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SEMI-ANNUAL PROGRESS REPORT

Report Prepared By: Dr. M. Michael Sigel Date: February 15, 1953

FILE 130-706

For Period: 7/1/52 to
12/31/52

CONTRACT: N8onr-72601

PRESENT ANNUAL RATE:

CONTRACTOR: The Children's Hospital of Philadelphia

PRINCIPAL INVESTIGATOR: M. Michael Sigel, Ph. D.

Assistants: Ralph Pollikoff, Graduate Student
Alan Bernstein, Graduate Student

TITLE OF PROJECT: "Studies on the Psittacosis-Lymphogranuloma
Venereum Group"

- Objectives:
1. To search for a specific complement fixing antigen. At present, there is no satisfactory serologic procedure available for differentiation between infections caused by members of this group.
 2. To study mode of growth and multiplication and other biologic characteristics of this group of agents. These studies may have a bearing on the problem listed under (1). Furthermore, knowledge obtained from these studies may help define the position of these agents in the microbial world.
 3. To study the effect of therapeutic agents

(antibiotics) on these viruses and on the diseases caused by them.

Abstract (or Summary) of Results:

Since Start of Project:

Work on Biological Properties of the Psittacosis-Lymphogranuloma Venereum Group.

1. One phase of this study was completed. It may be summarized as follows:

a. The pattern of growth of meningopneumonitis virus in vitro seemed to be similar to that occurring in ovo and thus the initial stages of development, the adsorption and the latent periods, were investigated by the use of tissue culture procedures.

b. The initial increment of infectivity in allantoic membrane suspensions following virus inoculation in ovo was due to prolonged adsorption of virus and not to immediate virus reproduction. The length of the adsorption period varied with the virus dilution employed.

c. The reduction of virus titer in allantoic membrane suspensions subsequent to adsorption was due to a change of infectious virus to a non-infectious form and this seemed to be a part of the normal developmental cycle of the virus.

2. In connection with the studies on cultivation of MP virus in cancer cells, the following observations were made:

a. Krebs ascites tumor cells survived longer in complete tissue culture (Tyrode, + human serum + chick embryo extract) than in Simm's fluid.

b. MP virus was found to inhibit or prevent the development of this tumor in mice.

c. We have as yet no definite evidence, however, that MP virus multiplies in the tumor cells. Studies aimed at elucidation of this point are now in progress, including attempts at breaking up tumor cells.

d. A new type of tissue disintegrator (manufactured by H. Mickle Company, Hampton, Middx., England) was found to break up all tumor cells in about 30 minutes without producing any decrease in the infectivity of MP virus.

Effect of Therapeutic Agents on These Viruses.

1. Work with lymphogranuloma venereum patients was moving very slowly because of the difficulties enumerated in the last report (see Supplemental Report).

2. One phase of the studies with antibiotics in chick embryo infected with MP virus was completed and may be summarized as follows:

a. Data indicated that the drug had no in vitro effect on the virus particle itself -- that is, aureomycin was not capable of

altering the extra-cellular virus.

b. The drug appears to affect virus multiplication by causing an extension of the "latent" period (see work on Biological Properties, 1a and 1c).

c. It was found that complete inhibition of growth during the time interval corresponding to the first cycle of growth occurred only if aureomycin was administered during the first 6-8 hours of virus growth. This would seem to indicate that after this time virus synthesis had passed beyond the process or stage in development which could be blocked by the drug.

d. The chief role of the antibiotic appeared to be one of virostatics, for the virus was able to resume its growth process when a critical, low level of the drug in the allantoic membrane was reached.

e. In connection with prolonged survival of treated embryos, it is especially interesting that virus was not found in the brains of treated embryos up to at least 192 hours after inoculation of virus. This is in contrast with the findings in allantoic membranes and livers of such embryos; these organs showed virus at 120 and 144 hours, respectively. (In untreated controls, virus appeared in membranes at 24 hours, in the liver at 48 hours and in the brain at 72 hours.)

B. During Current Report Period:

Work on Biological Properties of the Psittacosis-Lymphogranuloma
Venerum Group.

Continued studies with the Meningopneumonitis virus and Krebs-2 ascites tumor cells showed that the virus could prevent or inhibit solid tumors initiated by depositing tumor cells under the skin of the mouse. It was found that suppression of tumor was of a lower order of magnitude when mice immune to MP virus were employed, indicating that inhibition of virus by the immune process of the mouse partially protected the tumor cells from the effect of the virus. Similarly, administration of aureomycin allowed for the development of tumors in mice despite inoculation of virus.

Preliminary experiments suggest that it is possible to quantitate the virus-tumor cell system as regards concentration of the two components and the time relationship of their activities. The enclosed photographs show the changes in the size of tumors following administration of virus at different time intervals.

Effect of Therapeutic Agents on These Viruses.

With the completion of the experiments on chick embryos, greater emphasis was placed on field trials in patients. A large scale study was therefore commenced in Jamaica where the incidence of lymphogranuloma venereum is relatively high. One of the phases of the study is an investigation of the comparative values of different antibiotics, including aureomycin, in the treatment of lymphogranuloma venereum. The initial activity has been described in a

separate outline. The several phases of this study are as follows:

I. Effect of different procedures of treatment on acute lymphogranuloma venereum.

V. D. Clinic patients.

Patients at University College of the West Indies Hospital.

II. The pathogenesis of lymphogranuloma venereum, especially in the woman and the frequency of inapparent infection.

Women at Jamaican Knitting Mills.

Workers at D & G Brewery at Kingston.

III. Age at which antibodies to psittacosis-lymphogranuloma venereum group of viruses first appear. (Are viruses of this group other than lymphogranuloma venereum present in Jamaica?)

IV. The importance of conjunctivitis in relation to lymphogranuloma venereum.

V. The effect of yaws and leprosy on the specificity of the complement fixation test for lymphogranuloma venereum.

VI. The effect of therapeutic procedures on chronic or "old" cases of lymphogranuloma venereum.

Work on Antigens (Search for a Specific Complement Fixing Antigen)

The continued studies on absorption of serum with various preparations of viruses of the psittacosis-lymphogranuloma venereum group and on various types of treatment of antigens of this group have yielded results which may be considered as indicating the presence of

a specific complement fixing component. The most efficacious absorption method for the revelation of specific complement fixation activity consisted of using heterologous, heated, unwashed virus. The monospecificity of the serum was determined by using homologous, unheated, washed virus. Potassium periodate brought out a specific complement fixing component in live virus. Experiments involving purification of virus and determination of susceptibility of crude and purified preparations to heat and KIO_4 , suggested that there are at least two group reactive components, one enhanced by heat and fairly resistant to periodate and the other not enhanced by heat but susceptible to periodate.

PLANS FOR THE FUTURE:

Some of the studies involving tumor cells will be continued on a reduced scale. The major emphasis will be placed on the field studies which have been commenced in Jamaica. These, of course, will entail certain laboratory evaluations which will be carried out at The Children's Hospital of Philadelphia and in Jamaica.

PUBLICATIONS:

Studies on the psittacosis-lymphogranuloma group. II. A non-infectious phase in virus development following adsorption to host tissue. A. J. Girardi, E. G. Allen and M. M. Sigel. J. Exp. Med., 96, 233, 1952.

Mr. Ralph Pollikoff, one of the Graduate Students working under the ONR Contract, was awarded the Ph.D. degree at the February 1953 Commencement at the University of Pennsylvania.



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