DNA and DNase not only promoted the establishment of virulent cell types *in vitro* but also exerted an effect on the virulence of S pneumococci *in vivo*. A single subcutaneous (dorsal) administration of 450 μg of DNA and 200 μg of DNase per mouse, either at the time of intraperitoneal infection, 24 hours prior to infection, or 24 hours after infection significantly reduced the survival time and the LD_{50} (Table 2). This virulence-enhancing effect of DNA and DNase appears to be quite different from previously reported effects of purines on the virulence of purine-requiring mutants of *Salmonella, Erwinia, Klebsiella,* and *Agrobacterium* (*8*), for the injection of a purine pool did not affect the survival time of mice infected with pneumococci.

In studies with *Brucella*, desoxyadenosine and sonic extracts of S *Brucella* cells have shown some antagonistic activity toward the S-selecting effects of DNA and DNase *in vitro*. Further studies with these and other antagonists, as well as studies on the chemical nature of the active DNA breakdown product and its mode of action, are now in progress (*9*).

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Paper Electrophoresis of Avian and Mammalian Hemoglobins

As was reported previously (*1*), chick hemoglobin reveals two components on electrophoretic analysis. These components are present in varying proportions, depending on the age of the chick. In view of these findings, it was considered of interest to investigate the electrophoretic behavior of the hemoglobins of birds for comparison with hemoglobins of certain mammalian species. Accordingly, the hemoglobins of the pigeon (*Columba livia*), duck (*Anas*), guinea fowl (*Numida melagris* Linn.), and chick (*Gallus gallus*) and of man (one normal and two cases of Hb-E-thalassemia), cow, goat, and rabbit have been investigated in an LKB paper-electrophoresis apparatus using barbiturate buffer of *p*H 8.6 and of ionic strength 0.05.

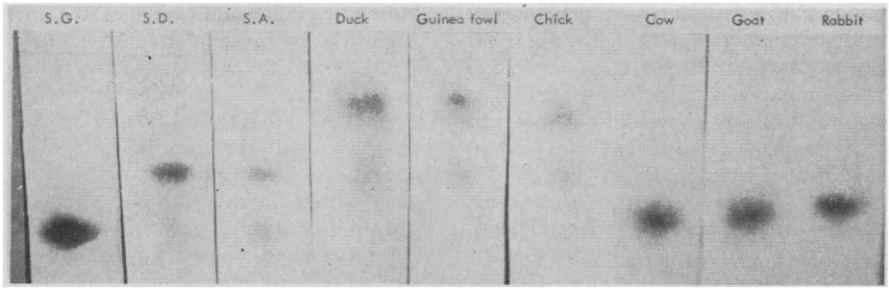

Fig. 1. Paper electrophoresis of avian and mammalian hemoglobins in barbiturate buffer (*p*H 8.6; ionic strength, 0.05) at 220 v applied for 15 hours. Samples S.A. and S.D. were taken from human patients with hemoglobin E-thalassemia; sample S.G. was taken from a normal human adult.

Blood was collected from the jugular vein and was washed with isotonic saline and treated in the usual way (*2*). The hemoglobin solutions thus obtained were centrifuged at 10,000*g* for 15 minutes at 5°C and diluted to a 5-percent solution before electrophoresis. The solutions were kept at −15°C and thawed prior to the electrophoretic runs. The electrophoretic runs were conducted at 220 v for 15 to 18 hours. The electrophoregrams were scanned photometrically at 540 mμ by means of a Photovolt densitometer model 525.

Figure 1 represents the relative positions of the hemoglobins of the rabbit, goat, cow, chick, guinea fowl, duck, two men suffering from hemoglobin E-thalassemia (S.A. and S.D.) and a normal human adult (S.G.).

During this study, it was observed that the blood of the mammals, including the normal human adult, showed only one component (Hb-A), while that of the different birds investigated showed two hemoglobin components. The one moving more slowly toward the anode may be called component 1 and the one moving faster may be called component 2. The percentage composition of each of the two components was evaluated from the density curve by means of a planimeter, and it was found that the proportion of the component 2 was always less. None of the hemoglobin components of the avian blood is identical with the mammalian hemoglobins. Component 2 of avian hemoglobin appears to be identical with hemoglobin E—that is, the special hemoglobin component which is present in the blood of the patients with hemoglobin E-thalassemia. Confirmation that the slower moving component in the blood of these patients referred to here is hemoglobin E (*3*) has been provided independently (*4*). The proportions of components 1 and 2 in avian blood vary from one species to another. The relative mobilities of hemoglobin A in cases of rabbit, goat, cow, and the human beings S.A., S.D., and S.G. were found to be 3.4, 3.6, 3.6, 3.5, 3.5, and 3.6, respectively. The relative mobilities of component 1 and component 2 in chick, guinea fowl, duck, and pigeon blood were found to be 1.0, 1.0, 1.0, and 0.7, and 2.5 2.5, 2.5, and 1.2, respectively. The relative mobility of hemoglobin E in the two cases referred to here was found to be 2.5. The relative mobilities were calculated as centimeters per volt, per second.

Experiments on the rates of alkaline denaturation of the hemoglobins of different species as carried out according to the technique of Singer *et al.* (*5*) indicate that there is no special relationship in this respect between the mammalian and the avian hemoglobins. Even the closely related groups, such as chick and guinea fowl, which are classified under the same order, show varying resistance to alkaline denaturation, the chick hemoglobin being more resistant than guinea-fowl hemoglobin.

Further electrophoretic studies on the hemoglobins of birds such as koel (Cuculidae) and parakeet (Psittacula) revealed the presence of only one hemoglobin component which corresponds to the component 1 of avian hemoglobin, whereas the crow (Corvidae) hemoglobin behaves similarly to the chick hemoglobin. Although the frog and the chameleon hemoglobins undergo a great deal of denaturation during the electrophoretic runs, they reveal the presence of two components. Comparative study of avian hemoglobins by means of paper electrophoresis provides us with the relationship and the evolutionary trends maintained in the chief groups of the birds (*6, 7*).

It is of interest to speculate whether the presence of hemoglobin E in thalassemic patients and in the different avian species is an indication of a common ancestry of mammals and birds, hemo-

globin E being retained as a genetic trait in thalessemic human beings, while it has practically disappeared from the blood of mammals generally, or whether it is the result of similar type of physiological adjustment that occurs in birds and in thalassemic human beings.

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Multiplication of Poliovirus in Reticuloendothelial Cells without Generalized Cytopathogenic Effect

With a technique previously described (*1*), we obtain from the peritoneal exudate, artificially produced in cynocephalus monkeys, living *in vitro* cultures of reticuloendothelial cells. These cultures, prepared in flattened tubes, show after 4 to 5 days a quite homogeneous population of histyocyte-macrophage type of cells established on the surface of standard-size cover slips that are introduced into the flattened part of the tube. The number of cells in 1-week-old cultures is approximately 10^5; this is of the same order of magnitude as that obtained in cultures of trypsinized monkey kidney cells prepared in the usual way in the same kind of tubes (*2*).

One monkey can provide enough exudate to prepare 40 to 50 cultures at one time, and it can be used again as a source of cells after a 7- to 10-day period of rest. Experiments with poliomyelitis virus were performed with the Mahoney type I strain. One thousand $TCID_{50}$ were introduced into each tube in 1 ml of medium, usually on the first or the second day of the culture.

No definite cytopathogenic effect was seen in the infected cultures observed during 8 and, in some cases, during 13 days, after introduction of the virus. Moreover, the acidification of the medium was progressing in these cultures at the same rate as it was in the noninfected controls. In cultures of monkey kidney cells that were infected simultaneously with the same virus concentration, the acidification of the medium was inhibited, and all cells were destroyed in 3 days.

In fixed and stained preparations of the cultures of reticuloendothelial cells 4, 6, 8, and 13 days after introduction of the virus, one can observe a nearly normal population of histiocytic cells, most of them having clear nuclei, distinct nucleoli and numerous cytoplasmic ramifications (Fig. 1). No typical poliomyelitis lesions, as they were described in human fibroblasts (*3*) or in human and monkey epithelial cells (*4*), are present.

Titrations performed with the supernatant fluid of the exudate cell cultures reveal that, despite the apparent lack of cytopathogenic effect, the virus is multiplying and is released in the medium. The virus introduced at the start at a concentration of 10^3 ID_{50} per milliliter is no longer detectable after 48 hours in control tubes without cells, while in the presence of exudate cells, this concentration rises to 10^5 to $10^{5.5}$ ID_{50} per milliliter and remains at a level of 10^5 to 10^6 ID_{50} per milliliter during at least 8 days despite complete renewal of the medium on the third and the sixth days. It is apparent, therefore, that release of virus by the cells is nearly continuous during this period.

It is permissible to conclude from these experiments that a culture of monkey reticuloendothelial cells reacts in a quite different way from a similar monolayer culture of dispersed epithelial or fibroblastic cells when infected with the same concentration of virus. Two possible explanations can be put forward:

1) Only a small proportion (less than 10 percent) of the cells in the reticuloendothelial cultures is available for infection and virus reproduction. If so, the specific destruction of the virus-infected cells would be difficult to observe even if present (*5*). One would then have to admit that, during the culture period, new cells are permanently coming to maturity in the sense of receptivity to virus. The mechanism of this possible maturation is not clear, but a similar phenomenon was noted *in vitro* with other freshly explanted tissues (*6*).

2) The other possibility is that the individual infected reticuloendothelial cells produce and release the virus in a more continuous and less explosive way than the epithelial or fibroblastic cells, and that virus reproduction in these cells is not necessarily associated with cell destruction.

Fig. 1. Eight-day culture of monkey peritoneal exudate cells, 7 days after introduction of 1000 ID of poliovirus. The virus titer of the culture fluid on the day of cell fixation was 10^6 ID_{50} per milliliter. (Top) normal histiocytic cells; (bottom) normal giant cell.

Anyhow, these experiments afford direct proof that polio virus can multiply *in vitro* in a population of reticuloendothelial cells that have been taken from a polio-sensitive species without any generalized cytopathogenic effect. These observations corroborate by an *in vitro* test the numerous data (7) obtained on the living animal concerning the massive poliovirus multiplication localized during the first stage of infection, most probably in the elements of the reticuloendothelial system that are connected with the alimentary tract without evident histological lesions.

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