were substantially the same as those shown in Table II.

Comment. The observation of hemolysis of human cells by complement from the same individual, and the various conditions under which this reaction occurs, are of theoretical interest and suggest possible new approaches to the problem of complement's role in hemolysis. The complement fixation test itself may prove to be of practical value under some circumstances. There are certain advantages in a serological test which involves an homologous hemolytic system, instead of the 3 or more mammalian species which are represented in the usual complement fixation test. Moreover, the method makes it possible to do complement fixation tests under conditions where one or another of the usual reagents cannot be obtained.

There are certain limitations to the use of this method which should be pointed out. In Wassermann tests, the frequent finding of an anticomplementary effect of antigen presents a technical disadvantage. Moreover, the test is not feasible with antigens for which many human sera possess antibodies in low titer, such as influenza virus in allantoic fluid, since the complement employed for the test may be partially fixed due to the presence of antibody in the same serum. For the same reason, it is probable that certain tissue antigens, with which human sera may react non-specifically in low dilution cannot be tested by this method. However, in those systems which lend themselves to the homologous test, the results appear to be clear-cut and are reproducible.

Summary. Human erythrocytes undergo lysis in the presence of tannic acid and human complement, even when cells and complement are obtained from the same blood sample. This reaction is dependent upon an optimal concentration of 0.7% sodium chloride, and an optimal concentration of between 0.06 and 0.03% tannic acid. When these substances are present in proper concentration, human complement can be titrated with reproducible results.

Specific fixation of human complement by three separate antigen-antibody systems has been demonstrated, employing homologous erythrocytes sensitized by tannic acid. Antibody titers determined by this method were comparable to those obtained in the standard complement fixation test.

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Effect of Chloromycetin on Experimental Infection with Psittacosis and Lymphogranuloma Venereum Viruses.

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The results of the search for chemical and antibiotic substances which might be effective in the treatment of infections of viral and rickettsial origin have been reviewed recently by several groups of workers.[1-5] The contribution of Hamre and Rake[5] is of especial interest as regards the current subject since it deals particularly with viruses of the psittacosis-lymphogranuloma group. The pres-

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ent paper amplifies our earlier report[6] on the use of Chloromycetin[7] in experimental infections with psittacosis virus and summarizes our observations on the treatment of embryonated eggs and mice infected with the virus of lymphogranuloma venereum.

Materials and Methods. Virus material. The 6-BC and P-4 strains of psittacosis were employed in this work. The former, isolated in 1941 by Dr. K. F. Meyer from a parakeet, was obtained from Dr. E. Lennette as lyophilized yolk sac material; it is well adapted to growth in embryonated eggs and is lethal for mice when inoculated by either the intracerebral or the intraperitoneal route. The latter, isolated in 1942 from a sick pigeon,[8] also grows profusely in the yolk sacs of embryonated eggs but is lethal for mice only when inoculated intracerebrally. The L. A. strain of lymphogranuloma venereum used in the present experiments was obtained in 1943 from the inguinal gland of a patient;[9] it has been maintained since in this laboratory by passage in the yolk sacs of embryonated eggs or brains of mice.

Preparations of the 3 agents used in the present work consisted of 20% suspensions of infected yolk sac tissue triturated in sterile Difco skimmed milk media pH 7.2; these were cleared of large particles by light centrifugation and stored in 2.0 ml amounts in rubber stoppered tubes at −70°C. The infectivity of each of the stored suspensions was determined by titration in embryonated eggs inoculated by the yolk sac route. In addition, each suspension was titrated intracerebrally in mice, and the preparation containing the 6-BC strain was also titrated intraperitoneally in mice. Titers were calculated by the 50% end point method.[10]

Chemotherapy experiments in eggs. The procedure developed in this laboratory for testing the efficacy of drugs on the growth of rickettsial organisms in embryonated eggs[11] was followed in the current studies. In this technique, groups of 24 embryonated eggs which had been incubated for 7 days were inoculated by the yolk sac route with 0.1 or 0.2 ml of a solution containing the desired amount of drug; the control group received 0.2 ml of buffered saline. Then all the embryonated eggs were again injected into their yolk sacs with 0.2 ml of that dilution of standard virus suspension which would be expected to kill the majority of the embryos in the control group in 4 to 5 days. Usually 30 to 45 minutes elapsed from the time an embryo was treated with drug until it received the infectious inoculum. In certain tests therapy was delayed for 24 to 48 hours after infection. Eggs were kept from the time of setting and throughout the experiment in an incubator regulated to 37°C. Following inoculation they were candled daily and the time of death of the embryos recorded. Deaths occurring in the first 2 days were not counted in the results. The mean day of death of embryos in each group was calculated. An estimate of the efficacy of the drug was obtained by subtracting the mean value of the control group from that of the treated group; this gave a figure which was taken as the average prolongation of life of the treated eggs. The methods for statistical analysis of the present data were the same as those used previously.[11] Dr. Ross L. Gauld performed these calculations for us.

Chemotherapy experiments in mice. Swiss mice of the Bagg strain, weighing 14-18 g were used in the present experiments. Mice infected by the intracerebral or intraperitoneal route received 0.03 ml or 0.2 ml, respectively, of an appropriate dilution of standard frozen virus suspension. Chloromycetin was administered to mice by mouth or injected intra-

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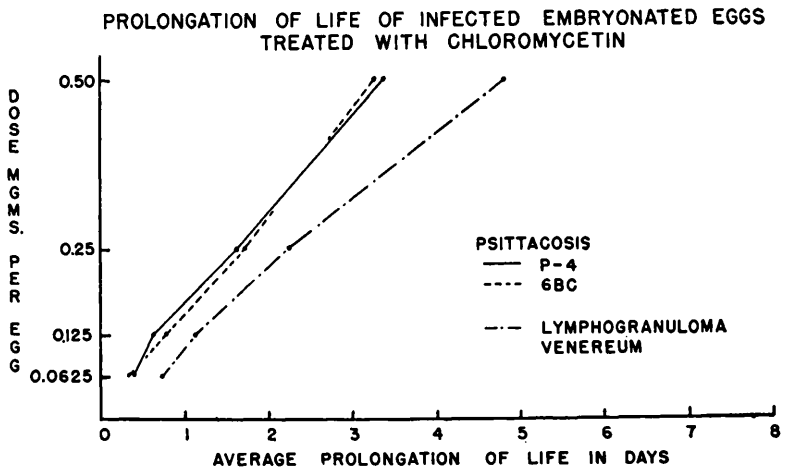
FIG. 1



peritoneally. When the former route was used, sufficient drug was dissolved in distilled water so that each 1.0 ml of solution contained ½ the desired daily dose for a mouse. Daily at 5:00 p. m. each group of 10 mice was offered 20 ml of such solutions in a drinking bottle. This amount was usually consumed by morning unless the mice were moribund. During the day the animals were offered tap water. For therapy by the intraperitoneal route sufficient drug was dissolved in physiological saline solution so that each 1.0 ml contained the desired daily dose; this was given each morning at 9:00 a. m.

The lethal titers of the virus suspensions were calculated by the 50% end point method[10] for each set of control mice, and where feasible, for the groups of drug-treated mice.

Results. Prolongation of life of infected embryos treated with varying doses of drug and at different times. Fig. 1 summarizes experiments in which doses of drug varying from 0.0625 mg to 0.5 mg were injected into the yolk sac just prior to infection with several hundred M.L.D.'s of one of the 3 agents. In this figure the average prolongation of life of the treated groups expressed in days is plotted against the amounts of drug given. The values for prolongation of life obtained in tests with doses of 0.0625 mg and above were statistically significant. It is apparent that a direct relationship exists between the amounts of drug employed and the prolongation of life of the treated embryos. Furthermore, the results obtained with the 2 strains of psittacosis virus are essentially identical. In this method of testing, lymphogranuloma virus would appear to be slightly more susceptible to chemoprophylaxis with the drug than psittacosis virus.

Chloromycetin has a chemotherapeutic effect in infected embryos even when treatment is delayed for 24 or 48 hours. The results obtained in tests with the P-4 strain of psittacosis are illustrated in Table I; these are typical of the data obtained with each of the 3 agents. The prolongation of life in the group treated 24 hours after infection was, in each instance, slightly greater than that obtained when treatment was given ½ hour before infection. While the values for these differences in some of the 5 experiments with the 3 agents were such as might have occurred by chance, the fact that they all trend in one direction is significant. In the experiment illustrated, and in those with the other strains, the effect induced by treatment given 48 hours after infection approached that recorded for the group treated just prior to infection. In another experiment embryos were infected with approximately 1,000,000 M.L.D.'s of the 6-BC virus. The average day of death in the control group in this test was 3.1 days. Even when injection of the drug was delayed for 48 hours there was still a significant prolongation of life of

TABLE I.
Chloromycetin in Experimental Psittacosis in Eggs (Infecting Dose 500 MLD's of P-4 Strain).

No. of eggs in group	Drug treatment: mg/egg	Drug treatment: Time given (in hours)	Mean day of death	Prolongation of life (days)
24	None		4.83	
24	0.5	½ Pre	8.13	3.30
24	0.5	24 Post	8.77	3.94
24	0.5	48 Post	7.64	2.81

TABLE II.
Chloromycetin in Experimental Psittacosis in Mice (Infecting Dose 80 MLD's of 6-BC Strain Intraperitoneally).

Route	Drug treatment: mg/day mouse	Drug treatment: Day begun	Dilution of infectious inoculum: 10-5	10-6	10-7	10-8	10-9	10-10
	None		10/10	7/10	8/10	6/10	2/10	0/10
I.P.	2.5	1 Post		1/10				
	2.5	3 "		1/10				
	2.5	6 "		1/10				
	1.5	1 "		0/10				
	1.5	3 "		2/10				
	1.5	6 "		1/10				
	0.75	1 "		0/10				
	0.75	3 "		4/10				
	0.75	6 "		7/10				
Fed	5.0	1 Pre		0/10				

TABLE III.
Chloromycetin in Mice Infected by Different Routes with 6-BC Strain of Psittacosis.

Exp.	Drug treatment: Route	Drug treatment: mg/day mouse	Drug treatment: Day begun	Route	Infectious inoculum Dilution: 10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10	Titer
		None		I.P. 0.2 cc	10/10	10/10	10/10	6/8	8/10	6/10	5/10	0/10	10-8.3
1a	I.P.	2.5	1 post		3/10	0/9	2/9	0/8	0/9				<10-3
	Fed	5.0	1 pre		5/10	2/10	2/10	0/8	0/10				10-3.4
		None		I.C. 0.03 cc		10/10	10/10	10/10	10/10	10/10	5/10	0/10	10-9.0
1b	I.P.	2.5	3 hours pre					10/10	10/10	10/10	4/10		10-8.8
	Fed	5.0	1 pre					10/10	9/10	9/10	2/10		10-8.5

the treated embryos, *i.e.*, 1.5 days.

Effect of Chloromycetin in mice infected by different routes. Mice infected by the intraperitoneal route with approximately 80 M.L.D.'s of the 6-BC strain of psittacosis were treated with Chloromycetin by the intraperitoneal or oral routes. Data from such an experiment are illustrated in Table II. In this and subsequent experiments the antibiotic was given to the mice in each test group from the time indicated until death occurred or until the 12th day after inoculation of the infectious material; treated and control mice were observed until the 21st day when the experiment was terminated. Seven of the 10 control mice which received the same concentration of challenge virus used to infect the treated mice died between the 6th and 10th

days. It is apparent from the tabular data that the daily intraperitoneal administration of 1.5 mg of Chloromycetin beginning 6 days after infection was effective since only one of the 10 mice in the group died. Half this amount of drug was ineffective when treatment was begun on the sixth day but did protect the entire group when given from the first day after infection. Finally, the data in Table II show that all mice which received approximately 5.0 mg of drug per day by mouth survived the infection.

A series of experiments was next performed in which mice were infected by the intracerebral route with the P-4 strain of psittacosis or the L. A. strain of lymphogranuloma venereum virus and treated with Chloromycetin either by mouth or by intraperitoneal injection. In none of these experiments was a beneficial chemotherapeutic effect demonstrated. The question then arose as to whether Chloromycetin would under any circumstance protect against intracerebral infection with a member of the psittacosis-lymphogranuloma group. The 6-BC strain of psittacosis was chosen for the next experiment because it is lethal when given either intraperitoneally or intracerebrally; this is in contrast to the P-4 strain of psittacosis and the lymphogranuloma virus which are lethal only when given by the intracerebral route.

In the experiment summarized in Table III, groups of mice were injected by one of these 2 routes with serial 10-fold dilutions of the 6-BC suspension and treated with Chloromycetin by daily injection or by feeding. It is apparent from the data presented in this table that treated mice survived inoculation with enormous amounts of virus injected by the intraperitoneal route but displayed no resistance to intracerebral inoculation of the virus.

Effect of Chloromycetin on the virus in vitro and in vivo. Chloromycetin possesses little or no direct virucidal action against the agent of psittacosis. This was demonstrated in an experiment in which 2 portions of a 10^{-5} dilution of a standard suspension of 6-BC strain were prepared; one contained the virus in ordinary milk diluent whereas in the other the virus was suspended in milk media in which was dissolved 2.5 mg of drug per ml. These 2 portions were held at room temperature for 2 hours and then titrated in embryonated eggs. The lethal titers were $10^{-9.6}$ and $10^{-10.3}$, respectively. Furthermore, the average day of death of the embryos inoculated with dilutions of the control and drug treated suspensions of virus were essentially the same. As was to be expected the lives of the embryos which received the original virus suspension containing 2.5 mg of drug per ml were prolonged.

In the present experiments none of the embryonated eggs inoculated with even a few hundred M.L.D.'s of virus survived to the time for hatching even when treated with 0.5 mg of drug. Yolk sac tissues from moribund treated eggs were as rich in elementary bodies as were those from controls.

In the experiment summarized in Table II, practically all of the mice which survived when treatment was begun later than the first day after infection developed obvious signs of disease. Those least affected showed ascites but otherwise appeared fairly healthy. No apparent illness was noted among the mice given the drug by mouth beginning the day before infection. In this experiment the spleens from 2 animals of each treated group in which all mice survived were tested for virus by passage to normal mice; each suspension of splenic tissue contained active virus. Furthermore, the remaining animals in these groups resisted an intraperitoneal challenge inoculation containing several hundred M.L.D.'s of the 6-BC strain. Thus, infection was not prevented in mice treated prophylactically nor were the tissues freed of virus when the drug was used for chemotherapy.

Direct evidence that Chloromycetin has a suppressive effect on the growth of virus in treated mice was obtained in the experiment summarized in Table III. Two mice from each of the groups which received a 10^{-6} dilution of virus were exsanguinated on the sixth day, care being taken to obtain the blood without contamination from the peritoneal cavity. At this time the treated mice looked healthy but the controls were sick. The

infective titers of the pooled hearts' bloods determined in mice were as follows: controls, $10^{-2.5}$; group treated with injections of 2.5 mg of drug, $10^{-1.3}$; group fed 5 mg of drug, less than 10^{-1} (1 of 5 mice receiving a 10^{-1} dilution of blood succumbed).

Discussion. Penicillin and drugs of the sulfonamide series have a therapeutic effect on experimental infections caused by a number of agents of the psittacosis-lymphogranuloma group. Furthermore, these substances have been employed in the treatment of patients with lymphogranuloma venereum or psittacosis (see [5]). The present data obtained in infected embryonated eggs and mice treated with Chloromycetin suggest that the new antibiotic possesses about the same degree of activity as that displayed by penicillin and sulfadiazine in similar types of studies.[5,12] It is of interest that none of the drugs are really effective in treatment of mice infected by the intracerebral route with these agents.

Conclusion. Chloromycetin possesses considerable therapeutic activity in embryonated eggs and mice infected with the viruses of psittacosis or lymphogranuloma venereum. This activity is comparable in amount to that demonstrated by others for sulfadiazine and penicillin tested under similar conditions.

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Inhibition of Multiplication of Influenza Virus by Tannic Acid.*

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Recently it has been reported[1-8] that various substances inhibit multiplication of one or more viruses of the mumps-influenza group as well as the pneumonia virus of mice. In some instances these substances inhibit both virus multiplication and virus hemagglutination. Moreover, of considerable interest is the fact that some of these substances are themselves capable of agglutinating erythrocytes. For the most part, when tested *in vitro* they exert little or no direct effect upon the viruses against which they are active *in vivo*. With the exception of the enzyme described by Burnet,[8] which apparently acts upon the receptor substance of the cell, the mode of inhibitory action and the relationship of inhibition of hemagglutination to that of multiplication are obscure.

The fact that tannin agglutinates erythrocytes has been known for some time.[9] During the course of studies on hemagglutination it was observed that tannic acid in concentrations as low as 45 μg per cc agglutinates chicken erythrocytes. Subsequent investigation showed that smaller amounts of tannic acid actually inhibit the agglutination of chicken erythrocytes by influenza A virus. Furthermore, tannic acid inhibits the multiplication of influenza A virus *in vivo* and inactivates the virus *in vitro*.

* Aided by a grant from the United States Public Health Service.

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