and A_2 have roughly similar total contents of saturated chains in both PL and NL fractions, and similar monoenoic contents in the PL fraction, but the PL fraction of A_2 is richer than that of A_0 in polyenoic acids. In addition, the ratio of monoenoic to polyenoic acids of NL is much higher for A_0 than for A_2. The PL fraction of influenza B has about the same level of total saturates as A_0 and A_2, but the NL fraction has much more, and the monoenoic levels are lower for both PL and NL fractions of influenza B than they are for either of the other viruses. The NCP shows much lower levels of total saturates than any of the viruses do and a much higher NL monoenoic level. It is possible that the NL fraction of the NCP is contaminated by egg-yolk lipids which contain large amounts of unsaturated acyl groups [notably 18:1 (18 carbon atoms: 1 double bond)] in the form of triglycerides, cholesterol esters, and free fatty acids. It is also not clear whether the fatty acid composition of NCP is representative of the overall host surface membrane, or whether localized and possibly transient fluctuations occur which may be related to release of NCP.

The greatest differences between the viruses are found in the NL fraction. The yield of lipid in this fraction is substantially increased after extraction with boiling ethanol; this suggests that some neutral lipid molecules are more strongly bound into the viral envelope, and the wide variations in NL fatty acid composition reflect distinct differences in the nature of these strong binding sites in each of the viruses.

Peptide maps of tryptic digests of the nucleoprotein (nucleocapsid) of two strains of type A influenza virus show but a single difference, whereas the surface antigens, for example, the hemagglutinin, of strains of the same type show many differences (*18*). Immunological cross-reactivity is not found for either nucleoprotein or surface antigens of types A and B influenza virus. We feel the observed variance in fatty acid composition reflects differences in the conformation, dependent upon the amino acid sequence, of the envelope proteins. If the nucleocapsid were the directing agent, we would expect similar compositions for A_0 and A_2; if no direct selection of lipids took place during formation of the envelope, all three viruses and NCP should have the same composition.

Since the viruses contain the same range of acyl groups as do NCP, the mechanism of selection of lipids may involve either rearrangement of existing membrane lipids in the vicinity of the area bearing viral envelope proteins, or exchange of acyl groups to achieve a more sterically favorable conformation of lipid molecules. The possibility exists that viral infection induces a specific enzyme (for example, an acyltransferase) that could produce these changes. Recent work has shown that many molecular species of lecithin exist in a single cell (*19*). It is possible, therefore, that a sufficient variety of lipids is already present in the cell to permit assembly of different enveloped viruses.

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Survival of Germfree Rats without Vitamin A

Abstract. *Weanling, germfree rats, transferred to a conventional animal room and fed a vitamin A-deficient diet, died in 23 to 54 days. In contrast, their littermates, kept germfree and on the same diet, survived for as long as 272 days. The rats kept in germfree conditions stopped growing after 1 to 4 months but responded to supplements of retinoic acid.*

In a previous study of vitamin A deficiency in the germfree rat, Beaver (*1*) noted no difference in the survival time of deficient rats, whether in the germfree or non-germfree condition. In contrast, we have found that germfree, vitamin A-deficient rats survive many months after their littermates, placed in a conventional animal room, have died.

A diet deficient in vitamin A (*2*) was fed to Sprague-Dawley strain females, kept in the germfree production unit of the National Institutes of Health, (i) at the beginning of pregnancy, and (ii) during the lactation period when the young were 10 days old. The young rats were weaned when they were 21 days old, and some were transferred to a metal germfree isolator, while the others (termed ex-germfree rats) were placed in a conventional animal room. All rats were caged singly on raised wire floors with free access to food and water. The vitamin A-deficient diet of the ex-germfree rats was steam sterilized under the same conditions (30 minutes at 121°C) as the diet fed to the germfree rats. Positive control rats received the same diet, with the addition of stabilized retinyl acetate (6 mg/kg).

In the first experiment [young rats whose mothers were fed the vitamin A-deficient diet at the beginning of pregnancy], both germfree and ex-germfree rats fed the vitamin A-deficient diet had slower rates of weight gain than the vitamin A-supplemented rats by the fourth week. The ex-germfree male deficient rats reached a weight plateau in 32 to 35 days (Table 1) and all were dead by 46 days. In contrast, the germfree male deficient rats continued to gain slowly. These rats, after about 70 days, developed nervous symptoms characterized by head wobble, slow gait, and hind-leg weakness. The latter was shown by the rat's difficulty in righting itself when placed on its side. This condition became more

Table 1. Time for germfree and ex-germfree vitamin A-deficient rats to reach a weight plateau and their periods of survival.

Type and sex	No. of rats	Weight plateau		Survival time (days)
		Time (days)	Weight* (g)	
		First experiment		
Germfree ♂	4	45–74	192–265	145–202†
Germfree ♀	4	67–135	187–225	170–272†
Ex-germfree ♂	3	32–35	144–186	42–46
Ex-germfree ♀	2	18–25	122–132	23–45
		Second experiment		
Germfree ♂	5	28–32	163–217	56–150‡
Germfree ♀	5	28–41	160–189	58–150‡
Ex-germfree ♂	5	23–28	154–195	32–54
Ex-germfree ♀	5	28–35	141–204	34–50

* Average weaning weights of males and females were, respectively: first experiment, 30 and 25 g; second experiment, 47 and 45 g. † Not all rats died; some were cured by feeding retinyl acetate. ‡ After 150 days, four males and one female were still alive.

pronounced for about 1 week, then underwent no further change. At this time, there was depilitation around the eye (so-called "spectacled eye") but no porphyrin pigment was apparent. Germfree, vitamin A-deficient female rats showed similar growth retardation and nervous symptoms 2 to 8 weeks later than the males.

The deficient, germfree rats, after reaching a weight plateau, generally maintained a constant weight for prolonged periods. The only change in condition was an accumulation of porphyrin around the eyes. Eventual death was always preceded by weight loss for about 2 weeks and cessation of eating. An autopsy showed that all rats had urinary bladder stones. Death was attributed to intestinal strangulation (volvulus) in three rats, urinary blockage in two, and was undetermined in two. None of the germfree rats supplemented with vitamin A died, but grew and appeared normal in all respects.

In the second experiment [young rats whose mothers were fed the vitamin A-deficient diet during lactation], the deficient rats and the vitamin A-supplemented control rats were kept in separate isolators to preclude the possibility of the deficient rats having access to traces of vitamin A. These rats were considerably heavier at weaning than in the first experiment, presumably because their mothers were changed to the deficient diet during the lactation period rather than before the young were born. Both male and female germfree deficient rats reached a plateau earlier than in the first experiment (Table 1), probably because of a faster growth rate, and they died sooner. Causes of death, when ascertained, were the same as in the first experiment. After 150 days, five rats were still alive and were maintaining weight.

To test the possibility that the casein in the deficient diet may contain a trace of vitamin A that could be sufficient for survival in the germfree state, the 22 percent casein was replaced with a mixture of 4 percent casein and 10 amino acids, to give an essential amino acid composition equivalent to that of 18 percent casein (*3*). Two germfree rats, deficient for 126 days, were fed this diet for 3 weeks. After an initial adjustment period of 1 week, during which slight weight loss occurred, they maintained weight for 2 weeks with no change in appearance.

One germfree deficient rat whose weight (256 g) had been constant for 5 weeks was given retinoic acid in the diet (12 mg/kg). Weight gain began in 3 days and continued for 14 days when the retinoic acid was withdrawn (weight, 304 g). No change in weight occurred during the next 30 days. A second addition of retinoic acid to the diet for 14 days promoted a weight gain of 24 g. When the supplement was withdrawn, the weight gain again ceased promptly, and a constant weight was held for the next month.

These experiments indicate that vitamin A is not essential for prolonged survival (*4*) of the germfree rat that has been weaned with low tissue stores of the vitamin (*5*). Early death in conventional deficient rats must be a consequence of bacterial infection.

Note added in proof: In a third experiment, the vitamin A-deficient diet had an L-amino acid mixture substituted for casein and sucrose substituted for starch. The diet was sterilized by irradiation. Three ex-germfree deficient rats died after 46 to 54 days. One germfree deficient rat died on the 68th day and three others were still alive after the 100th day.

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2. Composition of the diet (percent): vitamin-free casein (Nutritional Biochemicals Corp.), 22; Wesson salt mix, 4; corn oil, 4; vitamin mix in sucrose, 2; cornstarch, 68. The vitamin mix had three times the normal amounts of all vitamins except A.
3. This diet could be autoclaved satisfactorily, whereas a diet containing 19 L-amino acids and no casein became dark brown and hardened on cooling.
4. Drs. N. Raica and H. E. Sauberlich of the U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado, have also observed prolonged survival in germfree, vitamin A-deficient rats (personal communication).
5. Analysis of livers from two rats when weaned for the second experiment gave 5.6 and 8.2 μg of vitamin A per liver.

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Amoebic Meningoencephalitis: A New Amoeba Isolate

Abstract. *A strain of* Naegleria *sp. was isolated repeatedly from the spinal fluid of a boy who died of acute meningoencephalitis 5 days after the onset of the first symptoms.*

Since 1962 sixteen cases of fatal amoebic (limax-type) meningoencephalitis have appeared in Northern Bohemia (*1*). The causal agents were detected in histologic preparations, but never isolated in culture.

In 1968 spinal fluids and nasal swabs of all patients with the meningeal syndrome hospitalized in the department of neuroinfections of the North Bohemian district hospital were cultivated on agar slants [2 percent weight by volume of Bacto-Agar (Difco) in distilled water] coated with a suspension of thermally killed *Aerobacter aerogenes* culture before the beginning of therapy. The ability of our strain of *Aerobacter aerogenes* to support the growth of *Naegleria* even after the thermal preparation described (*2*) was also demonstrated with the pathogenic HB-1 strain of *Naegleria* sp.

Positive cultures were obtained from three spinal fluid samples collected on 27, 28, and 29 June 1968 from a 12-year-old boy. This patient developed