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VIRUS SURVIVAL AS A SEASONAL FACTOR IN INFLUENZA AND POLIOMYELITIS

by

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INTRODUCTION.

In endemic areas the morbidity of many virus diseases shows a pronounced seasonal variation. For influenza and poliomyelitis this is especially true in moderate climates. In the Netherlands poliomyelitis is a "summer disease" and the difference between highest and lowest morbidity is almost a factor fifty (Fig. 1) which is much more than for most bacterial diseases. For influenza, which is a "winter disease" the variations are of the same order. Since no satisfactory explanation of these opposite seasonal variations has been given a closer examination of this problem seemed worth while.

Environmental factors play an important part in the spread of virus diseases. In those respiratory infections which spread mainly or partly by droplet nuclei the survival of the virus in the airborne state is obviously important.

Influenza is a disease which spreads at least partly by droplet nuclei. Atmosferic influences on the survival of influenza virus in the airborne state might be a possible explanation of the seasonal variation in this case.

Poliomyelitis may be spread by faeces or by pharyngeal secretations. In proportion to the decline of faecal contamination as a consequence of sanitary measures, the respiratory tract and mouth

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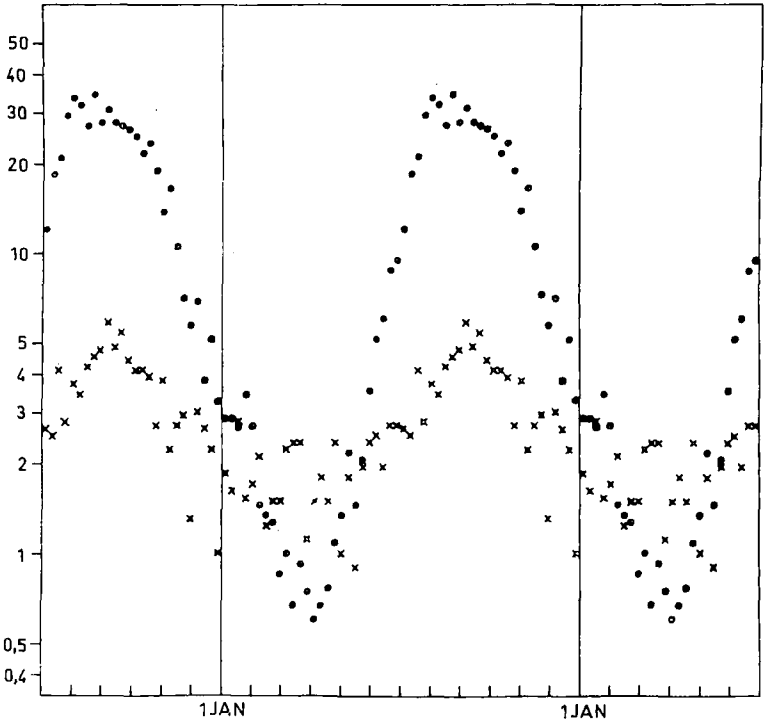

Fig. 1. Mean number of cases reported per week in the Netherlands. Poliomyelitis (1946–1957): • ; Typhoid fever (1948–1957): ×.

become more important as sources of infection. In countries with poor sanitary conditions poliomyelitis will probably spread mainly with faeces and seasonal variation is generally absent. In areas with better standards of hygiene spreading by air or by oral contact may be more important. It is interesting that more pronounced seasonal variations in morbidity are generally found in countries of the latter group. Consequently climatological influences on the survival of airborne poliomyelitis virus might be a cause of seasonal variations.

Furthermore the influence of atmosferic conditions on the survival of virus in the dry state – in dust and on contaminated objects – might be comparable to that on airborne virus, as it is for streptococci (LIDWELL and LOWBURY, 1950). The same reasoning might be applied to other enteroviruses.

Considering all this experiments were started about the influence of temperature and humidity on the survival of influenza virus and poliomyelitis virus in aerosols.

A preliminary report has already been published (HEMMES *et al.*, 1960).

MATERIALS AND METHODS.

The experimental method used was identical to that used to test bacterial aerosols (HEMMES, 1959).

A e r o s o l p r o d u c t i o n. Virus suspensions were atomized from an all-glass, indirect type spray. The mean size of aerosol droplets 5 cm in front of the outlet was 5-6 μ, while 90% had a diameter $< 8\ \mu$. Particle size was determined by trapping the droplets on a slide coated with a vaseline-oil mixture (FUCHS and PETRJANOFF, 1937). The mean output of virus suspension was 54 mg per minute.

S t o r a g e s y s t e m. Since we were especially interested in long term survival of the viruses a static system was used. The volume of the test room was 4 m^3.

Air was circulated by an electric fan. The settling rate for the relatively big droplet nuclei resulting from aerosolization of a 10% sodiumchloride solution, under moderate conditions of relative humidity was $K_s = \pm\ 0.0020$.

Temperature was controlled during the whole of the experiments, while humidity was set before each experiment. UV-lamps were used in sterilisation.

S a m p l i n g. Air samples were taken by use of modified capillary impingers. The critical capillary tube was tangentially directed to the side of the round bottom of a 30 mm diameter tube. At the operating pressure-fall of $\pm$ 50% the flow rate was 11 l per minute. As sample medium 10 ml of a 1% peptone solution was used, to which 1 drop of olive oil was added as antifoaming agent. The absolute efficiency of our impingers was $90.9 \pm 0.5\%$, when tested with bacteriophage T_5 (diameter 50 mμ).

E x p r e s s i o n o f r e s u l t s. After an initial drop in the number of living organisms further decay takes a logarithmic course. The slope K of the survival curve is expressed as $\frac{\triangle \log N_t}{\triangle t}$ where N is the fraction surviving (= recovery) and t is time in

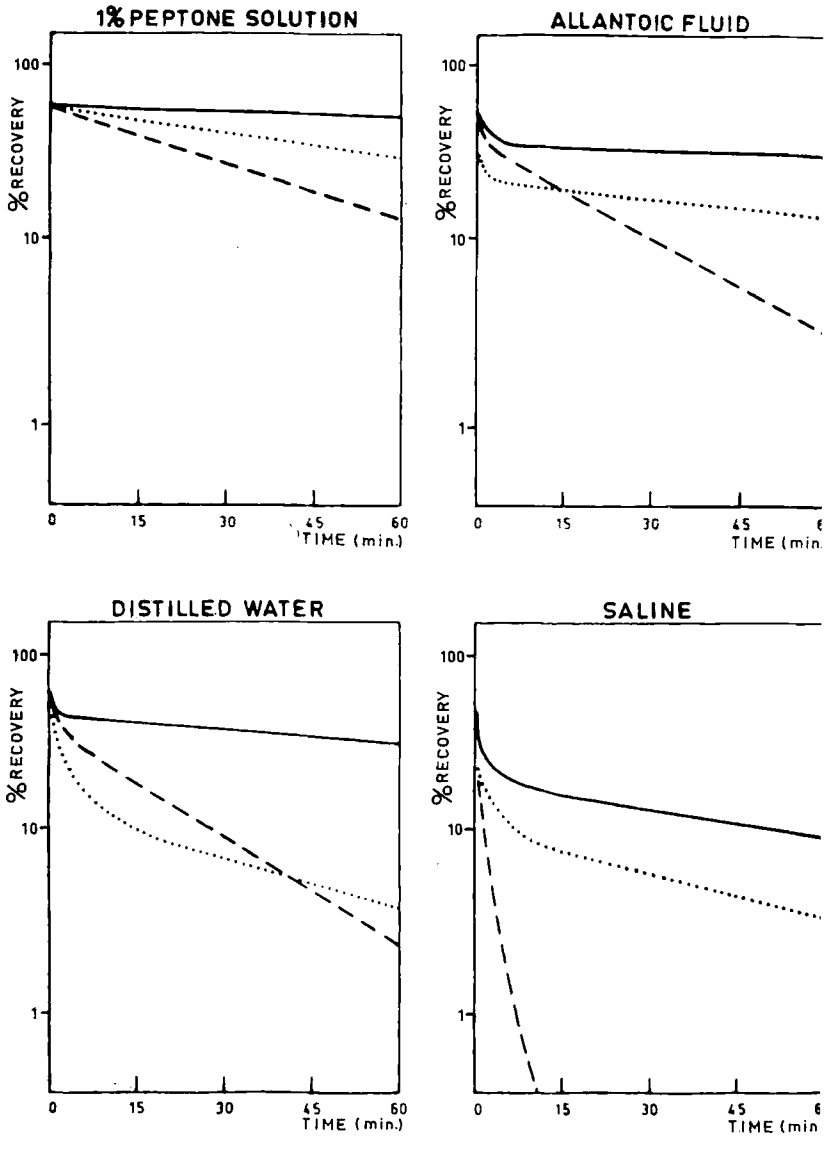

Fig. 2. The influence of relative humidity on the survival of bacteriophage T_5, aerosolized in media of different composition at 24° C.

———— 20% RH ..... 47% RH – – – – – 67% RH.

minutes. By extrapolating the survival curve during the logarithmical period to zero time N_0 could be calculated *i.e.* the fraction of organisms in the aerosolized population that was not affected by the processes in the initial period.

COLI-BACTERIOPHAGE T_5, PRELIMINARY EXPERIMENTS.

Since bacterial viruses are easy to work with, a series of model experiments was done with coli-bacteriophage T_5 propagated on *E. coli* B in nutrient broth.

Influence of suspension medium and humidity.

Bacteriophage suspensions (5×10^9 PFU/ml) diluted 1 : 25 in media of different composition were nebulized during 10 seconds each and 10 second samples were taken at adequate intervals of time. Surviving particles in the sample medium were counted in agar-overlays with the host bacteria *E. coli* B. As suspension media were used: 1% peptone (Difco), allantoic fluid, distilled water and physiological saline. The resulting survival curves for three different humidities are shown in Fig. 2.

The death rate of bacteriophage T_5 is sharply influenced by humidity. The composition of the suspension medium affects the level of decay materially, especially in the initial period. The suspension medium does not interfere however with the effect of humidity as the variations in decay rate with humidity follow the same trend in all the media used. The experiments in artificial media therefore seem to permit conclusions about the influence of humidity on the survival under natural conditions. As peptone seemed to have a protective influence and stabilized the pH at 7.0–7.2 a solution of 1% peptone was used in further experiments.

Influence of temperature and humidity.

Suspensions diluted 1:25 in a 1% Difco peptone solution were used for aerosolisation. Spraying, sampling and counting were conform the methods used before. Operating temperatures were 10, 20 and 30° C. The K-values of the resulting death-curves are shown in Fig. 3 and the mean N_0-values in different ranges of humidity are given in table 1. The theoretical value for N_0 (100% recovery) would be about 400 PFU/l.

Fig. 3 shows that the effect of relative humidity is most striking for all three temperatures. In each case a rather sharp transition

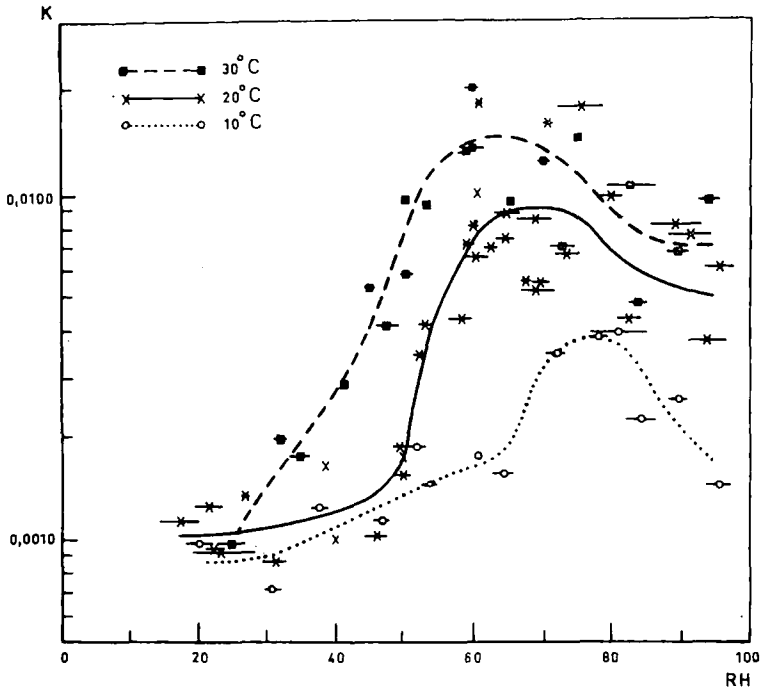

Fig. 3. Bacteriophage T_5, death-rate and relative humidity at 10°, 20° and 30° C.

of low death-rate at low humidity to high death-rate at high humidities occurs in the range from 40–60% RH. This means that relative humidity and not absolute humidity is the important factor.

TABLE 1.

Temperature	Relative humidity	N_0
10° C.	<50% and>50%	37%
20° C.	<50%	62%
	>50%	25%
30° C.	<50%	55%
	>50%	25%

The increase in death-rate per ten centigrades, the Q_{10} is about 2 to 3. At low death-rates the Q_{10} is smaller, probably because the apparent death-rate is partly sedimentation-rate. Compared

with relative humidity the temperature is obviously of minor importance. This would definitely be true in the range of variation of indoor temperature in moderate climates.

It can be seen from Table 1 that relative humidity is also of influence in the initial period before logarithmic decay begins. In accordance with previous results with bacterial aerosols the effect during the initial period – if any – is parallel to that during logarithmic decay.

Human viruses.

After the model-experiments with bacteriophage the influence of relative humidity at 20° C. on the survival of influenza virus and poliomyelitis virus was studied, influenza representing the winter diseases and poliomyelitis the summer diseases.

Influenza virus. A mixture of one part influenza virus PR_8 in allantoic fluid and one part 2% Difco peptone (between 10^8 and 10^9 ID_{50}/ml) was aerosolized for 2 minutes. The theoretical N_0 of the aerosol would then be between 2×10^3 and 2×10^4 ID_{50}/l.

The sampling fluids were titrated by amnion inoculation in embryonated hen's eggs or on surviving fractions of chorio-allantoic tissue *in vitro*. The resulting death-rates are given in Fig. 4.

At 50–90% relative humidity death-rates are about ten times those in the range 15–40% relative humidity. The transition is sharp. We had no opportunity to determine the N_0-values exactly, but they were of the order of 20% at all relative humidities tested.

Poliomyelitis virus. Poliomyelitis viruses were grown on human amnion cells line U in Hanks salt solution with lactalbumin-hydrolysate and 5% horse serum. The following 3 strains were used: CsL (type I, attenuated, Sabin), Leon 12ab (type III, attenuated, Sabin) and Mahoney (type I, virulent). Virussuspensions ($2–8 \times 10^7$ PFU/ml) mixed with equal parts of 2% peptone were sprayed for 2 minutes. The sampling fluids – one % peptone with Dulbecco buffer – were titrated by the plaque method on monolayers of the same amnion cells.

The survival curves are given in Fig. 5, while the K-values are plotted, together with those of influenza virus, in Fig. 4. Inactivation is slow at high relative humidities and very fast at low relative humidities; in fact below 45–55% no virus could be detected 30 seconds after spraying. The transition is rather sharp at 50–60% relative humidity.

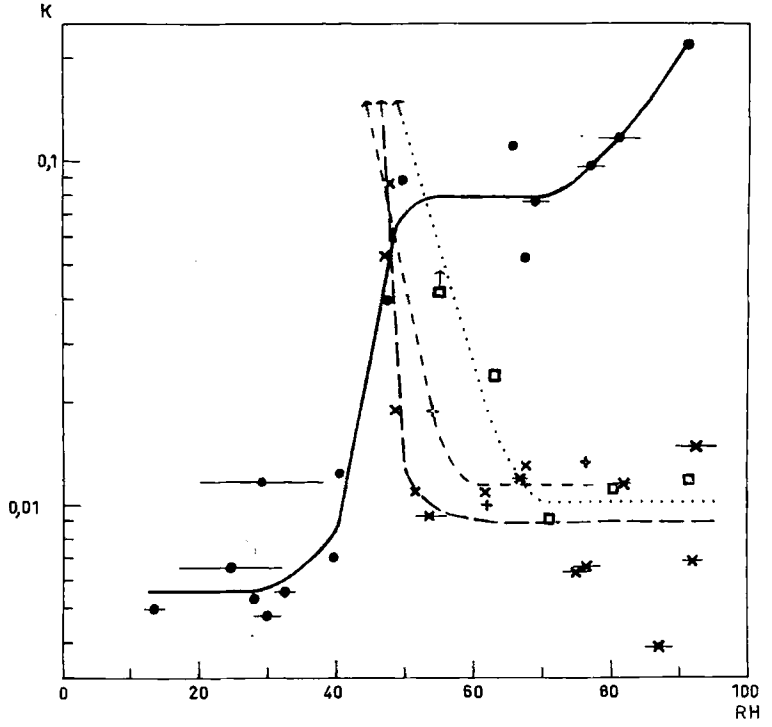

Fig. 4. Influenza and poliomyelitis virus. Influence of relative humidity on the death-rate at 20° C.

——— Influenza PR_8.
— — — Poliomyelitis type I CsL.
– – – – – – Poliomyelitis type I Mahoney.
. . . . . Poliomyelitis type III Leon 12ab.

Mixtures of influenza virus and poliomyelitis virus. Two parts of influenza PR_8-suspension, two parts of poliomyelitis CsL-suspension and one part of 5% peptone were aerosolized at ± 35 and ± 65% relative humidity. The sampling fluids were titrated selectively. The experiments were repeated three times. The mean survival curves are given in Fig.6.

The differences in survival under changing humidities at 20° C. are striking for influenza as well as for poliomyelitis viruses. For poliomyelitis the survival is optimal at high humidity, but no demonstrable virus is left at low humidity; for influenza just the reverse is found with a good survival at low humidity, but rapid

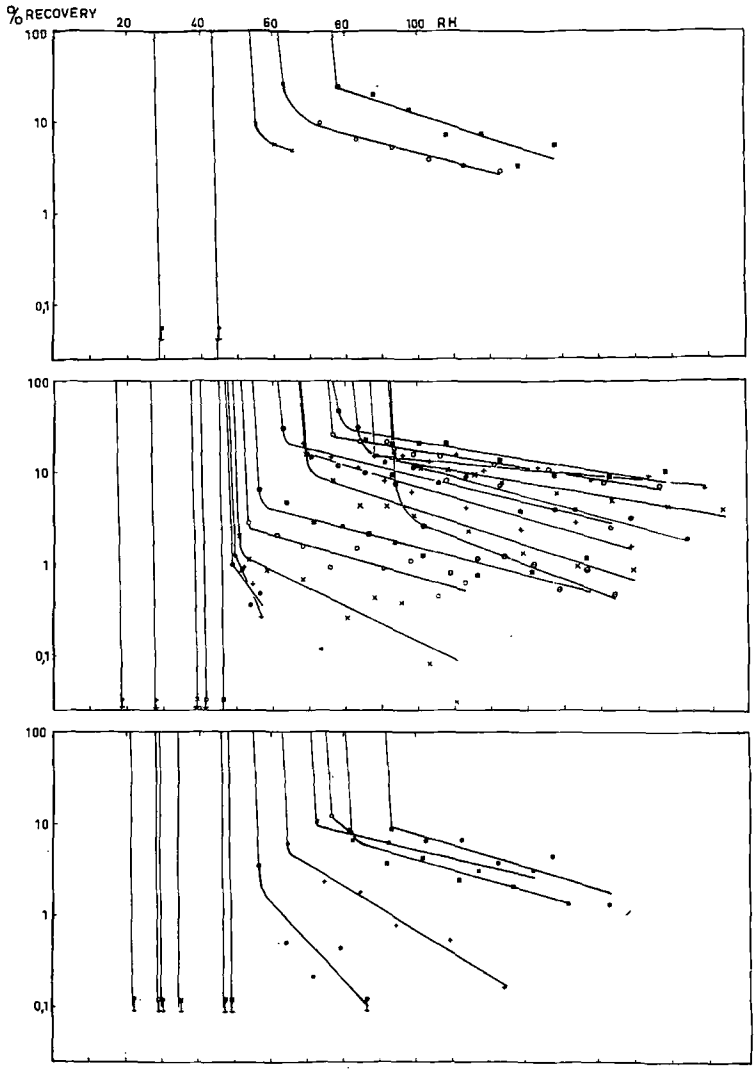

Fig. 5. Poliomyelitis survival curves at 20° C.
Start of each curve at RH tested. Time scale moving, one unit is 10 minutes. From top to bottom: type I Mahoney, type I CsL, type III Leon 12ab.

death at high humidity. These results for influenza virus are in accordance with the limited experiments published previously by EDWARD *et al.* (1943) and LOOSLI *et al.* (1943), they differ however

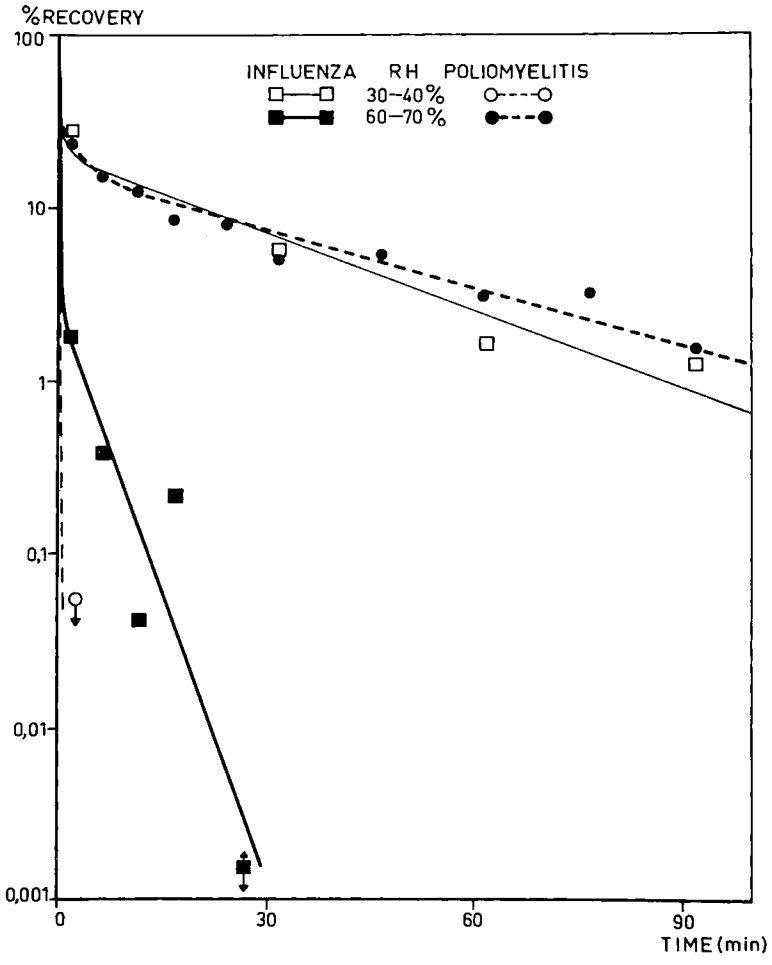

Fig. 6. Survival curves of poliomyelitis (CsL) and influenza (PR_8) virus, aerosolized in mixed suspensions at 20° C.

from the results of ROBERTSON *et al.* (1948), SHECHMEISTER (1950) and BORECKÍJ (1955). The result with mixed suspensions show, as was expected after the previous experiments with bacteriophage, that differences in the composition of the medium are not responsible for these contrasts. The similarity of the phenomenon using three poliomyelitis strains confirms that the principal behaviour of

the virus under the influence of relative humidity – analogous to that of bacteria – is linked to species and not only to type.

Death-curves of both type I strains, attenuated as well as virulent, are almost identical. As to the type III strain the transition is at a somewhat higher relative humidity and inactivation is a little more fast.

HARPER (1961) confirmed the opposite influence of relative humidity on the survival of influenza virus and poliomyelitis viruses. Furthermore he found better survival rates for poliomyelitis virus at 20% and 35% than at 50% RH. We were unable to detect this

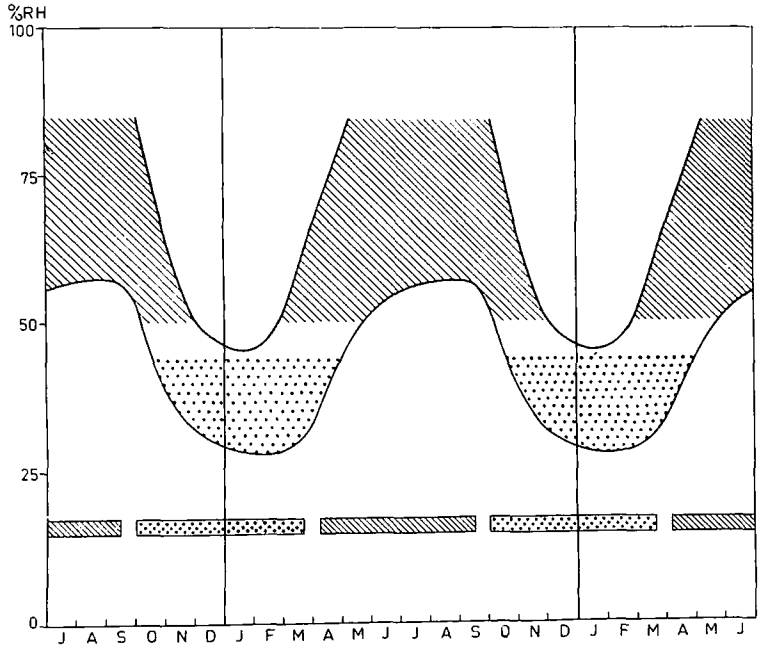

Fig. 7. Seasonal variation of relative humidity indoors in the Netherlands. The upper and lower curves represent mean maximal and minimal relative humidity as calculated from temperature and absolute humidity. The range for optimal virus-survival is stippled for influenza virus and hatched for polio virus. For polio-virus the CsL data were used. The other two polio-viruses have a narrower range of optimal survival. The period of increasing morbidity of influenza (data for England and Wales) and for poliomyelitis (Dutch data) are given at the bottom of the figure as stippled and hatched bars.

phenomenon as at low RH no virus was recovered in the first samples after spraying. This difference might be due to the lower concentrations of our virus suspensions or to differences in the suspension media.

Discussion.

The described experiments show that relative humidity is a more important factor than temperature with regard to the survival of aerosolised viruses. The effect of relative humidity is more or less independent of the suspension medium so that one may expect that relative humidity is also important under natural conditions as in airborne infections.

Since most infections occur indoors, the relative humidity indoors has to be taken in consideration. In moderate climates this relative humidity is high during summer but, due to heating, low during winter. The calculated mean range of variation in the Netherlands is for instance shown in Fig. 7.

The opposite effect of relative humidity on the survival of aerosols of influenza virus and poliomyelitis virus seems in striking relation to the epidemiological pattern of the corresponding diseases. The periods of increasing morbidity for influenza (Fig. 7, stippled bars) coincides nicely with the period of relative humidity best suited for virus survival. The same is true for poliomyelitis morbidity (Fig. 7, hatched bars) and the period for optimal survival.

This seems to indicate that relative humidity may be an important seasonal factor in the epidemiology of influenza and poliomyelitis; whether this is also true for other virus diseases remains to be determined.

Summary.

The influence of temperature and humidity on the survival of airborne viruses was studied in a static system. Preliminary experiments with a bacteriophage showed that relative humidity was more important than temperature and absolute humidity. The effect of relative humidity was not dependent on the composition of the medium surrounding the virus particles.

The survival of influenza and poliomyelitis virus are sharply

influenced by relative humidity but in an opposite way. Influenza virus survives much better at lower humidities, poliomyelitis virus at higher humidities.

In countries with moderate climates the period of increasing morbidity for influenza, in winter, coincides with indoor conditions of relative humidity which are optimal to virus survival. For poliomyelitis the same is true during summer (Fig. 7).

Indoor relative humidity is considered an important "seasonal factor" in the epidemiology of poliomyelitis and influenza and probably of other diseases.

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