

PRELIMINARY AND SHORT REPORT

CONTAMINANT FILTERABLE AGENT DERIVED FROM A HUMAN WART*

BENJAMIN V. SEGEL, PH.D.

Bivins (1) has reported the isolation of an agent from the single primary wart of a series of 17 warts which had developed on his right hand during a period of about 2 years. He accomplished this by inoculating a broth suspension of the homogenized verrucous material onto the chorio-allantoic membrane (CAM) of 10-day-old chick embryos. Whitish "pearl" growths appeared on the CAM after several days incubation, and he was able then to pass this agent serially in eggs with material filtered through a Berkefeld V candle. An eleventh egg passage of this agent received from Bivins has been serially egg-passed 18 times in this laboratory.

Because previous reports (2) and our own experience (3) indicated that the developing chick embryo does not provide a favorable medium for culturing human wart virus, it seemed of interest to investigate this agent further. The evidence presented in this report strongly indicates that this agent, thought to have been derived from *verruca vulgaris*, is a tripathogenic strain of avian-pox virus, most likely of canary-pox origin.

Chicken pullets, which had been inoculated with virus suspension by means of a stiff-bristled brush on scarified skin and comb, evinced discrete pock lesions (Fig. 1) in 5 to 7 days followed by rapid healing within 1 to 2 weeks after their first appearance. Suspicion that this might be fowl-pox virus was negated by the mildness and transiency of the lesion, the absence of typical intracytoplasmic inclusions (Bollinger bodies) in the lesions of the CAM and chicken skin and comb, and the failure of the agent to afford cross-protection *in vivo* with a known fowl-pox virus strain obtained from Dr. Charles C. Randall in Dr. Goodpasture's Laboratory at Vanderbilt University. Similarly, preliminary neutralization tests with Bivins' virus chick antiserum and Bivins' virus and fowl-pox virus (Table I) indicated that these two were independent agents. The virus was additionally inoculated into the scarified skin of pigeons, canaries, turkeys, quail, and pheasants. Of these inoculations typical pocks were produced in canaries and turkeys. In the case of the turkey, as with the chicken, the lesions were nonspecific, no inclusion bodies being seen in any of the histological sections. However, in the canary lesions the proliferating epithelium contained intracytoplasmic inclusions (Fig. 2), very similar to those described by Burnet (4) in the lesions produced by the Kikuth canary-pox virus (5). The virus was likewise lethal to the canary, the predominant post-mortem pathological feature being a marked hyperplasia of bronchial epithelium with the formation of numerous cytoplasmic inclusions.

Since this agent presumably was of dermal origin and produced readily detectable focal lesions on the CAM of chick embryos, the first attempts at identification were by way of association with one of the human dermatropic viruses which are known to produce membrane pocks, namely, variola, vaccinia, and herpes simplex. A 6 lb. male rhesus monkey was inoculated intradermally with 0.2 ml. of a membrane suspension of virus in the cheek, chest, and prepuce. The monkey inoculation had a twofold purpose, one as a test for variola and secondly because of a report of a successful transfer of human wart material to monkey prepuce (6). The absence of any lesions subsequently eliminated the possibilities of variola, and further observations over a period of 8 months revealed no takes in the prepuce or other areas inoculated. To test for the likelihood of vaccinia, virus was inoculated both intracerebrally and intraperitoneally into 1-day-old Swiss albino mice. No effect on the suckling mice was observable. A similar passage of brain material harvested on the fifth day was likewise

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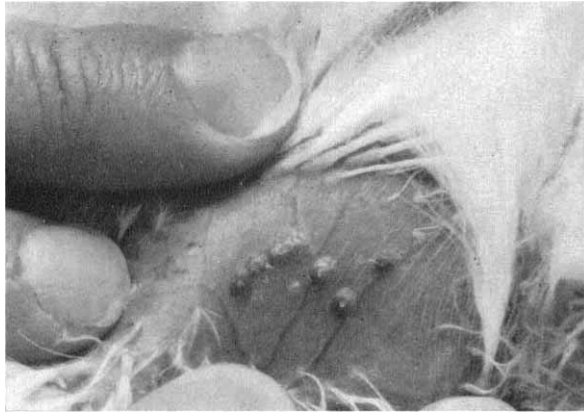


FIG. 1. Typical lesions produced by inoculation of virus in scarified skin of 2-month-old chicken. Infection initiated 10 days previously shows crusting in early healing process.

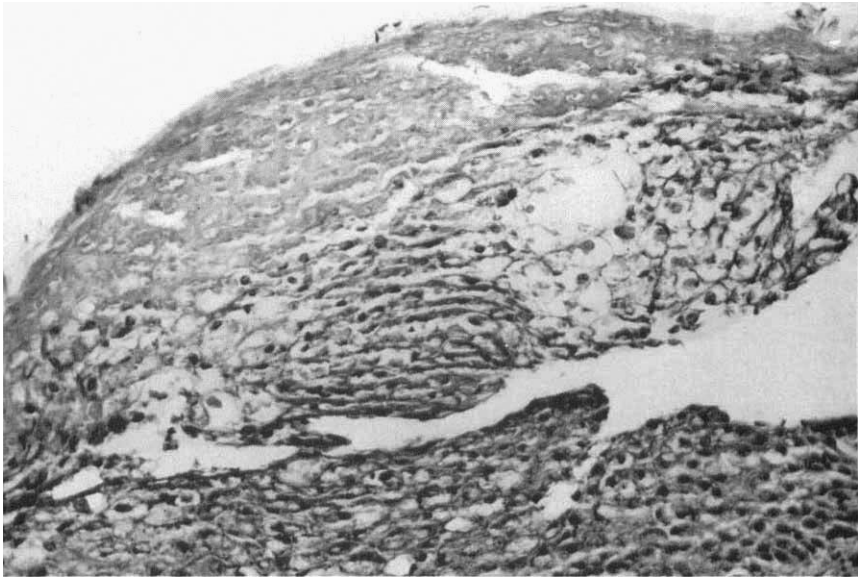


FIG. 2. Skin lesion in the canary showing intracytoplasmic inclusions in proliferating epithelial cells. Stained with hematoxylin and eosin. $\times 380$.

TABLE I

Neutralization tests with Bivins' virus and fowl-pox virus against Bivins' virus chick antiserum

Virus	Normal chick serum	Immune chick serum
Bivins.....	$2.7 \times 10^{6*}$	8.8×10^5
Bivins.....	1.1×10^6	4.4×10^5
Fowl-pox.....	4.2×10^6	2.6×10^6

* Infectivity titer

negative. Vaccinia was further ruled out by inability of the agent to agglutinate "susceptible" chick erythrocytes (7, 8) and by failure of a high titer vaccinia rabbit antiserum to neutralize the virus in egg tests. Intracerebral inoculation of the virus into 4-week-old mice and 3 subsequent serial brain passages were all uneventful, and this together with the failure of a high titer herpes simplex rabbit antiserum to neutralize the virus eliminated the herpes virus from consideration. Human gamma globulin neutralization tests were likewise negative.

Among the animal viruses producing poeks on the CAM, mouse ectromelia was ruled out by negative findings with mouse inoculations and chick cell hemagglutination; while negative findings following intradermal, subcutaneous, and scarified skin inoculations of rabbits contraindicated infectious myxomatosis. Failure to induce infection in mice and rabbits ruled out vesicular stomatitis. In addition, as part of a broad host range study, administration of the virus into the scarified skin of 4-week and 4-month-old rats, hamsters, and guinea pigs produced no lesions beyond healing.

That the virus could cause a cutaneous papilloma in man had still to be proven, and such an experiment was carried out in collaboration with Dr. Clinton Beirne. On November 3, 1955, 4 young male adults were inoculated intraepidermally in the flexor surface of the right upper arm with 0.2 ml. of a CAM suspension of the virus, while 0.2 ml. of a normal CAM suspension was inoculated into the left arm for control. In all this time, no reactions of any kind have been noted.

SUMMARY

In summary, the observations to date make it appear that this virus may have arisen as a laboratory contaminant. Its induction of a disease in chickens, turkeys, and canaries characterized by papular cutaneous eruptions, and the presence of intracytoplasmic inclusions in parasitized cells of the canary suggest the classification of this agent as a tripathogenic strain variant of canary-pox virus (9).

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