dilutions of culture. Constancy of virulence was ascertained in each test by infecting control eggs with culture alone in decimal dilutions from 10^{-6} to 10^{-9}. A series of not-infected eggs were kept as viability controls; this is an important precautionary measure to insure the tests against interference by unforeseen damaging influences. 50% endpoints of survival were calculated according to the method of Reed and Muench.[9]

The chick embryos are rather uniformly susceptible to infection, but protection is subject to considerable individual variation. Thus, it was found to be advisable to use 8 or, better, 10 chick embryos at each test level. Normal horse and rabbit sera and, similarly, antipneumococcic horse and rabbit sera were found to be devoid of any appreciable protective value in volumes of 0.02 ml.

The immune sera employed in these tests consisted in the main of pooled sera from rabbits that had been treated with increasing volumes of *H. influenzae* type b suspension. Tests were also made with a refined and concentrated horse immune serum 18R37, that had been found by Dr. Heidelberger[10] to contain 1.25 mg agglutinin and 1.15 mg precipitin N/ml.

By way of exemplification, we give in Table I. the results of some of our experiments in which immune sera in 5-fold decreasing dilutions were tested against 2 dilutions of culture. It will be seen that small volumes of immune serum afford protection against infections in the order of 10^3 and 10^4 lethal doses. This latter level of infection is about the upper limit of effectiveness of immune protection. As can be seen from line 5 of the table, infection with well above 10^5 microorganisms is evidently so overwhelming that immune protection is of no avail anymore. This experience is paralleled by that with *Enterobacteriaceae*.[4, 5]

A similar method can also be used for chemotherapeutic experimentation; for instance, the LD_{50} of chick embryos treated by a single injection of 5 mg of sulfadiazine into the amnion immediately before infection with *H. influenzae* was found to be 120,000 microorganisms, the LD_{50} of the controls in this experiment being 2 microorganisms.

Summary. Ten-day-old chick embryos are maximally susceptible to infection with *H. influenzae* type b, the average LD_{50} being between one and ten microorganisms.

Protection against many thousand lethal doses can be obtained by immune serum.

A similar technic can also be employed for chemotherapeutic testing as exemplified by tests with sulfadiazine.

[10] Personal communication.

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Properties of the Etiologic Agent of Infectious Hepatitis.*

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Although *infectious hepatitis* has been transmitted experimentally to human volunteers by feeding[1] and inoculating parenterally[2-5] serum which is bacteriologically sterile, the exact nature of the icterogenic agent is yet to be defined. It is believed to be a virus.

During the past few years, numerous reports have appeared, describing the occurrence of jaundice in man following the administration of human blood products and indicating that the icterogenic agent (or agents) of such

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homologous serum jaundice[6-9] and post-vaccinal jaundice[10-15] are filtrable through a Seitz filter and can withstand heating to 56°-60°C for 30-60 min. More recently, certain planned experiments[1, 16, 17] on the transmission of these diseases to human volunteers have corroborated these facts.

The present report describes certain properties of the icterogenic agent of the *naturally* occurring disease, *infectious hepatitis*.

Serum employed in this experiment was obtained during the first 5 days of the experimental disease (pre-icteric phase), which had been induced in 2 human volunteers by feeding fecal material obtained from naturally occurring cases.† As such it constitutes *first human passage* material. The serum (designated here as serum M.K.) was stored at dry ice box temperature for 2 months.

For the experiment this serum M.K. was thawed at room temperature, pooled, and filtered through a Chamberlain No. 2 filter, which was demonstrated to withhold a 24-hr broth culture of *Sal. cholera suis*. Immediately afterwards, the filtered serum was heated at 56°C for 30 minutes and stored in the ice box overnight. Cultures of the serum before and after filtration and heating were sterile.

The heated filtrate of serum M.K. was administered in amounts of 1 cc to each of 8 men. These men, who were residents in a penal institution, had volunteered for this experiment. During the experimental period of observation, they were isolated from their fellows. Four men received heated filtrate of serum M.K. orally (and intranasally) and 4 were inoculated sub- and intracutaneously. These men have been observed 125 days. Three of the 4 men who *ingested* the heated serum filtrate contracted moderately severe infectious hepatitis with marked constitutional reaction and jaundice after 23, 24, and 34 days repectively. One of the 4 men who were *inoculated* parenterally with the heated serum filtrate contracted moderately severe hepatitis with marked constitutional reaction and jaundice after 31 days. There had been no hepatitis in the institution for the preceding year, and no other cases have occurred during the period of the experiment.

Summary. An experiment on the transmission of the naturally occurring disease, *infectious hepatitis,* to human volunteers has demonstrated certain properties of the etiologic agent. As in homologous serum jaundice and post-vaccinal jaundice the icterogenic agent of infectious hepatitis is filtrable and withstands heating to 56°C for 30 minutes. The icterogenic agent of infectious hepatitis can be transmitted in serial passage in human volunteers.

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† These naturally occurring cases were in American and British troops serving in the Middle East.