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A virus, probably of rabbit origin, encountered during intratesticular transmission experiments.

By C. H. ANDREWES and C. PHILIP MILLER, Jr.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

As inoculation into rabbit testicles has been found to be a useful method for the propagation of certain viruses, this procedure was employed in an attempt to transmit a hypothetical virus of rheumatic fever to animals. Whole blood obtained from patients during the acute stage of the disease was injected into the testicles of rabbits. These were removed aseptically after 4 days, ground with sand and Locke's solution, and centrifuged at low speed for 2 minutes; 1 to 2 cc. of the supernatant fluid was injected intratesticularly into other rabbits. In 7 series transfers were carried on in this manner every fifth day for five or more generations. The rabbits used in the first few generations of each series had been previously injected subcutaneously with benzol in order to lower their resistance; later, normal rabbits were employed. Aerobic and anaerobic cultures of the testicles made at each transfer were almost invariably sterile; and dark field examination revealed no spirochetes.

In three series the rabbits showed evidences of an infection which appeared between the third and seventh transfer. This infection was manifested clinically by fever and by swelling and congestion of the testicles. Microscopic examination of sections of these testicles stained with eosin and methylene blue showed profuse interstitial accumulations of endothelial cells, lymphocytes and polymorphs in varying proportions; also marked disturbance of spermatogenesis. A characteristic feature was the presence of pink-staining bodies within the nuclei of large cells in the interstitial spaces. These bodies were indistinguishable from the so-called nuclear inclusion bodies found in the lesions produced by the herpetic and other ultramicroscopic viruses.

What appeared to be a virus could thus be propagated indefinately in either benzolized or normal rabbits. Intradermal inoc-

ulations of testicular emulsions produced a slightly elevated erythematous lesion after 5 or 6 days. Intrathoracic inoculations near the heart led to an acute fibrinous pericarditis and a localized myocarditis. Nuclear inclusion bodies were found in the lesions of the skin, pericardium, and myocardium.

The serum of rabbits inoculated 14 days previously was found to neutralize the virus *in vitro* while that of normal rabbits and of patients convalescent from rheumatic fever failed to neutralize it. The virus could be preserved in 50 per cent glycerine for 20 days and for at least 5 days after freezing and drying.

The technique employed was the same as that described by Rivers and Tillett[1] in their studies on Varicella. The apparent identity of the clinical and histo-pathological pictures in our rabbits with that produced by the virus isolated by them suggested the possible identity of the two viruses. With their coöperation, therefore, we conducted a series of cross immunity experiments. It was then found that rabbits inoculated with one virus were subsequently immune to the other, and that sera of rabbits immune to one virus would neutralize the other *in vitro*. We were, therefore, forced to the conclusion that we were dealing with the same virus, which almost certainly bore no relationship to rheumatic fever.

In order to determine whether the virus was of rabbit origin, the same technique was employed with six control series of rabbits in which normal rabbit blood was used as the original inoculum. Since Rivers and Tillett[2] had encountered evidence suggesting that the infection might be contagious amongst rabbits, every possible precaution was taken to prevent contamination of this control stock with the virus previously studied. All infected animals were killed, the cages sterilized, and the premises thoroughly cleaned with disinfectants. Three of the control series received benzene subcutaneously; three did not. What was apparently the same infection appeared in two series, after the third and fourth transfers respectively. One positive series had received benzene, while the other had not. The identity of this virus with that previously encountered has been established by finding similar nuclear inclusions and by cross immunity tests.

It thus seems highly probable that the lesions induced by suc-

[1] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1923, xxxviii, 673.

[2] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1924, xxxix, 777.

cessive transfers of rabbit testicles originally inoculated with blood from rheumatic fever patients, and from normal rabbits are identical; and that the virus inciting these lesions is of rabbit origin. Its identity with the virus discovered by Rivers and Tillett seems also to be established; these authors on other grounds have already suggested a possible rabbit origin.[1, 2] These facts must be kept in mind in undertaking any future work in which this technique is used, as well as in interpreting past work in which the method of testicular transmission of the so called filtrable viruses has been employed.

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The prevention and cure of rickets by means of bile.

By ROBERT KAPSINOW and DEBORAH JACKSON.*

[*From the Department of Surgery and the Department of Pediatrics, Yale Medical School, New Haven, Conn.*]

Since it has now become well established that cod liver oil prevents and cures rickets, the question naturally rises whether products of mammalian liver, in particular, bile, might not exert a similar action. The bile used was freshly obtained gall bladder bile from the pig. The animals used in the experiments as test subjects for the effects of bile were rats about 32 days old. The rats were divided into two groups.

In group I bile was added to the diet No. 3143 used by McCollum, Simmonds, Shipley, and Park[1] for the production of rickets in the rat.

The diet with the bile added was then fed for a period of 35 to 36 days with the object of determining whether the bile would prevent the development of rickets. In group II the rickets producing diet without the addition of bile was fed for 35 days, by which time, as is now well known, well marked rickets develops in the rat. At the end of that time bile was added to the diet

* Introduced by Edwards A. Park.

[1] McCollum, E. V., Simmonds, Nina, Shipley, P. G., Park, E. A., *J. Biol. Chem.*, 1921, xlvii, 507.