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**ANTIBODY ASSAYS FOR JAPANESE ENCEPHALITIS VIRUS
IN BOVINE SERUM BY ENZYME-LINKED
IMMUNOSORBENT ASSAY**

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The preliminary results of Enzyme-Linked Immunosorbent Assay (ELISA) for detection of a specific antibody against JaGAR-01 strain of Japanese encephalitis (JE) virus in bovine serum were presented. The criterion to detect a specific antibody by ELISA was determined according to the neutralization test (50% plaque reduction) on the bovine sera collected in Kagoshima as an epidemic area of JE and in northern Hokkaido as a non-epidemic area.

The results obtained were as follows :

- 1) With virus infected BHK-21 cell monolayer being used as a solid-phase antigen, ELISA antibody was specific in rabbit sera, but not in bovine sera because of a high level of non-specific reactions.
- 2) With sucrose-acetone (SA) extracted antigen which had been prepared from a suckling mouse brain (SMB) infected with virus and adsorbed in the wells of the polystyrene microplate, the titration of specific ELISA antibody was successful in both bovine and rabbit sera.
- 3) No marked differences were seen in the simplicity of procedure and the reproducibility of antibody titer between the use of conjugates prepared from anti-bovine IgG rabbit IgG labeled alkaline phosphatase (ALP) and horseradish peroxidase (HRP) for bovine sera. For rabbit sera, the conjugates being used were prepared from anti-rabbit IgG sheep IgG labeled ALP or HRP, the results of which were the same as for bovine sera.
- 4) ELISA antibody titers were not lowered by acetone treatment of either bovine or rabbit sera.
- 5) JaGAR-01 antibody titers against JaGAR-01 SA antigen in 486 sera in northern Hokkaido were accumulated at a peak of 1:10, with the rate of sera showing 1:40 or more was 4.3%. On the other hand, the titers in 204 sera in Kagoshima were distributed around 1:40, with the rate of sera showing 1:40 or more was 26.0%. The neutralizing antibody titer 1:10 in Kagoshima sera was almost equivalent to that of ELISA antibody titer 1:40.
- 6) ELISA antibody titers against normal SMB-SA antigen in 208 sera in Hokkaido was similar to that against JaGAR-01 SA antigen derived from infected SMB. The mean antibody titer against normal SMB antigen was as low as 1:10, with the rate of sera showing 1:40 or more was about 2.0%.
- 7) The cross-reactors between JaGAR-01 and Negishi virus in flaviviruses were found in 20.1% of Kagoshima sera by ELISA, and the specific ELISA antibody against JaGAR-01 which was four times or more greater

than that against Negishi was found in 9.8%, and in the same relation the specific ELISA antibody against Negishi in 5.4%.

**EXPERIMENTAL STUDIES ON TRANSFUSION INTO DOGS :
CLINICAL AND HEMATOLOGICAL FINDINGS ON
TRANSFUSION WITH BLOOD STORED IN
CPD ON DOGS AFTER BLEEDING**

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The present study was undertaken to document the clinical and hematological findings on transfusion with blood stored in CPD into hypovolemic dogs. For this purpose, the changes of the components of canine blood stored in CPD solution at $4 \pm 2^\circ\text{C}$ for up to 6 weeks were observed, and after bleeding of 20% of the circulating blood volume, the dogs were transfused with the homologous blood stored for 1 day, 3 weeks and 6 weeks as a replacement.

The results were summarized as follows :

1) During the 6 weeks of blood storage, the following changes were observed: the occurrence of decreases in the pH and Po_2 , osmotic fragility of the erythrocytes, blood glucose, 2,3-DPG and ATP in the erythrocytes; increases in the plasma hemoglobin and potassium and the breaking up of the white blood cells. There were no changes observed in the RBC, Ht, T.P. and plasma sodium. The 2,3-DPG contents in the erythrocytes of the stored blood maintained 68% of the values of fresh blood after 3 weeks of storage and 26% after 6 weeks; and the ATP contents were 82% after 3 weeks and 50% after 6 weeks.

2) While hemorrhagic dogs were transfused with the blood stored in CPD to an equal volume of bleeding, the arterial pressure was increased gradually with infusion but not recovered to the initial values even after the completion of transfusion. Thereafter, however, the arterial pressure was maintained favorably, and there followed an improvement in clinical findings. The circulating blood volume was approximately 91% of the initial value during the 10-day post-transfusion period.

3) After transfusion, the Ht, Hb, T.P. and plasma sodium were maintained approximately at the initial values, but thereafter, the Ht and Hb were decreased gradually up to 3 days after transfusion. During the 3-hr post-transfusion period, the arterial and venous pH and blood glucose were restored approximately to the initial values.