FURTHER EXPERIMENTS RELATING TO THE PROPAGATION OF VIRUS IN THE BOVINE MAMMARY GLAND

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In a previous paper (1) data were presented which indicated that three viruses, namely duck, Newcastle disease and human influenza type A persisted in the mammary gland for approximately two weeks after injection into the lactiferous sinus. The data concerning the first agent were not sufficiently precise and consequently only of superficial value. However, it was shown that the injection of Newcastle disease virus and the virus of human influenza was followed by a rise of virus titre in the milk after the first day. About ten days later a permanent descent commenced and in a short time the agent was no longer present in the milk. Results were consistent with the propagation of virus within the mammary gland. However, because no basic information was available relating to the behaviour of viruses within the gland, it was felt undesirable to draw conclusions from the evidence at hand. Therefore, the matter has been investigated further. That which follows indicates the results.

Comparison of the Behaviour of Active and Inactive Virus within the Mammary Gland:— It has been mentioned that following the inoculation of active virus into the lactiferous sinus that the titre rose and persisted for several days in spite of daily milking. If for some unknown reason the mammary gland might retain virus at a high titre in the absence of propagation, it seems reasonable that the injection of inactivated virus would be followed by similar results. If, on the other hand, the titre of the inactivated virus descended rapidly, it should furnish almost incontrovertible proof that propagation of the active agent had taken place. It was thought that a trial using Newcastle disease virus would suffice to test this point. The viability of this agent is somewhat easier to determine and in addition the conservation of large animals has to be considered.

The virus was propagated in chick embryo, the fluids harvested, 0.25% formalin added and the mixture allowed to stand for 10 days at 37°C. The mixture was then inoculated into embryos and chickens. No evidence of active virus was found. Also, it gave satisfactory results when used in the haemagglutination test.

A suitable cow was chosen, the milk from each quarter was examined for a number of days and found to contain non-specific haemagglutination substances in only a low titre (1:20). One quarter was then selected and 2 cc. of the egg fluids containing inactivated virus injected through a teat tube into

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the lactiferous sinus. Milk was collected daily and subjected to the haemagglutination test. The results ars shown on Chart 1.

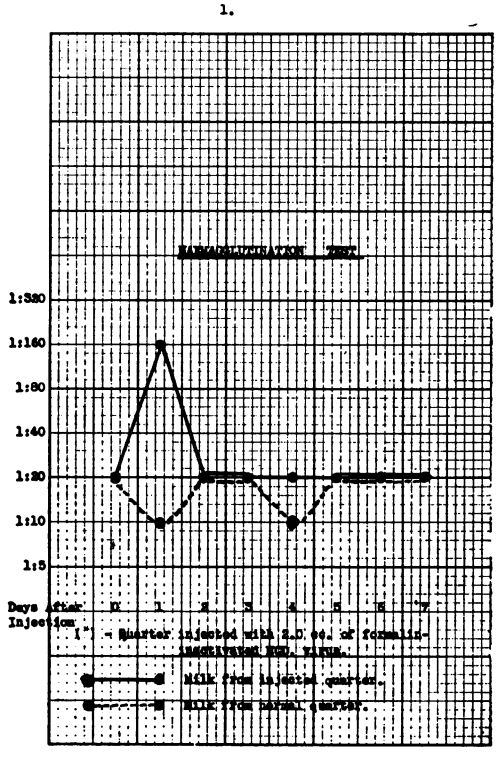

Chart No. 1

It will be noted that on the second day following injection the haemagglutination titre had dropped to the normal level where it remained. This was in direct contrast to the behaviour of the active virus in the gland.

Susceptibility of Injected Quarters to Re-injection:— If the viruses injected into the quarters had not propagated and had disappeared because of physical reasons, it is likely that no difficulty would be found in repeating the results by re-injection. Therefore, the appropriate quarters were again injected with respective viruses using the same amounts as were first employed. Samples of milk were taken daily and examined for the presence of virus by the inoculation of chick embryos. It was found that twenty-four hours after the injection of the quarters the viruses had entirely disappeared.

Neutralizing Antibody in Milk and Blood:— Milk and blood samples were collected from the animal at frequent intervals following the injection of the quarters described in the former paper (1). These samples were examined later for the presence of neutralizing antibodies. Two methods were employed — the haemagglutination inhibition test (beta method) and the chick embryo protection test. Charts 2, 3, 4 and 5 indicate the results.

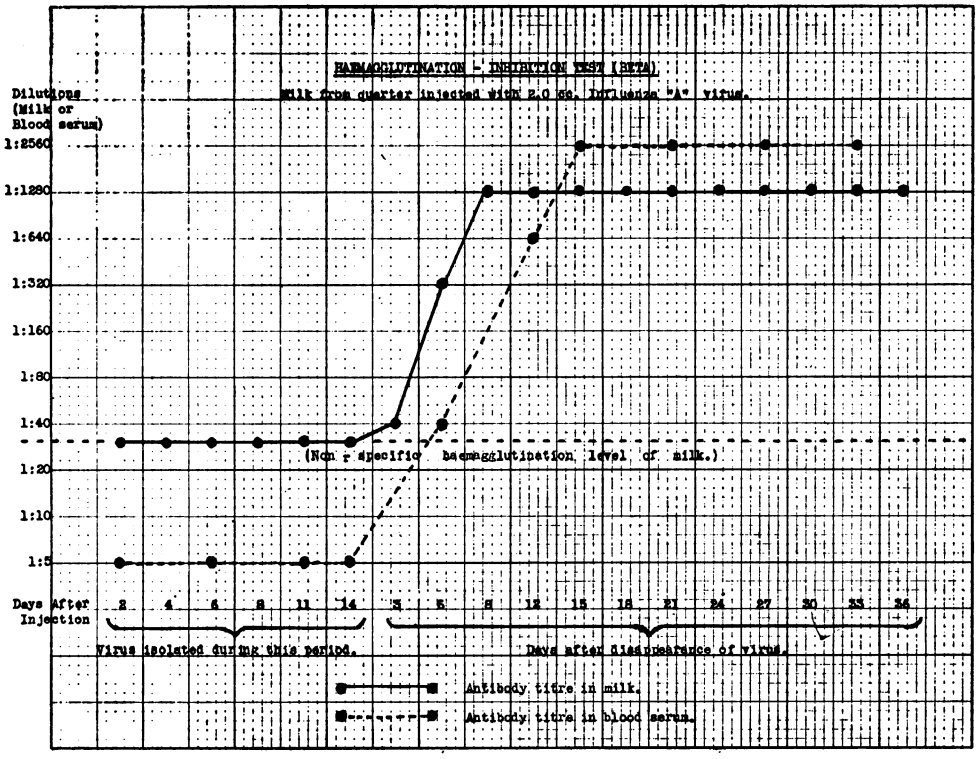

Chart No. 2

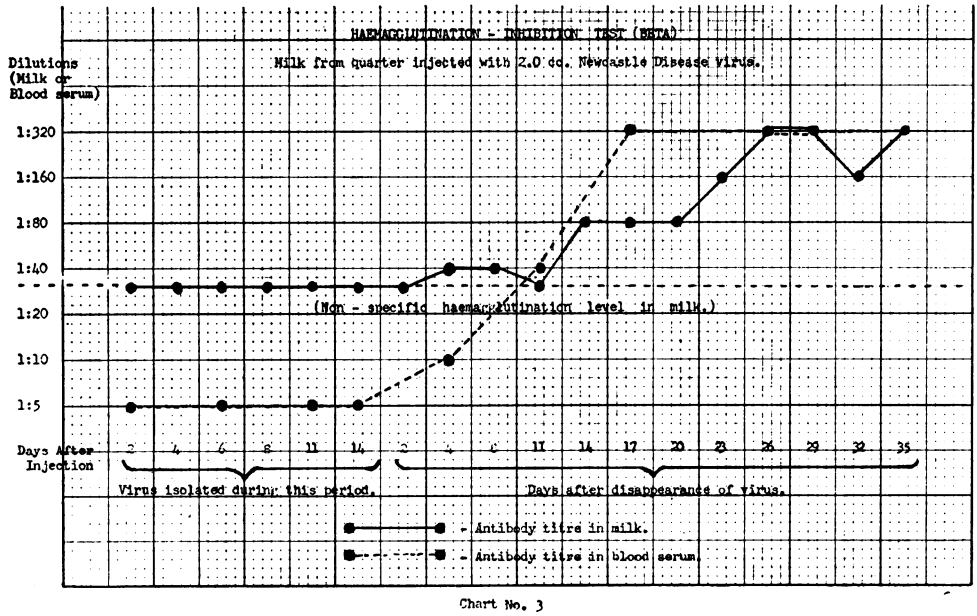

Chart No. 3

It will be noted that antibody was not demonstrated in the milk of the injected quarters nor in the blood until after the viruses were no longer present in the gland. A short time later however antibody could be demonstrated in each injected quarter and about the same time in the blood. In each of these the titre quickly rose. No chart relating to the two control quarters has been

Charts 4 and 5.

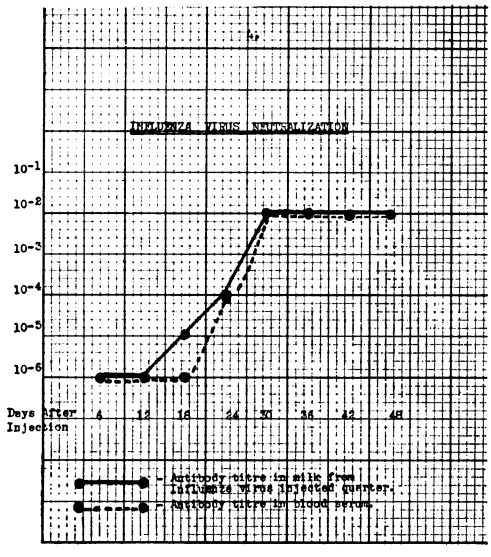

Chart No. 4

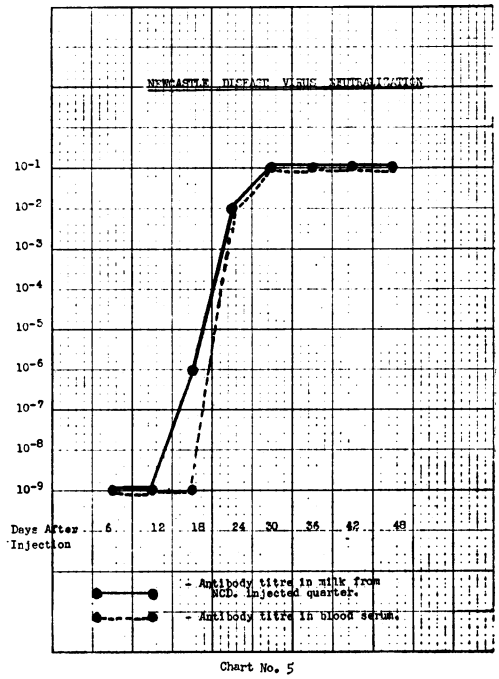

Chart No. 5

prepared. A small amount of influenza antibody was present in the adjoining longitudinal quarter six days after the virus had disappeared from the site of injection. It gradually increased until a level of almost 1:160 was reached. Antibody in the quarter adjacent that injected with Newcastle disease virus at no time rose about the non-specific level (1:40).

DISCUSSION

To decide whether virus propagated in the mammary gland it is necessary to review briefly the evidence presented. When active virus was injected into a quarter the titre of virus contained in the milk rose quickly and persisted for several days. There is no apparent explanation for this except propagation within the gland. In case some unknown factor might be involved the experiment was repeated using virus inactivated with formalin. In 48 hours the virus level in the milk was diluted to a point where its presence could not be determined. The two trials considered together would appear to demonstrate conclusively that virus propagated within the gland.

The reason for the disappearance of the viruses had therefore to be con-

sidered and if possible explained. It had been shown previously that agglutinins for *Brucella abortus* were formed within the gland if the organism colonized in the organ either during the course of natural infection or after being injected into lactiferous sinus (2). An examination of milk and blood samples from the animal injected with the viruses demonstrated that neutralizing antibodies made an appearance soon after the virus had disappeared. This provided an explanation for the rapid recovery of the quarters from infection. Failure to produce infection by re-injection emphasizes this point.

The authors believe that approximately the following took place. When the viruses were introduced into the lactiferous sinus they invaded appropriate cells, perhaps the columnar or cuboidal cells of the sinus. Here propagation took place unhindered for a number of days, then neutralizing antibodies commenced to form locally. This, in turn, was followed by a decline of virus titre until a balance was struck with a consequent disappearance of virus. Immediately thereafter neutralizing antibodies gained ascendancy and could be demonstrated in the injected quarters in gradually increasing titres. Simultaneously with the formation of antibodies in the quarters some were taken into and stored in the circulating blood.

Obviously there are many avenues to be explored. A determination of the viruses that will propagate in the mammary gland, the tissue invaded, if this tissue will lend itself to tissue culture technique, does residence in mammary tissue alter the pathogenicity for the natural host and can the production of antibody in the gland be exploited for practical purposes are some of the many points that arise. A few of these are now under investigation.

CONCLUSION

Newcastle disease virus and the virus of human influenza type A PR 8 strain propagated for approximately two weeks in the bovine mammary gland after injection into the lactiferous sinus. This propagation was followed by the development of neutralizing antibodies.

REFERENCES

(1) MITCHELL, CHAS. A., WALKER, R. V. L. and BANNISTER, G. L. Preliminary experiments relating to the propagation of viruses in the bovine mammary gland. Can. J. Comp. Med. and Vet. Sci. 17, Mar. 1953.

(2) MITCHELL, CHAS. A., HUMPHREYS, F. A. and WALKER, R. V. L. Agglutinins in the milk and blood serum of cattle following injection of *brucella abortus* into the mammary gland. Can. Pub. Hlth. Assoc. Abstracts of Laboratory Section papers, Dec. 1935.