CHAPTER IV

DISCUSSION

It should also be borne in mind that immunoelectrophoresis gives primarily qualitative results although immunoelectrophoresis can also be used for quantitative determinations but it does not allow the precise quantitative determination of plasma proteins. In this test what we hope for is only qualitative results.

There exists in the human at least five major classes of immunoglobulin, the IgG, IgM, IgA, IgD, and IgE globulins. All of these except IgD and IgE have known functions in virus-host interactions. The most thoroughly studied of the serum immunoglobulins are the 7s rG globulins which contain most of the antiviral activity in serum. The rA globulins have been somewhat less well studied, mainly because of technical difficulties encountered in their characterization. Recently, however, it has been possible to demonstrate rA viral antibody in the serum. One of the most important recent discoveries in immunology is that a unique form of rA is found in the external secretions of the body, the external secretory system. These secretory antibodies have been shown to be an important part of the protection of the host against viruses, particularly those which produce localized infections. (4)

The rM globulins are the largest in size of the immunoglobulins and include antiviral antibodies produced early in the immune response. These globulins have also been shown to be phylogenetically and ontogenetically primitive forms of antibody which are characteristic of the fetal or newborn antibody response in man. (4)

Following immunization or infection with viruses, there appears to be a sequential appearance in the molecular varieties of antibody. Initial antibody is usually associated with the rM class and is followed later by rG antibody. (5)

So that in the acute stage IgM level should be higher than IgG and should be lower than IgG in the convalescent stage. From the symmetrical arcs in the results they showed the IgG and IgM were both found in both stage. The interpretation can be as follow.

1. In the acute serum there are both IgG and IgM, this may be because it is not really acute serum. The patient usually came to the hospital many days after the onset of fever. The serum might be in the convalescent stage and should have the IgG.

2. In the convalescent serum there still are both IgG and IgM like the acute serum. It may be that it is the antigen nature to stimulate the host to produce the immune response of both types, IgG and IgM, in both stages. Tuchinda (1971) (41) found that in the determination of serum immunoglobulins, the mean values of IgG and IgM levels detected in paired sera of 31 dengue hemorrhagic fever patients was that, in acute and convalescent serum there were both IgG and IgM, and IgG levels are higher than IgM levels in both stages.

From this, it can say that filter paper strips are good enough to use in the sense that they don't absorbed immunoglobulin parts (IgG and IgM). When they are eluted, the IgG and IgM are eluted too. So the antibody titers which were detected were reliable.

HI titers against dengue infection obtained by filter paper and syringe method didn't correspond well to each other. When comparing standard syringe method and filter paper method which stored at 4°C for 2 weeks, the HI titers obtained by the filter paper method showed higher than those by the syringe method about 2- 8 folds, and few cases equal to each other. (Table 2) If the inhibitors still remained after kaolin treatment they would appear more markedly where HI antibodies were not so high. Differences seen in this case might be caused by remaining inhibitors in the

specimens of the filter paper method or it might caused by the errors in absorbed volume of filter paper.

This filter paper strips were developed to have the absorbed volume of each piece of 0.1 ml blood (serum: 0.04 ml) as in the blood sampling paper operation given by Toyo Roshi Kaisha, Ltd., Japan. In the extraction method, the extracted solution corresponds to the 1:20 dilution when eluted with 0.8 ml PBS. If the absorbed volume is higher than 0.1 ml, when we extract by the same volume of PBS, the dilution of the extracted solution will be lower than 1:20 and the end point will be in the higher dilution. We will prove about this in later experiment.

Oppelaar (1966) (32) found that elution with a uniform quantity of PBS might cause a variability in the quantitative serological reactions of at least two dilutions.

Karstad, et al. (1957) (29) suggested that the great variation observed in titers of whole blood samples collected on paper, may have been due in part to deposits of unequal quantities of coagulated blood. Some of the discs saturated in the laboratory carried small clots of dried blood.

When comparing HI titers of filter paper method which stored at different temperature between 4° C in refrigerator and room temperature (20° C) and at different time of storage between 2-15 wk (Table 3), it was clearly seen that the titers of the eluates of blood strips decreased when keeping at room temperature comparing with those keeping at 4° C in refrigerator up to 2 weeks (14 days), and the titer continued to decrease rapidly according to time of storage up to 15 weeks. So the best condition of storing blood dried filter paper was at 4° C. Higher temperature at room temperature will cause a deterioration of the antibody titers.

The titers of filter paper kept at 4° C were compared between 2 weeks and 4 weeks of storage (Table 3) and were found that there were 1 case equal dilution,

6 cases 2 fold dilution lower and 4 cases more than 2 fold dilution lower. When compared between 2 weeks and 14-15 weeks of storage, it was found that there were 3 cases equal and 2 cases 2 fold dilution lower.

The blood dried filter papers could be kept at 4° C in refrigerator with some changes but could be detectable titers up to 15 weeks. Although the HI titers of the paper eluates were higher than those derived from fluid serum controls, the increasement did not impair the validity of the test or render interpretation difficult. The method simplified the collection and handling. A minimum of storage and shipping facilities were required and very small quantities of blood and serum could be tested. The procedure appears to be of value in epizootiological and epidemiological studies.

Although a serum with a low titer might be undetected by the paper absorption method, low titer sera have been rare and have not contributed information of significance to the present study of dengue infection.

Worth (1964) (49) found that mumps HAI titers of eluates of dried frozen blood discs stored for 4 to 6 months did not differ significantly from titers of discs stored for only 1 to 2 months.

Gaggero and Sutmoller (1965) (22) found that bovine serum can be dried on blotting paper and stored without refrigeration for at least 60 days without interfering with the detection of antibodies against foot-and mouth disease virus. The same technique has been applied successfully to blood, but without determination of the storage time.

The virus-neutralizing activity of the dried specimens was, on the average, lower than that of the control frozen serum samples, but the difference was of no practical importance in the interpretation of individual results.

Oppelaar (1966) (32) found that the titers of the eluates of blood and serum discs remained unchanged up to 70 days of storage, alternately at room temperature and at 37°C.

Karstad, et al. (1957) (29) showed that samples of serum dried on paper may be stored for considerable periods of time without deterioration.

Adams and Hanson (1956) (1) found that exposure of serum on paper discs to 56°C for 1 hour or to 37°C for 7 days resulted in no appreciable loss in titer of vesicular stomatitis virus neutralizing antibodies.

Brody, et al. (1964) (7) compared measles HAI titers for serum and disks of 15 individuals which are presented on Table 4. In eight instances, titer was identical between serum and disk of the same individual. In one instance the disk titered one dilution higher and in three instances the serum titered one dilution higher than the corresponding disk. Three individuals did not have titer by either method. These disks remained at room temperature for 6 months before testing. Similar results were achieved on larger series in which the disks were stored for up to 1 year at -20°C before testing.

From this study, and those of other investigators it may be said that the higher titer of the filter paper method may caused from the error in absorbed volume of absorbing part of the filter paper strip. It should not caused from the remaining of the nonspecific inhibitor after kaolin treatment. The variation in volume of absorbed blood on filter paper strip may be more or less than 0.1 ml. The fourth experiment was the detection of the error of blood volume absorbed by filter paper strips.

This filter paper strips as described by Toyo Roshi Kaisha, Ltd., can absorb 0.1 ml of blood (0.04 ml serum) and will be 1:20 dilution if add 0.8 ml of PBS. But from this result (Table 5) if we need a serum dilution 1:20 we will add a 1.2-1.4 ml PBS (female) or 1.2-1.6 ml PBS (male).

In the former experiment the filter paper was added with 0.8 ml PBS so the serum dilution received must be lower than 1:20 and then the HI titer of the serum may be shifted to the higher dilution. These made a great variation in titer between syringe and filter paper method.

The papers absorbed with positive dengue infection blood of the same person were sent from 8 different provinces (4 from the south, 2 from the north, and 2 from the north-east part of the country). The time of transportation was different and the average titer are shown in Table 7.

This experiment showed the practical value of filter paper strips that even sented in the climatic condition, exposed to both temperature and humidity, the titer of the eluates did not change much even took a long time of transportation (11 days) and when took 12 days of shipping the titers change only 2 fold dilution lower. So if the transportation took time lower than 11 days the detected titers would be reliable but if it took more than 11 days the detected titers might be varied in some.