

the complement-fixing substances present in the sera of syphilitic patients could not be applied to specific complement fixing substances obtained after protein injections, in view of the fact that, in the former case, the antigens employed were non-specific. This has led us to study the rate of destruction by heat of *specific* complement-fixing antibodies.

The mode of immunization as well as the complement fixation tests were conducted as indicated in the first paper of this series. The tests were carried out in each case with unheated serum and the same immune serum heated to varying temperatures, beginning with 5 minutes at 56 degrees C. and ending with 1 hour at 65 degrees C. It was soon found, however, that these temperatures did not lessen the antibody content of the rabbit serum, and that the thermal destructive point of these complement fixing antibodies existed apparently at a higher temperature level.

The sera were then diluted 1-10 with saline in order to raise the protein coagulation level (Ebersson) and placed in the water bath for 2 hours at 65 degrees C.; 1 hour at 70 degrees C.; and $\frac{1}{2}$ hour at 75 degrees C.—without any apparent effect on the antibody content. The sera were then subjected to temperatures of 80 degrees C. and 85 degrees C. for 15 minutes. At the former temperature the antibodies were practically destroyed, while at the latter, completely destroyed.

The fact that the so-called complement fixing antibodies present in the sera of patients suffering from syphilis are destroyed when subjected to temperatures ranging between 60 and 65 degrees C. and that specific complement fixing antibodies withstand a temperature of 75 degrees C. suggests the possibility that there exists inherent biological differences between the two types of antibodies and opens a suggestive field for research.

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The use of blood plasma in the imbedding or the dissection of small organisms.

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Various methods have been used in order to overcome the difficulties attendant upon the carrying of a minute organism,

such as a protozoön, through the numerous reagents necessary for imbedding in paraffine preliminary to microtome sectioning. It has been found that the phenomenon of coagulation in blood plasma makes it of use in work of this type. In carrying out this method, a few drops of blood plasma is placed on a depression slide under a binocular microscope and the specimen at once placed in it with a pipet. After the plasma coagulates, which will take place in a very few minutes, the specimen will be found to be firmly imbedded in a resistant fibrin clot which can be taken through the various reagents, imbedded in paraffine, sectioned in any desired plane and finally mounted on a slide. In brief, the clot containing the specimen may be treated as a regular piece of tissue of the same size.

The orientation of the specimen in the clot may be accomplished before the clot is fully formed or at the time when the clot is in the clearing fluid just prior to imbedding. In the clearing fluid the clot becomes transparent and can be examined under a microscope and the imbedded specimen located in it. The clot can then be trimmed so as to indicate the orientation of the specimen.

Plasma of various animals can be used. I have generally used frog plasma and have secured it by the method previously described.¹

Blood plasma has also been found to be useful in holding small animals firmly in a certain position so that they can be dissected. In this connection it may be noted that the dissection can proceed as far as desired and then no matter how fragile the dissected parts may be, additional plasma can be added and the fragile dissected part or parts imbedded in a clot in the same manner as described above. If desired, the clot can then be taken through the regular reagents and imbedded in paraffine for sectioning.

There is no doubt that blood plasma can be put to many other similar uses depending upon the nature of the problems and the ingenuity of the investigator. The securing and keeping of the plasma will not be found too difficult after the operation has been performed a few times.

¹ *Journal of Experimental Medicine*, 1915, xxi, 456.