

Recent work suggests that PHA-P may function as an antigen<sup>5,6</sup>. Cells are known to divide in the presence of antigen *in vivo*<sup>7</sup>. PHA is not unique in its ability to induce cell division in tissue culture. A wide variety of heterologous substances have been demonstrated to stimulate mitosis *in vitro*. PHA has several unusual features: ribonuclease inactivates it<sup>8</sup>. Its activity is associated with a leuco-agglutinating property or substance<sup>9</sup>. Primary immunization to PHA has not been proved necessary to leucocyte proliferation. The precise mechanism by which PHA stimulates cell division is still uncertain.

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## PATHOLOGY

### Establishment of a Turkey Flock Free of 'N' Strain *Mycoplasma*

*Mycoplasma* of the 'N' strain are frequently isolated from turkey poults affected with airsacculitis but free of *Mycoplasma gallisepticum*<sup>1</sup>. Research on the 'N' strain has been impeded by the presence of a high proportion of turkey poults with airsacculitis in all breeding flocks examined<sup>1,2,4,5</sup>, by the difficulty of culturing and identifying the *Mycoplasma* strains, and by the lack of a practical serologic test. This communication describes the establishment of a small nucleus of 'N'-free turkeys from a commercial breeding flock known to carry 'N' *Mycoplasma*. Hens and toms for the production of 'N'-free poults were selected from the parent flock by serology and culture.

An antigen was prepared from an 'N' *Mycoplasma* designated 'strain 529'. This strain was isolated from the sinus exudate of a turkey negative to *Mycoplasma gallisepticum*. Unlike other 'N'-type isolates which grew poorly and on agar medium only, this isolate grew readily in broth, facilitating antigen production.

Virgin female and male turkeys were screened serologically at 14 and 6 days prior to the first insemination. Swabs of the mucosa from the female oviduct taken by eversion of the cloaca, and semen from the males, were also collected for culture. In a test pen, 12 females diagnosed as negative by serology and culture were inseminated artificially with semen from males which were also negative. In a control pen, 19 females diagnosed as positive by serology or culture were mated to males which were positive.

Because a previous experience indicated that airsacculitis possibly caused by 'N' *Mycoplasma* was lowest in the poults from early hatches<sup>3</sup>, only the first hatches were considered. All 'dead-in-shell', piped eggs, cull poults and half the normal poults from the first hatch from each group were killed and dissected, and specimens taken for culture. Ten eggs candled out at 14 days, four cull poults, six piped eggs, and 21 normal poults were examined from the negative test pen. No evidence of airsacculitis was found in the poults, and all cultures were negative for *Mycoplasma*. The remaining 19 birds in the hatch were raised in isolation and serologically tested at frequent

intervals with 'strain 529' antigen. At 27 weeks of age, and after 11 separate bleedings, nine females and six males were negative. The four birds which died or were culled from this group were free of airsacculitis and 'N' *Mycoplasma* as diagnosed by examination and culture.

The culture of ten eggs candled out at 14 days incubation from the positive control pen revealed one isolation of an 'N' strain *Mycoplasma*. Four cull poults examined were found negative for airsacculitis and *Mycoplasma* on culture. Twenty 1-day-old poults were examined; one had airsacculitis from which 'N' *Mycoplasma* was cultured. Of 17 additional poults held for further investigation, one of nine that died within a 21-day period had airsacculitis from which *Mycoplasma* was recovered. The remaining 8 poults killed at 21 days of age were all negative for airsacculitis and *Mycoplasma* of the 'N' strain.

The fertility of the eggs in the second hatch from the negative test females was compared with that of the positive controls. The percentage of eggs candled out as infertile at 12 days incubation was 4.5 for those laid by the negative hens and 13.2 for those from the positive hens. In 'break out' examination of these, 1/11 from the negative hens was considered truly infertile, compared with 12/37 from the positive group. The remaining eggs were considered to be cases of early embryonic death.

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### Inhibition of a Transplantable Murine Leukæmia by a Lymphocytopenic Virus

IN our laboratory the isolation of a viral agent with lymphocytopenic action *in vivo* directed our attention to its possible anti-leukæmic action. Anti-tumour properties of this virus are briefly described herein.

We have described the physical and chemical properties and the pathology of this lymphocytopenic virus which was isolated from a non-tumorigenic tissue culture line of Ehrlich's ascites carcinoma<sup>1</sup>. Parenteral introduction of this virus into adult mice of Swiss and several inbred strains (*C<sub>3</sub>H*, *DBA/1*, *A/He*, *C<sub>57</sub>Bl/6* and *Balb/c*) produces an acute infection within 7-10 days, involving primarily a reduction of lymphocytes in lymph node, thymus and spleen, secondarily some fatty degeneration of the liver, and is accompanied by the development of small amounts of ascitic fluid. The most susceptible Swiss strain usually develops both ascitic and pleural fluids and was chosen for routine passage of this virus in its 28th passage on August 18, 1964. The transplantable lymphoid leukæmia<sup>2</sup> in its 166th passage, on August 18, 1964, was used as a source of leukæmic cells. This lymphoid leukæmia was originally induced by 3,4,9,10-dibenzpyrene treatment to *Balb/c* mice in 1960. The concentration of leukæmic cells was determined with a Coulter Counter, followed by serial dilution with sterile phosphate-buffered saline (pH 7.4) to reach the desired number of cells in a 0.25-ml. inoculum.

Each experimental group was concurrently injected with a standard dose of the lymphocytopenic virus. A standard dose of lymphocytopenic virus was arbitrarily set as 0.25 ml. of a filtrate obtained from a 1:5 dilution of liver homogenate with phosphate-buffered saline (pH 7.4) obtained on the 7th day of infection in Swiss mice. Experimental mice were compared with control groups