in a loss of the majority of the bacilli which were collected in the first step by precipitation. (d) Slide losses are reduced by the use of milk as a fixative.

These findings, in extension of our previous work,[2] demonstrate again that tubercle bacilli cannot be collected efficiently by direct centrifugation. Although the urate sediments in the first stage are extremely bulky, they contain more bacilli per unit volume than the very slight sediments obtained by direct centrifugation. For purposes of guinea pig inoculation or of cultivation, it should be noted that the total bacillary content of the urate sediments exceeds those from centrifugation by approximately one hundred times.

To wash, or to dissolve and recentrifuge a sediment, once collected by any method, results in a marked diminution of the numbers of bacilli collected.

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Attempted Transformation of Rabbit-Fibroma Virus into the Virus of Infectious Myxoma.*

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The following experiments were undertaken in an attempt to duplicate the results obtained by Berry and Dedrick,[1] who reported the transformation of fibroma-virus into that of infectious myxoma. The method of procedure followed throughout the experiments was identical with that of the original investigators[2] and consisted in inoculating domestic rabbits with a mixture of active fibroma-virus and heat-inactivated myxoma-virus.

The suspensions of viruses were prepared by grinding 10 to 20 g of virus-bearing tissue with alundum and 100 cc of Locke's solution. Five to 10 minutes before use, the suspension was centrifuged at about 1000rpm. Ten cc ampoules were filled completely with the

2 Hanks, J. H., Clark, H. F., and Feldman, H., *J. Lab. and Clin. Med.*, 1938, **23**, 736.

* The work reported in this paper was carried out under the direction of the late Earl B. McKinley.

1 Berry, G. P., and Dedrick, H. M., *J. Bact.*, 1936, **31**, 50; **32**, 356.

2 Berry, G. P., personal communication.

suspension of myxoma-tissue, sealed in flame, and immersed for a total period of 35 minutes in waterbaths kept at 60°C, 75°C, and 90°C.

Each experiment, run in duplicate, consisted of 3 groups of rabbits; one group as a control to show that the fibroma-suspension alone produced "orthodox" lesions, one group as a control to prove that the myxoma suspension had been completely inactivated, and one group in which transformation was to take place.

In the group used as a control for the fibroma-suspension, 2 rabbits were inoculated in the following sites with a fresh 10 to 20% fibroma suspension diluted with an equal volume of Locke's solution:

A. Rt. side, head end, 1 cc.
B. " " tail " 1 "
C., D., E. Left " 1 cc in each of 3 places.
T. Rt. testicle, 1 cc.

In the group used to check inactivation of the myxoma-suspension, 6 rabbits were inoculated, 2 with the suspension inactivated at 60°C, 2 with the suspension inactivated at 75°C, and 2 with the suspension inactivated at 90°C. The following injections were made on each rabbit:

4 subcutaneously, ventral surface, 1 cc each.
1 intratesticularly, 1 cc.
1 intraäbdominally, 10 cc.

At intervals of 5 to 15 days, the inoculated testicles were removed, emulsified, and the suspension was injected into the testicles of a second group of 6 rabbits which in addition had received subcutaneous and intraäbdominal injections of the heat-inactivated material. This procedure was again repeated with a third set of rabbits.

In the group in which transformation was to take place, 6 rabbits were used. Two of these received the following injections:

A. Rt. side, head end, 1 cc of a 10 to 20% fibroma-suspension diluted with an equal volume of Locke's solution.
B. Rt. side, tail end, 1 cc of a 10 to 20% myxoma-suspension inactivated at 60°C, diluted with an equal volume of Locke's solution.
C., D., E. Left side, 3 cc of a 10 to 20% fibroma-suspension mixed with an equal volume of 10 to 20% myxoma-suspension inactivated at 60°C.
F. Rt. testicle, 1 cc of solution used in C, D, and E.

Two other rabbits received the same injections with the exception that the myxoma-suspension was inactivated at 75°C, and the last 2 received myxoma-suspension inactivated at 90°C. This experiment was carried out 3 separate times, and in each series the rabbits were observed for a period of at least one month. At no time has any of the animals in the control group for the inactivated myxoma-

suspension shown any evidence of myxoma. The fibroma-control groups have always been positive, and there has been no indication of myxoma in any of the animals in the "transformation" group.

We next tried a variation in pH, adjusting the inactivated myxoma to pH 9.4. Since earlier workers failed to get any transformation using myxoma inactivated at 90°C, the remaining experiments utilized only suspensions inactivated at 60°C and 75°C. The fibroma-control groups were positive; the groups inoculated only with inactivated myxoma-suspensions of pH 9.4 were negative, as were those in the so-called "transformation" group. Three animals of the myxoma-control group were later inoculated with a living suspension of myxoma and died within the customary period. A shift of pH to 3.8 was next tried and, as before, with negative results.

In a final set of experiments, to the inactivated myxoma-suspensions were added respectively, normal horse serum, trypsin, and a suspension of killed staphylococci. The normal horse serum was used undiluted. The trypsin-solution was made up with 1.4 g of trypsin per gram of myxoma-tissue and the mixture digested 48 hours. As a control normal rabbit tissue was also digested with trypsin. The staphylococcal suspension was heated for 35 minutes at 75°C.

These experiments were set up as before with the exception of the addition of the various substances named above to the inactivated myxoma-suspensions. Two rabbits were also inoculated with untreated myxoma-suspension inactivated at 60°C and 2 with untreated myxoma-suspension inactivated at 75°C. The fibroma-control group was positive, both the treated-inactivated and the untreated-inactivated-control groups were negative. Nor was there any indication of myxoma among the rabbits inoculated with the combined treated-inactivated myxoma- and fibroma-suspensions.

These results seem conclusively to show that the fibroma-virus (Shope) with which we were working could not be made to exhibit activity characteristic of the virus of myxoma by inoculating normal domestic rabbits with a mixture of active fibroma-virus and heat-inactivated myxoma-virus (Hyde-Moses). Hyde[3] also attempted to activate heated myxoma-virus by the addition of fresh fibroma-virus, but without success. In his words we are "at a loss to account for the difference in the results" between our findings and those of Berry and Dedrick,[1] which were confirmed by Hurst,[4] who states: "This remarkable observation was confirmed at the first time." It

[3] Hyde, R. R., *Am. J. Hyg.*, 1936, **24**, 217.

[4] Hurst, E. W., *Brit. J. Exp. Path.*, 1937, **18**, 23.

is not thought that the strain, age, or diet of the rabbits used can be the factor involved, for in the controls typical myxoma and fibroma developed. Fibroma-virus derives from Shope and in America myxoma-virus traces through Hyde or Rivers to Moses in South America. While originally identical it is likely there is a difference of many passages between the viruses used by Berry and Dedrick, Hyde, Hurst, and ourselves. It is possible that different viral strains or even the same strains kept in different laboratories may vary in their capacities to undergo alterations and that this may be the explanation for the contradictory findings on fibroma-myxoma inter-relationships.

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Decomposition of Sodium Mucate by *Aerobacter cloacae*.

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Brown, Duncan, and Henry[1] stated that the sodium salt of mucic acid is apparently not decomposed by *Aërobacter cloacæ*. Thus this singular property served to aid in the separation of this species of organism from the rest of the coliform group. In their study of the fermentation of salts of organic acids by various bacteria, a 1% peptone broth containing 1% of sodium mucate was used. To detect decomposition of sodium mucate, a solution consisting of 0.4 cc of glacial acetic acid and 0.6 cc saturated lead acetate was added to 5 cc of the culture. If the mucate was unaltered, a dense turbidity resulted which ultimately settled down in a small precipitate; but if decomposition of the salt had occurred, no precipitate followed upon the addition of the lead-acetate solution. However, they noted that on decomposition of mucate by other bacteria, sodium bicarbonate was one of the end-products formed. Thus, on the addition of lead acetate, a small precipitate of lead carbonate was formed. To overcome this disadvantage, a small amount of acetic acid (see above) was necessarily added to dissolve the lead carbonate and prevent false reactions. This is a tedious procedure.

In the author's study[2] on the decomposition of organic acids by

[1] Brown, H. C., Duncan, J. T., and Henry, T. A., *J. Hyg.*, 1924, **23**, 1.

[2] Hajna, A. A., *J. Bact.*, 1935, **29**, 253.