of the above-mentioned decrease of antibodies in the animal body.

Effectiveness of cortisone or ACTH in preventing and curing neuroparalytic accident has been reported mainly on the experimental basis by some prominent workers, but if cortisone were utilized for fear of neuroparalytic accident,
the anti-rabies treatment seems to lose largely its significance in view of an apparent decrease of antibody formation and increase of susceptibility to the virus.

Preferability of reducing the number of injections has been emphasized by some workers (Habel, Jervis, Yaoi, etc.). In accordance with such views, we have been carrying out recently an anti-rabies treatment with 7 doses in 5 days, and in all 26 cases treated so far no failure or accident has been observed up to date. It appears to us that the “7-dose” anti-rabies treatment in every case of human rabies is the goal which has been already reached. However, in view of the present experimental data, the completion of anti-rabies treatment with 2 to 3 doses of vaccine in combination with PVL seems to be not an inordinate ambition.

Moreover, it is to be hoped that the antiallergic nature of PVL may settle the problem of neuroparalytic accident as well.

**SUMMARY**

1) By the usual mouse protection test, group-A which received the 3-dose-vaccine together with PVL showed a MLD protective value of 8-times greater than the group-B which received the 6-dose-vaccine.

2) On the contrary, group-C which received the 3-dose-vaccine together with the 3-dose-cortisone showed 1/100th the MLD protective value of group-A. And, group-D which received the 3-dose-vaccine and later the 3-dose cortisone showed a protection of 1/52 of the group-A.

3) Also in the immunization experiment on rabbits, a rapid formation of neutralizing antibodies was shown by the combined vaccination with PVL. For example, the vaccine-PVL-group showed N.I. values of 4 times larger at 10 days and 2 times larger at 21 days, compared to those of the simple vaccine-group.

4) In the similar immunization experiment, a group which received cortisone of 4 mg/kg of body weight in successive days showed low N.I. values like 1/10 of the vaccine-PVL-group and 1/3 of the vaccine-group at 10 days, and 1/6 of the vaccine-PVL-group and 1/2 of the vaccine-group at 21 days.

5) Cortisone gave rise to the increase of susceptibility of mice to rabies virus and emaciation of rabbits.

**REFERENCE**

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