Susceptibility to *Haemophilus influenzae* Infection in Relation to the Age Factor

Generalized infections with *Haemophilus influenzae* in man are most frequent during the first two years of life[1]. The higher resistance of later age groups is usually interpreted as the result of humoral immunity due to bacteriolysins of amboceptor-complement type[2], which were found to increase with age[3]. However, the increase in antibodies becomes apparent at a later age than the age of decrease in case incidence. Thus, we have felt some doubt whether the humoral factor is solely responsible for the age-induced resistance, particularly as recent research on this problem in virus infections has shown that the interdependence of cause and effect is quite complicated[4]. As the mouse is not a natural host to true *H. influenzae*, at least acquired humoral immunity in the adult animals cannot be of importance. We, therefore, undertook to examine this problem by the inoculation of mice of different ages. The capsulated smooth *Haemophilus influenzae* strain type "B" used in our experiments had been isolated from the pericardial exudate of an infant who died from purulent pericarditis.

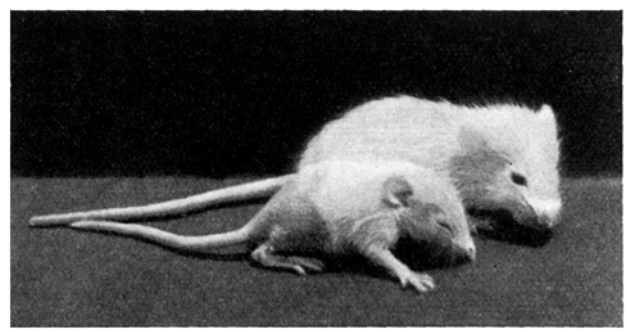
In 20 adult mice (average weight 25 g) the subcutaneous injection of half a slope blood agar culture caused only a local swelling which subsided after 1–2 days and all the animals remained alive. There was no damage to the skin and the fur at the site of injection. However, a transient bacteraemia quite often followed the injection. When groups of 6 mice were inoculated in the way described and killed after 3, 24 and 48 h, the injected organisms could be cultured after 3 h from the liver of 4 animals but in no instance after 24 and 48 h. Thus, the adult mouse quickly overcame this bacterial flooding and showed no subsequent damage.

Table I
Effect of *H. influenzae* infection in infant mice of different age

Age in days	Number of mice	Dead within 48 h	Recovered
5	20	10	10
10	20	13	7
15	20	3	17
20	20	2	18
30	20	0	20

In infant mice, the infection took quite a different course. Groups of 20 mice were subcutanously injected with 1/50 slope agar culture per gram body weight at the age of 5, 10, 15, 20, and 30 days. The results are given in Table 1. About half of the animals in the first two age groups died quickly and the bacteria could be recovered from liver and spleen of all the dead animals. The histological examination of the internal organs revealed necrotic foci in the liver with marked leucocytic infiltration. In the two later age groups the death rate dropped considerably and became nil in the last one.

The survivors of the first group, still naked and blind at the time of infection, were markedly stunted in their general development and showed inhibition of the growth of hair over a wide area around the site of injection as well as similar smaller defects on other parts of the fur (Fig.). Histological examination of the affected skin, ten days after injection, revealed a heavy polymorphonuclear infiltration of the subcutis, thickening of the derma and, in places, rarefaction of the epidermis with blocking of the hair follicles by keratin plugs (follicular hyperkeratosis). Three weeks later, the infiltration had diminished and young hairs were growing, but the cutis was still thickened. This skin involvement, analogous to the dermo-necrotic effect of *H. pertussis* toxin[1], was obviously due to endotoxins, liberated from the bacteria, against which the adult animal appears to be more resistant. The skin lesions cleared after about one month but a certain irregularity of the hairy coat remained visible. In the other age groups, where the fur had already started to grow at the time of infection, no such effect on the skin was observed but the general development in the second age group was also markedly retarded.



Infant mouse, 10 days after infection, injected with *H. influenzae* in right hindquarters at the age of 5 days. Large bald area over posterior part of body, a smaller one above the right eye. General retardation of growth, as compared with control mouse of same age and stock behind it (Natural size).

These experiments demonstrate that infant mice are considerably more sensitive to Haemophilus infection than older ones. After the 15th day of life the resistance approaches that of the adult and reaches that level at the 30th day.

The pathogenicity to embryos is still higher than to postnatal animals. As far back as 1937, GALLAVAN[2] reported irregular pathogenicity of *H. influenzae* to chick embryos by chorio-allantoic inoculation, which caused encephalitis, meningitis and occasional ectodermal necrosis. GALLAVAN and GOODPASTURE[3], inoculating *H. pertussis*, observed also ectodermal necrosis, increase of mesodermal cells and pulmonary lesions reproducing those of human pertussis. SHAFFER and SHAFFER[4] demonstrated the marked superiority of yolk sac inoculation over the chorio-allantoic route with *H. pertussis*.

Our Haemophilus strain killed chick embryos by yolk sac inoculation regularly in less than 24 h, and corresponding effects were observed in mice. 8 females in the middle of pregnancy were injected subcutaneously with half a slope agar culture and all aborted within 36 h but remained otherwise healthy. Out of 8 others, in-

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jected towards the end of pregnancy, two aborted and six delivered sickly litters. Many of these baby mice were eaten by their mothers but a few survived and showed patchy defects of the fur similar to those brought about by direct injection. The most regular damage of the aborted foetus, as revealed by histological examination, were scattered necrotic foci in the liver without leucocytic infiltration.

In a further experiment, 10 females in the middle of pregnancy were subcutaneously injected with half slope agar cultures and killed 24 h later. In six of them abortion had already begun by that time. Cultures were performed from the liver and spleen of the females on the one hand, and from the embryos on the other. No *Haemophilus* was found in the organs of the mother animals nor was there any subplacentar hemorrhage. The abortion could not, therefore, be related to maternal injury. Out of 66 embryos, 13 yielded a rich confluent growth, 8 showed isolated colonies and 45 were sterile. The distribution of these findings is given in table No. II.

Table II
Presence of *Haemophilus* in embryos of pregnant mice infected 24 h before examination

Serial No. of female	Embryos			
	Total number	Confluent growth	Isolated colonies	Sterile
1	7	7	–	–
2*	6	–	1	5
3*	4	–	4	–
4	10	–	–	10
5*	7	–	–	7
6*	8	6	2	–
7*	6	–	–	6
8	7	–	–	7
9	4	–	–	4
10*	7	–	1	6
Total	66	13	8	45

* Abortion had already started before examination.

The bacteriological findings of the placentas, cultured separately, did not differ from those of the corresponding foetus. Experiments with intravenous injections of 1/10 slope agar cultures gave even more impressive results. After 24 h, the organs of all the females were sterile whilst the Haemophilus could be recovered from the embryos in most of the litters.

In conjunction with our previous results, these findings may be interpreted as follows: The embryos are killed by the liberated endotoxins to which they are more sensitive than the adult animals. If bacteria penetrate through the placental barrier into the foetus, they establish a foothold in the impaired embryonic and placentar tissue, whilst they are quickly destroyed in the adult animal by cellular defence mechanisms. Humoral antibodies would have acted equally in both, mother and foetus, since they pass unhindered through the placenta in rodents, apes and man[1].

The susceptibility of infants to *H. influenzae* infections cannot, therefore, be explained solely by a lack of humoral immunity. The primary cause appears to be a particularly high vulnerability of the immature organism to the endotoxins of Haemophilus which seem to paralyze its cellular defence. The exact nature of this process still requires elucidation. It would be even more interesting to investigate, whether a difference in vulnerability, similar to that between foetus and mother, pertains also to other immature tissues within a mature organism, i.e. in tumor-bearing animals. Should this be confirmed, the Haemophilus toxin might be considered for trial as an anticancerous agent.

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Zusammenfassung

Mäuse zeigen in den ersten zwei Wochen ihres Lebens, im Vergleich mit erwachsenen Tieren, eine ähnlich erhöhte Empfindlichkeit gegenüber Allgemeininfektion mit *Haemophilus influenzae* wie menschliche Kleinkinder in den ersten zwei Jahren. Diese Erscheinung beruht vermutlich in erster Linie auf einer besonders hohen Empfindlichkeit des infantilen Organismus gegenüber den Endotoxinen, welche die zellulären Abwehrfunktionen hemmen. Noch empfindlicher sind Föten in der Mitte der Gestationsperiode, die sämtlich abgetötet werden, während das Muttertier keinerlei Schädigung erleidet.

Es wird angeregt, zu untersuchen, ob eine ähnliche Verschiedenheit der Empfindlichkeit zwischen den unreifen Zellen von malignen Tumoren und den reifen ihrer Träger besteht.

Der Rucktanz als wesentlicher Bestandteil der Bienentänze

Vor einigen Jahren berichteten wir über richtungsweisende Bienentänze bei Futterplätzen in Stocknähe[1]. Es stellte sich heraus, dass bei einer Entfernung des Futterplatzes von zum Beispiel 30 m sowohl Sicheltänze (BALTZER, TSCHUMI) als Acht- und Schwänzeltänze (v. FRISCH) nebst Übergangstanzformen ausgeführt wurden. Dazu kamen noch regellos eingestreute Rundtänze und ungerichtete Sicheln. Trotz der starken Variation schienen die Tänze, wie dies auch TSCHUMI[2] feststellte, eine ausgesprochen richtungsweisende Funktion zu erfüllen. Letztere kann deshalb unseres Erachtens nur auf den im Jahre 1950 von uns beschriebenen «Rucktänzen» beruhen, die unter anderem auch die «Wendepunkte» BALTZERS[3] markieren. *Nur diese Rucktanzstrecken können durch Länge und Richtung die Lage der Futterquelle anzeigen*, entsprechend den von v. FRISCH entdeckten Regeln. Die zwischen den Rucktänzen eingeschalteten Laufstrecken haben, ebenso wie die Rundtänze und die ungerichteten Sicheln, eine aktivierende Bedeutung. Ihr Auftreten ist einerseits durch örtliche Umstände auf der Wabe bedingt, andererseits durch das Temperament des Volkes. Letzteres kann verschieden sein, und zwar sowohl *vorübergehend* durch Einflüsse, wie Wetter, Qualität der Futterquelle, Stärke und Entwicklungszustand

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