ment-fixation reaction which is almost always associated with syphilis. If it is a specific antibody, its absence in all cases of active rheumatoid spondylitis without peripheral joint involvement and in psoriatic arthritis is suggestive of different etiologies. The modified test, by eliminating the natural amboceptor and by more closely reflecting the titer of the rheumatoid arthritis factor, is more sensitive than the original hemagglutination test. A comparison of the 3 tests employed indicates that a significantly higher incidence of positive reactions can be obtained by this procedure.

Summary. 1. The hemaggutination test for rheumatoid arthritis is modified by the selective absorption of non-specific factors. The details of this modification are described.

2. Of 39 cases with active peripheral rheumatoid arthritis with x-ray evidence of joint changes, 35 were positive by this test.

3. All cases of inactive peripheral rheumatoid arthritis, rheumatoid spondylitis without peripheral joint involvement and psoriatic arthritis tested were negative.

4. In presumptive cases of early rheumatoid arthritis without x-ray evidence of joint changes, the test was negative.

5. In 67 control cases, including a variety of non-rheumatoid arthropathies, the test was negative except in two cases of infectious hepatitis.

6. The modified test was positive in 90% of the proven cases of active peripheral rheumatoid arthritis tested; the Rose test in 61%; the streptococcus agglutination test in 58%. In all cases in which the modified test was negative, the Rose test and the streptococcus agglutination test were also negative.

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Methyl Alcohol Purification of the Rabbit Papilloma Virus.*† (17421)

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Rabbit papillomatosis is an endemic virus disease of cottontail rabbits readily transmissible to domestic rabbits. Serum from infected cottontail and domestic rabbits contains antibody which is capable of neutralizing the virus *in vitro*.[1] The virus can be obtained easily from cottontail rabbit papillomas, but ordinarily it is not recoverable from domestic rabbit growths.

The demonstration that extravasated fluid from large confluent cottontail papillomatous growths was effective in neutralizing active virus led Kidd[2] to suggest that the neutralizing principle represented antibody derived through escape from altered capillaries. Kidd[2] found no evidence of such "masking" in naturally occurring papillomas of cottontail rabbits.

When Kidd[2] obtained no virus from the growths of 8 of 11 domestic rabbits and little from the other 3, and found that the sera of the 11 rabbits had little or no neutralizing capacity, he concluded that it was improbable that antibody was primarily responsible for the masking of the virus in the growths produced in domestic rabbits.

The method developed by Cox, van der Scheer, Aiston and Bohnel[3] for the purification and concentration of influenza and other

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1 Shope, R. E., and Hurst, E. W., *J. Exp. Med.*, 1933, **58**, 607.

2 Kidd, J. G., *J. Exp. Med.*, 1939, **70**, 583.

viruses by employing organic solvents at low temperatures to precipitate virus proteins led us to explore this technic in our efforts to characterize the agent responsible for the masking of the papilloma virus. Accordingly, experiments were designed to isolate the virus of infectious rabbit papillomatosis by precipitation (a) from crude tissue suspensions derived from naturally occurring cottontail papillomas, (b) from a suspension of virus artificially "masked" as the result of specific neutralization *in vitro*, and (c) from tissue suspensions derived from virus-induced domestic rabbit papillomas.

It is the purpose of this paper to report the results of our findings.

Methyl Alcohol Purification of Cottontail Papilloma Tissue. Four grams of cottontail papilloma tissue were ground with sand in a mortar to yield a 20% suspension in saline. After centrifugation in an angle centrifuge at 5,000 r.p.m. for thirty minutes at 4°C, the supernatant fluid was withdrawn. Ten ml of ether were added to 20 ml of the supernatant material. The mixture was shaken and centrifuged at 5,000 r.p.m. for 30 minutes at 4°C. After withdrawal of the lipid layer the remaining ether was removed *in vacuo*. The suspension was frozen rapidly at -20°C, thawed at room temperature, and centrifuged at 2,500 r.p.m. for 30 minutes at 4°C. The supernatant was drawn off, diluted to 200 ml with 0.1 M phosphate buffer, pH 7, cooled in a dry ice bath, and absolute methyl alcohol at –40°C was added drop by drop by means of a capillary pipette with constant stirring until the alcohol concentration reached 25%. The suspension was then kept in an ice bath for one hour at –2°C to allow time for the formation of the precipitate. The material was centrifuged at 5,000 r.p.m. for 30 minutes at 4°C. The alcohol was decanted and the precipitate washed with .02 M phosphate buffer, pH 5.1, to remove more non-viral protein. The addition of 4 ml of phosphate buffer, pH 7.0, to the resultant precipitate made available papilloma virus in a suspension of which 1 ml was equivalent to 1 gm of tissue. After elution for 15 minutes, the suspension was frozen, thawed, and centrifuged at 2,500 r.p.m. for 30 minutes. The final water-clear supernatant fluid was kept in storage at –20°C until used.

Tests for the Presence of Virus in Purified Material. The purified viral suspension was diluted so as to have 8 successive two-fold dilutions ranging from 1-10 to 1-5120 to test for infectivity by transfer to domestic rabbits. Each dilution was inoculated in triplicate by the application of 0.2 ml by crosshatch scarification to previously shaved areas of skin on 3 rabbits. The results as observed 4 weeks later are recorded in Table I.

For control purposes to learn the infectious titer of the papillomatous tissue which was employed for methanol precipitative purification, infectivity tests in triplicate of the non-purified virus suspension in dilutions of from 1-10 to 1-6400 were carried out. Papillomatous growths were present 4 weeks later at all sites of inoculation.

It appears from these results that virus was lost in the process of purification, since the limiting dilution for infectivity of the purified virus was found to be 1-2560, whereas an end point was reached for the control material in a dilution of 1-6400.

These findings were established when the same technic for purification, concentration and biologic testing of 5 additional samples of cottontail papilloma tissue that had been collected between 1938 and 1944 yielded similar results. In each instance the purified virus suspension produced growths in dilutions through 1-2560 and the control virus suspensions yielded tumors in slightly higher dilutions, in most cases 1-6400.

Methyl Alcohol Purification of Neutralized Papilloma Virus. Cottontail papilloma tissue that had been stored in 50% glycerin at 4°C was ground to a fine paste in a mortar, and saline was added to make a 20% suspension. This was centrifuged at 2,000 r.p.m. for 20 minutes, and the supernatant material removed. Blood was drawn by cardiac puncture from a domestic rabbit which had borne several large papillomatous growths for about 3 months, and the serum was separated by centrifugation. Ten ml of serum was added to

[3] Cox, H. R., van der Scheer, J., Aiston, S., and Bolinel, E., *J. Immunol.*, 1947, **56**, 149.

TABLE I.
Growths Produced with Purified Virus.

Rabbit	Reciprocal of dilution							
	10	40	160	320	640	1280	2560	5120
1	+	+	+	+	+	+	+	—
2	+	+	+	+	+	+	+	—
3	+	+	+	+	+	+	+	—

(+) Visible papillomatous growth.
(—) No visible growth.

an equal volume of the 20% viral suspension. The mixture was incubated at 37°C for one hour followed by refrigeration at 4°C overnight. For control purposes a 20% suspension of unneutralized virus was treated in the same manner. Following the incubation and refrigeration, 20 ml of the neutralized virus were subjected to the methanol purification procedure described previously. Four ml of phosphate buffer, pH 7.0, was added to the final precipitate. This suspension was allowed to elute for 15 minutes, frozen, thawed, and centrifuged at 2,500 r.p.m. for 30 minutes. The final supernatant represented the test inoculum. Three normal rabbits were prepared for inoculation by shaving and lightly scarifying 3 dermal areas. Each rabbit was then inoculated by cross-hatch scarification with the methyl alcohol purified neutralized virus in 0.2 ml amounts, and with control suspensions of neutralized virus and of crude virus. The control suspension had been carried through the same incubation and refrigeration procedures as the methyl alcohol purified material. After an incubation period of 5 weeks the rabbits were examined for signs of papillomatous growth. No growth appeared on any of the areas inoculated with the neutralized virus or with the methyl alcohol purified neutralized virus. Control inoculations of virus alone resulted in confluent papillomatosis.

The results indicated that methanol purification was ineffective for the separation of infective virus from the virus-antibody combination, since no visible papillomatous growths appeared in 6 rabbits after inoculation with the purified material. Three additional experiments failed to show that the virus could be extracted from the combination.

Methyl Alcohol Purification of Domestic Rabbit Papilloma Tissue. Papilloma tissue was removed surgically from pure-bred Dutch domestic rabbits which had borne the growths for 6 weeks or more. The tissue was triturated with sand in saline to make a 20% suspension. This material was centrifuged at 5,000 r.p.m. for 30 minutes at 4°C. The supernatant material was drawn off and subjected to the same methyl alcohol purification procedure as was used on the cottontail papilloma tissue. A precipitate was obtained similar to that obtained with the purified cottontail papilloma tissue. Four areas on the ears and flanks of 4 normal pure-bred Dutch rabbits were inoculated by scarification with the purified material. The rabbits were examined for signs of growth at weekly intervals following the inoculation.

When no growths resulted on any of the rabbits inoculated over a 12-week period, it was concluded that the domestic papillomatous growths following treatment by the methanol technic for purification yielded insufficient active virus to cause visible papillomatous growths. Further attempts were made to extract virus from 4 samples of domestic papilloma tissue from rabbits which had borne the growths from 3 to 15 weeks. In no case was any demonstrable virus obtained.

Summary. The methanol precipitative technic of Cox, *et al.*, when applied to the purification and concentration of papilloma virus from naturally occurring cottontail rabbit papillomas, domestic rabbit papillomas and neutral virus-antibody mixtures readily yielded active papilloma virus from the cottontail papillomas but it did not yield virus from either domestic rabbit papillomas or from papilloma virus and its antibody in neutral mixture.

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