FACTORS INFLUENCING THE EVOLUTION OF VIRAL DISEASES AT THE CELLULAR LEVEL AND IN THE ORGANISM[1]

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"For in a sense every universal cognition is a cognition of a singular thing."
William Ockham
Quodlibeta (*circa* 1323)

CONTENTS

I. INTRODUCTION

A. The Problem

A virus is injected into an animal. The virus multiplies, a number of cells are destroyed, pathological processes take place, and essential organs are damaged. If the infected animal dies, there is no problem: everything is all right. Difficulties start when the infected host survives. In this animal which is going to survive, signs may be apparent or not, but the virus multiplies anyhow. Cells are infected and killed, lesions develop. One day, however, viral multiplication ceases, the cells stop dying, and health is finally restored. Why and how? What is the nature of the factors which control the evolution of a viral infection and the fate of a diseased animal? In an infected animal, the virus, the cell, and the organism interact in the most conspicuous way. The virus modifies the cell. The cellular alterations modify the organism, and the reactions of the modified organism in turn modify the cell-virus system.

We have to analyze, and if possible to understand, this highly complex situation. I propose first to introduce succinctly viruses. Then, the role of specific factors in viral infections, that is antibodies, will be considered briefly. This being done, the main subject will be attacked, namely the problem of the nonspecific factors influencing viral development in the cell and in the organism. A number of data will be discussed. My ambition is to interpret them in terms of a unifying concept concerning virulence and disease.

B. Viruses

Viruses are complex organized infectious entities which are reproduced from their genetic material only. The essence of a viral infection is the introduction into a cell or an organism of the genetic material of a virus. Viruses are strict intracellular parasites. In order to develop in an animal, a virus has to come in contact with sensitive cells. An organism is sensitive to a virus only

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if some of its cells at least are able to adsorb the virus and allow its reproduction.

When a flask containing a sensitive population of cells is infected with a virus, the virus multiplies and all the cells may degenerate within a day. The rate of viral multiplication is high. The number of infective particles may be increased by a factor of 10^9 in 24 hr. When a virus is injected into a sensitive animal, the virus also multiplies but the rate of viral multiplication is generally relatively low. In the animal something decreases the rate of viral multiplication. Among the specific factors which decrease or stop viral reproduction are antibodies.

C. The Specific Factors

It is well known that antibodies are produced in the course of infections and that they are, at least partly, responsible for immunity. But a certain number of observations leads to the idea that antibodies are not exclusively involved in the fate of the infected animal. A few examples will be given.

First. Agammaglobulinemic patients are unable to synthesize antibodies or they synthesize only a very small amount of globulins. They can nevertheless be immunized against smallpox (Gitlin *et al.* (22)).

Second. If the virus of choriomeningitis is injected into the suckling mouse 24 hr after birth, the virus develops but no antibodies are formed. A state of immunity nevertheless develops.

Third. It is known mainly from Bodian's work (6) that antibodies, even at high titer, cannot prevent the cell-to-cell spread of poliovirus which has succeeded in gaining access to the central nervous system.

Another type of observation deals with the disappearance of a virus in an infected animal. Rabbits infected by the corneal route with the virus of herpes may die of acute encephalitis between the 5th and 11th days. Occasionally no virus is found in the brain despite the presence of typical herpetic lesions (Gildemeister and Herzberg (21); Loewenthal (35)). This phenomenon is described as lethal autosterilizing neuroinfection. It is questionable if the titer in antibodies at the time of death is sufficiently high to account for the destruction of all viral particles.

To study the inactivation of a virus by antibodies in an animal is rather difficult. We may, however, study a population of cells in a flask. Let us infect the human K.B. cells with a poliovirus. Depending on the input of virus, the cells will degenerate in one day or in a few days. If specific antibodies are added, cell degeneration is prevented (table 1). The concentration of antibodies necessary to eliminate the virus completely is relatively high under experimental conditions: 3.3 per cent of the serum of an hyperimmune rabbit. With 2.5 per cent, a balance is maintained and the cell-virus system can be subcultured. With 1 per cent, the rate of cellular degeneration is markedly decreased, but the cells eventually disappear. Following a natural infection, the antibody concentration is much lower than in an hyperimmune animal. And it is questionable whether antibodies reach a high enough titer at the critical moment of the evolution of a primary viral infection to be a determining factor in the fate of the animal. This question will be discussed later. For the time being we can only suspect that factors other than the specific ones, that is, nonspecific factors, could play a role. Two of them will be considered: temperature and acidity.

TABLE 1

*Effect of antiviral antibodies on the fate of an infected population of cells**

	Conc of Hyperimmune Serum†
	%
Disappearance of the virus	3.3
Balance of cells and virus	2.5
Rate of cellular degeneration markedly decreased	1
Rate of cellular degeneration slightly decreased	0.25

* 1.5×10^6 K.B. cells. Poliomyelitis virus I, 1.2×10^5 infectious particles.

† Concentration in neutralizing antibodies calculated according to Dulbecco, Vogt, and Stickland: 10^{11}. Specific constant of inactivation: 300.

II. TEMPERATURE

Fever is an abnormally high body temperature. Fever is the functional evidence of many infectious diseases. It is one of the most constant symptoms of viral diseases, and, in some instances, the only one. The term fever has often

been used in the past as synonymous with disease. Fever is thus a sign of disease, but it may also be considered as a response of the diseased organism. Could its role in the viral infection be beneficial? In a century where purposiveness has been so ridiculed, I apologize for producing such a finalistic hypothesis. The important point here is not the philosophical aspect of the question: it is to know if the hypothesis can be tested. Actually, it can be tested in two ways: *in vitro* and *in vivo*. It is clear that if the hypothesis is correct, a 2 to 3 C increase should be able to influence markedly the development of viruses. The cycle of the polio virus is followed in a one-step experiment (figure 1), and a typical type of curve is obtained. At 40 C, the yield is 250 times

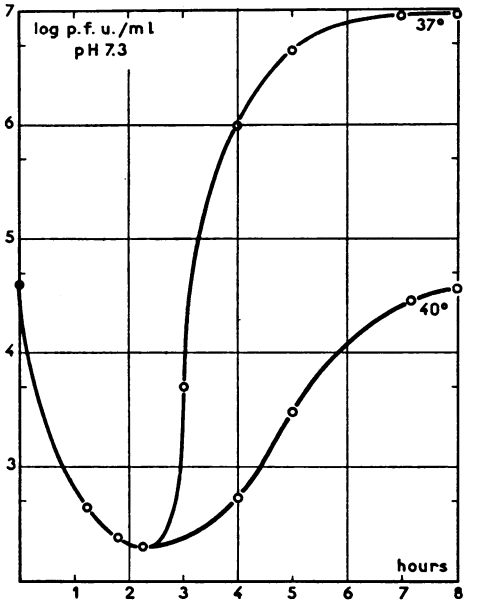

Figure 1. Development of poliovirus at 37 C and 40 C. A suspension of cells is mixed with the poliovirus type I (strain Brunhilde-Enders-Chimpanzee). After 5 min have been allowed for adsorption, the infected cells are washed to eliminate the free virus and suspended in a nutrient fluid, and aliquots are distributed into siliconed flasks. These are gently agitated in a water bath. Samples are withdrawn at intervals. The cells are crushed in a Van Potter grinder to release intracellular virus. The infectious particles are then estimated by one of the techniques derived from the classical Dulbecco method, that is, by counting the plaques produced in a layer of sensitive cells; pfu denotes plaque-forming units.

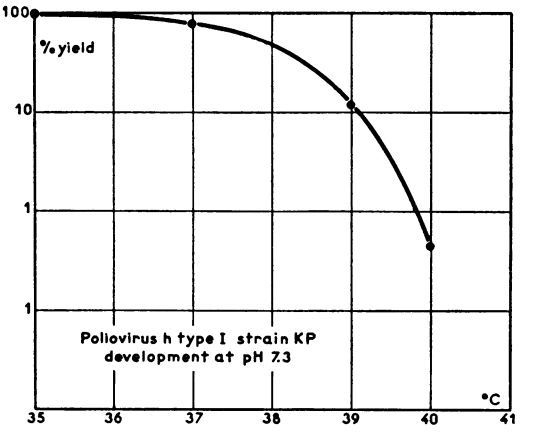

Figure 2. Viral yield as a function of temperature. The viral development at each temperature is followed in a one-step experiment (see legend of figure 1). The initial KP corresponds to the "Brunhilde-Enders-Chimpanzee" strain.

smaller than at 37 C.[3] As a matter of fact, the number of viral particles released per ml of cell suspension is smaller than the number of viral particles which have been adsorbed. For convenience, we may express the yield at different temperatures in terms of per cent of the maximal average yield (figure 2) (A. and M. Lwoff (36, 38)).

The reduced viral yield at "high" temperatures could be due to an alteration of cellular functions. Against this hypothesis it should be noted that the K.B. cell can be grown and subcultured at 39 C and retains a healthy appearance for at least 4 days at 41 C. Moreover, as will be seen later, some mutants of poliovirus are able to multiply at 41 C.

In order to know what the inhibited reaction could be, the following experiment has been made.

1. Infected cells are incubated at 40 C. At 3 hr, they are transferred to a water bath at 37 C. One and 2 hr later, the titer is not far from the titer of the control at 37 C. This means that the vegetative phase of the virus during the first 3 hr proceeds almost normally (figure 3).

2. The infected cells are incubated at 37 and

[3] In the experiments of Likar and Wilson (34) the total yield of poliovirus type I (Mahoney) was measured after a few days: the development was much lower at 30 C than at 37 C and lower at 40.5 C than at 39.5 C.

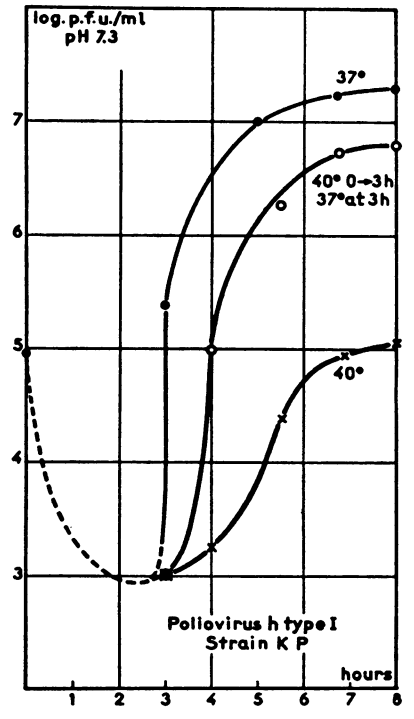

Figure 3. Effect of a 3-hr stay at 40 C on viral development.

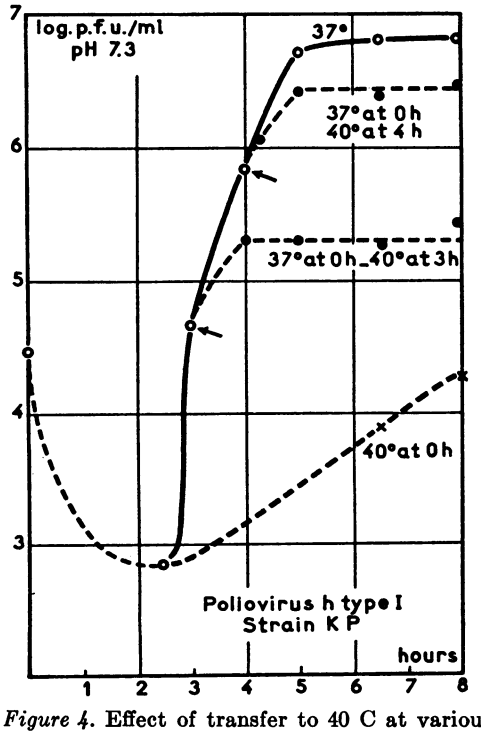

Figure 4. Effect of transfer to 40 C at various phases of the vegetative phase of viral growth.

transferred at 40 C after 3 and 4 hr. The titer increases slightly during the 1st hr. Then the production of infective particles is completely blocked (figure 4).

The thermal inactivation of viral particles in a few hours at temperatures ranging from 37 to 41 C is negligible. It is clear that we are not dealing with the destruction of viral particles, but with the effect of high temperature on viral development. The nature of the reaction blocked by high temperatures has not been disclosed. The experiments point toward the conclusion that it is a late reaction of the vegetative phase.

Thus temperature markedly affects the viral development. So does also the reaction of the medium.

III. ACIDITY

It was shown by Vogt *et al.* (48) that some strains of poliovirus can form plaques in media of reduced bicarbonate concentration, that is, in acid media, whereas some others are unable to do so. The viral development followed by Vogt *et al.* (48) in a one-step experiment proved to be the same at pH 6.8 and 7.4. The conclusion was that the cells are only slowly modified in acid media, and that more than 8 hr are needed in order for the alteration to become incompatible with viral development.

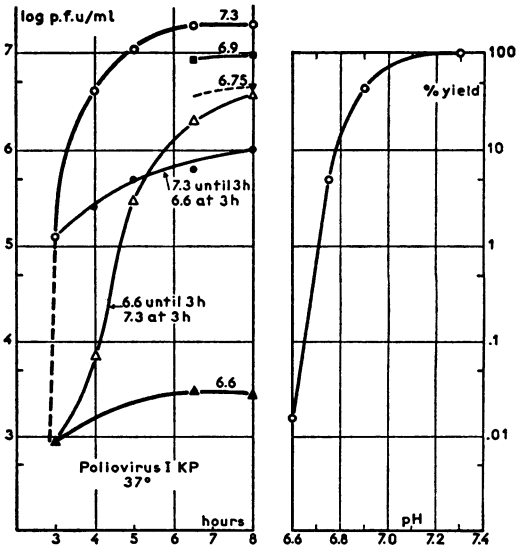

Figure 5. Development of virus as a function of pH. At 3 hr, the pH of one of the cultures at pH 6.6 was shifted to 7.3 and vice versa. Note the absence of lag in the pH effect.

Our first experiments were performed in poorly buffered media. It was observed that the viral yield was poor when the cells were abundant. This could be due to a drop of pH and the pH effect was systematically investigated in one-step growth experiments. The bicarbonate concentration varied between 1.1 to 2.2 g per L and the CO_2 concentration of the gaseous phase from 4 to 21 per cent. The pH was maintained constant throughout one viral cycle, which was followed until completion approximately 8 hr after the infection (figure 5). It is clear that pH immediately and markedly affects the viral yield. The effect is, however, important only below a critical value, which lies around 6.9. That the pH effect is not due solely to a cellular lesion produced by the acid medium is exemplified by the fact that different viral strains are unequally sensitive to the acidity of the medium; a viral property is involved. As shown by Vogt *et al.* (48) this property may be modified by mutations and is therefore controlled by a viral gene.

IV. THE CRITICAL STATE

We are interested in the viral disease of the organism. What has been considered up to now is the intracellular multiplication of a virus as it is affected by temperature and pH. The figures obtained represent an average. But as this average corresponds to a one-step growth cycle, we are entitled to consider that we have studied an "average cell." Before attacking the animal, let us, as a transition, consider a population of cells.

A population of approximately 10^6 cells growing in a flask is infected with a poliovirus, let us say 10^5 infective particles. The optimal temperature for the development of this particular virus is 36 C; the optimal pH, 7.3. Within 24 hr, at 36 C and pH 7.3, all the cells are killed. At 41 C, the viral yield is decreased by a factor of 1000. After 4 days at 41 C, the infected cellular population appears healthy. At pH 6.6, the yield is decreased by a factor of 500. If the CO_2 pressure is increased and the pH thus brought to 6.6, the cells remain normal for more than 2 days. In the one case as in the other, the infected population of cells in the flask has been protected.

The fate of a population of sensitive cells infected by a virus depends in the last analysis on the proportion of infected cells which will produce virus and on the number of virus particles produced by each cell. If the ratio of virus produced to infecting virus is greater than 1, the viral titer increases, more cells are infected, and the cells finally disappear. If the ratio is smaller than 1, the virus output is smaller than the virus input and it is the virus which disappears and the cells which persist.

The fate of a cell-virus system can thus be modified by factors altering viral multiplication. The balanced state in which cellular multiplication would exactly compensate cellular destruction is necessarily fragile. It will be called the critical state. Any environmental factor acting on one of the components of the system will shift the balance towards a direction either favorable to the cells and unfavorable to the virus or vice versa.

Animal virologists have for a long time been aware of the existence of an optimal temperature for viral development, and of a lower and upper limit. Burnet and Lush (9) noticed that the virus of ectromelia does not multiply in the chick embryo and does not produce lesions unless the temperature is below 39.5 C. The same is true for influenza virus which, as shown by Burnet (7), develops well in the chick embryo at 36 C and not at 39.5 C. Enders and Pearson (14) found that the influenza virus injected into the egg disappears from the chicken after hatching. The temperature of the chick reaches 40 to 41 C soon after hatching and the hypothesis was suggested that the body temperature of the chick may be a factor in resistance to infection with influenza virus. Thompson and Coates in 1942 (47) studied the influence of temperature on the development of three viruses: herpes, myxoma, and vaccinia. They showed that the yield drops by a factor of approximately 1000 between 37 and 40 C. In all the experiments just quoted, it is the total yield in the chick embryo which was estimated. Sigurdsson (44), however, gave beautiful one-step growth curves of the vesicular stomatitis virus developing in the chick embryo: the titer at 39 to 40 C is 1 per cent of the titer at 35 to 36 C.

Nevertheless, a flask containing a culture of cells, and the chorioallantoic membrane of a chick embryo can hardly be considered as organisms. They are just introductory models.

V. VIRAL INFECTIONS OF ORGANISMS AS AFFECTED BY TEMPERATURE

Our main interest lies in animals. It is, however, only honest to credit plant pathologists for

TABLE 2

*Temperature of the rabbit as affected by external temperature**

Temp of Air	Temp of Rabbit	
	Rectum	Skin
C	C	C
10–23	38.7–39.4	33–36
35–38	39.4–40.5	38–40

* From the data of Thompson (46).

the discovery of the temperature effect. Johnson (28), in 1921, observed that tobacco plants infected with tobacco mosaic virus develop typical manifestations at a range of temperatures from 20 to 30 C. Above 30 C, the sensitivity of the host to the disease is markedly decreased. If infected plants which have developed mosaic disease at 20 C are transferred to 36 C, the signs disappear. The same type of observation was made by Johnson for a potato virus. And in 1936, Kunkel (31) was able to demonstrate that peach trees can be cured from "peach yellow" infection by growing them at 36 C. A number of interesting experiments dealing with the effect of high temperatures on viral infection of plants has appeared since. The problem has been clearly and extensively reviewed by Kassanis (29).

We are dealing with viral diseases of animals. As early as 1934–1935, Wolf (50) attempted to prevent the development of poliomyelitis in monkeys by means of hyperpyrexia. Short waves were utilized in order to increase the temperature. Wolf stated that positive effects were obtained when the treatment was applied early enough.

A few years later, in 1938, Thompson (46) performed a convincing experiment. Rabbits inoculated with the virus of myxoma were kept at two temperature ranges: 10 to 23 C and 35 to 38 C. As seen from table 2, the high ambient temperature markedly increased the temperature of the skin. The effect of the increase was striking: at 35 to 38 C, 5 out of 10 rabbits survived, whereas all the controls at 10 to 23 C died. This was probably the origin of the already mentioned work of Thompson and Coates (47) on the action of high temperature on viral development. I would like only to quote the conclusion of their 1942 paper: "In all living organisms, one of the most important limiting factors for growth is temperature and in most acute diseases, including those caused by viruses, fever is a prominent symptom. It is important therefore to determine the effect of an elevation of temperature upon the growth and survival of infective agents."

The temperature problem did not succeed in capturing the attention of scientists, as exemplified by the fact that the subject is hardly mentioned or not mentioned at all in textbooks or treatises of animal virology. Why this work did not develop is a mystery. A scientist must not only have the right idea, do the right experiments, and give birth to a paper. He also must build a coherent doctrinal corpus and force it into reviews and textbooks. (And must force it also into the brains of his colleagues.)

Anyhow, during the same year, 1942, Armstrong (1) found that mice infected with herpes simplex virus generally die when kept at room temperature, whereas the majority of animals survive if kept at higher temperatures.

The subject was taken over again in 1958, in the United States, by Walker and Boring (49). Their experiments are elegant and impressive. Adult mice (14 to 16 g) are infected with a Coxsackie virus and put at three different temperatures: 4 C; room temperature, that is, 25 C; and 36 C. The internal temperature of mice at room temperature is "normal," that is, 37 to 38 C. In mice at 4 C, it is around 2 C below normal, and in mice at 36 C, it is 2 C above (table 3). In those mice kept at room temperature, the virus multiplies; all nevertheless recover their health. In mice kept at 36 C, the virus does not multiply, and the mice of course survive. In

TABLE 3

*Effect of temperature on mice infected with Coxsackie virus B-1**

Temp		Viral Multiplication		Lesions	Death
External	Internal	2nd Day	4th Day		
C	C				
4	36–37	++	++++	+	+
25	37–38	++	+	+	0
36	39–40	0	0	0	0

25 C, 4 days → 36 C: virus disappears
25 C, 4 days → 4 C: death
(antibodies +)

* Compiled from the data of Walker and Boring (49).

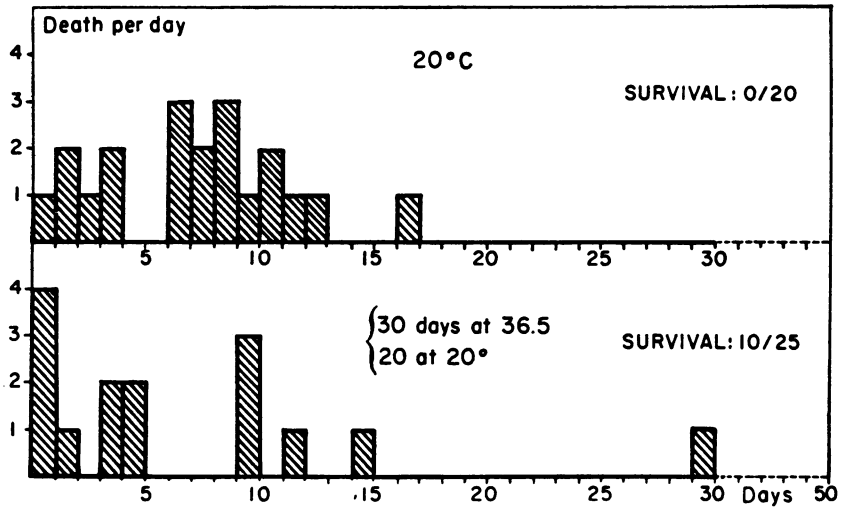

Figure 6. Influence of hyperpyrexia on experimental poliomyelitis of mice. Male R.A.P. mice (15 ± 1 g) injected intracerebrally with 240 pfu of poliovirus type II, strain MEF_1, are kept at 20 C or 36.5 C.

mice kept at 4 C, viral multiplication is important and all the animals die of an acute viral disease. In those infected mice kept at room temperature, antibodies are already present on the 4th day. They nevertheless die if transferred to 4 C. The determining effect of the animal's temperature on the evolution of the viral infection is beautifully exemplified by these experiments.

Similar types of experiments were performed by Lwoff *et al.* (39). Mice (R.A.P. mice 15 ± 1g) were injected intracerebrally with poliovirus type II; the highly neurovirulent MEF_1 strain of Sabin *et al.* (43) was utilized. The LD_{50} is 2.4 plaque-forming units (pfu). Death occurs in all animals, between the 5th and 12th day with 24,000 or 2,400 pfu, between the 8th and 16th day with 240 pfu, and between the 9th and 18th day with 24 pfu. The evolution of the disease is dramatically modified if, instead of keeping the inoculated animals at room temperature (20 C), they are placed in an incubator at 36 C (figure 6). In most of the mice, death occurs markedly later than in the controls; in some cases the infection remains latent as long as the mice are kept in the incubator; finally, in still other cases, infected mice survive. In one of the series injected with 240 pfu, 10/25 mice survived; in another, 10/20. Thus hyperpyrexia may save an animal infected intracerebrally by a high dose of a virulent strain of poliovirus, or transform an hyperacute disease into a latent infection.

VI. THE THREE-BODY PROBLEM

Thus, an increase of temperature and a decrease of pH markedly depress the multiplication of certain viruses. And a mere increase of temperature may protect an infected animal. We are now ready to attack the three-body problem. Three systems are interacting in the infected animal: cell and virus, virus and organism, organism and cell. In an infected animal, the environment of the infected cell is the surrounding tissue, the *milieu intérieur* in a broad sense. It is, however, not the normal *milieu intérieur* but the *milieu intérieur* as modified by the responses of the infected organism. For an animal reacts to viral infection.

One of the reactions is the inflammatory response: the standard features are dilatation of the capillaries and increase of permeability, venous stasis, and infiltration with a variety of cells. As a result, the oxygen supply is decreased. A more or less anaerobic state is created. The CO_2 tension is increased. The glycolysis is increased too and, as the cells are numerous, a large amount of lactic acid is produced; its concentration may reach 2.5 g per L. The pH of inflammatory zones has been measured: it varies, according to the intensity of the reaction, between 6.5 to 5.5 (Frunder (19, 20)). It should be added that cells infected by some viruses produce more acid than normal noninfected cells (Levy and Baron (32), Fisher and Ginsberg (18)). This would tend to increase

the acidity in an inflammatory zone of viral origin.

A short digression concerning the internal pH of the nerve cell would be useful here. It has not been measured in mammals but only in molluscs. The pH of the axoplasma of the giant neurone of *Sepia* (the ink fish) is 6.6, and the pH of the somatoplasm of the neurone of *Aplysia* (the sea hare) varies from 6.0 to 6.4. An increase of the CO_2 pressure to 10 per cent decreases the pH to 5.5 or 5.4 within 3 to 5 min (Arvanitaki and Chalazonitis (2, 3)). It would of course be important to know if these data apply to the mammalian nerve cell.

Another reaction of the infected organism is fever. The studies of Atkins and Huang (4) have led to the conclusion that the increase of temperature is due to a pyrogenic substance, probably of endogenous origin, produced by the infected organism as a reaction toward the viral infection.

Thus, as a consequence of viral development in an infected animal, the temperature is increased and, in inflammatory zones, the pH is decreased. Both factors act in the same direction: they both tend to depress or even stop viral multiplication. The experiment shows that the effects of a high temperature and a low pH are additive. If the inhibition due to temperature is 90 per cent and the inhibition to pH also 90 per cent, the inhibition produced when both factors are at play is 99 per cent.

I would like here to quote Bodian (6): "Although we cannot ignore the possibility that nonspecific viral inhibitors are produced by the inflammatory change, it is nevertheless clear that the decline of virus [poliovirus] in the spinal cord is independent of the intensity of the inflammatory response." Thus Bodian should be credited with the idea that nonspecific inhibitors might be involved in the outcome of a viral infection. To his preventive objection to our views, I would just say that what is important in an inflammatory reaction is not its intensity, it is the production of conditions which shift the state of the cell-virus system below the critical level.

It is known that viruses often disappear completely from an infected animal which has survived an infection. This could be due to the combined effect of specific and nonspecific factors. The inactivation of infectious particles by antibodies does not need to be discussed. However, as mentioned earlier, it is difficult to visualize a total sterilization by antibodies in the case of the acute lethal autosterilizing neuroinfections. It is also clear from the experiments dealing with poliomyelitis in mice that a viral infection can disappear as a result of an increased temperature. It seems as though a decrease of pH and an increase of temperature could be responsible for this phenomenon.

Two mechanisms can be considered: (a) the infected cell survives as a result of an altered metabolism; it is known that some neurons can recover after infection by the poliovirus (Bodian (5), Sabin (41)).

(b) the infected cell dies without infectious particles being produced. This type of abortive infection would eventually lead to the disappearance of the virus. It is known to exist "normally" in cells infected with poliovirus (Dunnebacke and Reaume (13)). A few experiments have shown (*unpublished data*) that the number of infectious centers decreases 20 to 30 per cent in 3 hr when cells infected with poliovirus are maintained at 39 C. It seems probable that a number of factors are able to turn a productive infection into an abortive one.

We have studied only two of the factors which are able to play a role in the evolution of a viral disease, but it would really be strange if pH and temperature were the only nonspecific factors affecting the evolution of viral infection. It is known that, as a result of viral development, substances are produced or liberated which inhibit viral multiplication. Such is "interferon" (Isaacs and Lindenmann (25)). And, as a matter of fact, an interferon-like substance is produced during the development of certain polioviruses, as shown by Ho and Enders (23). These authors have put forward the hypothesis that interferon might be produced in inflammatory zones and play a role in the mechanisms of resistance operative during the acute phase of viral diseases.

In an inflammatory zone many substances are produced or liberated and some of them are likely to modify the cell-virus system and influence viral multiplication. Moreover, hormones which modify the cellular metabolism could also act indirectly on the vegetative virus. A number of agents are in fact known to alter the response of an infected animal. This is the case for cortisone, which can confer sensitivity to a virus to an otherwise resistant animal. The action of cortisone is manifold: it is known to act on the mesenchymal tissue; it produces an involution of

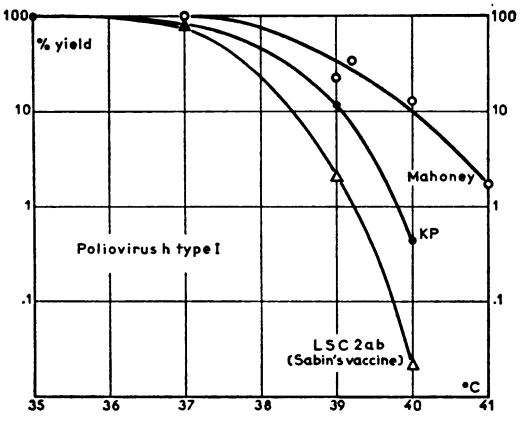

Figure 7. Development of three strains of poliovirus type I as affected by temperature.

lymph nodes and thymus and a circulatory lymphopenia; and it diminishes the antibody production. It also decreases the local manifestation of inflammation.

The mechanism by which cortisone influences viral infections (Thomas (45)) is certainly complex and is not yet understood. It seems possible that its effect is due in part to the decrease of the inflammatory response.

Dubos has discovered that the viability and the multiplication *in vitro* of the tubercle bacillus are unfavorably influenced at low pH by concentrations of lactic acid of the order of those encountered in inflammatory zones. In his already classical book, *Biochemical Determinants of Microbial Diseases*, Dubos (12) stresses the importance of inflammation and clearly shows how and why the inflammatory response helps overcome the microbial infection. His conclusions seem to apply also to viral infections.

VII. VIRULENCE

Within a given viral pathogenic species, some strains are virulent whereas others are devoid of virulence. Is there any correlation between virulence and sensitivity of viral development to high temperature? Three strains of poliovirus type I have been studied. One is the highly attenuated strain (LSc2ab) used by Sabin as live vaccine for immunization of humans. It is completely devoid of virulence for the monkey by the oral route as well as by intracerebral inoculation, which is a more severe test. It has only slight activity after inoculation of large doses directly in the anterior horn of the spinal cord.

The second (K.P.) is the Brunhilde-Enders strain which, according to Sabin (40, 41), is of relatively low virulence for the monkey by the intracerebral route but not by the spinal route. The third is the highly virulent Mahoney strain. It is clear (figure 7) that the curves expressing the sensitivity of the viral development to temperature differ with the strain. The more neurovirulent the strain, the less sensitive to high temperature is its development (A. and M. Lwoff (38)).

The various strains of poliovirus type I have without doubt derived by mutation from a common ancestor. By growing a given strain either at high or at low temperature, mutants can be selected which exhibit considerable difference from the original parent strain.

Dubes and Wenner (10) have studied the effect of growth at low temperature. By selecting polioviruses able to develop at 23 C, strains were obtained, the development of which according to Dubes and Wenner is "blocked" around 37 to 38 C. The same operation has been performed with the Brunhilde-Enders strain (N. Groman and A. and M. Lwoff, *unpublished data*). After a few passages at 30, 27, and 25 C, a strain was obtained which is already inhibited at 39 C (figure 8). It is markedly more sensitive to high tempera-

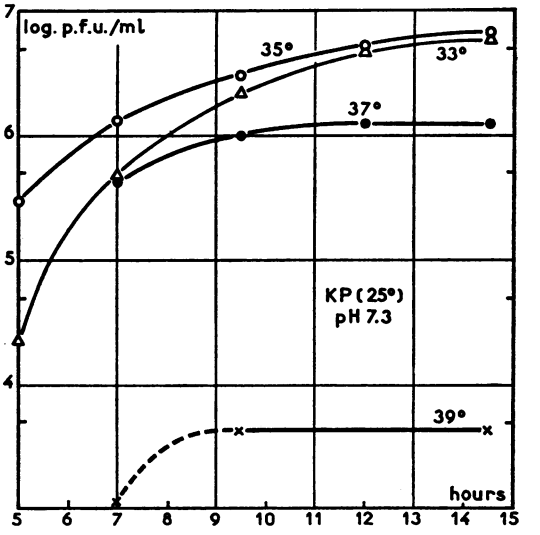

Figure 8. Development of a "cold" strain of poliovirus as a function of temperature. The Brunhilde-Enders strain has been selected by serial transfers at 30 to 27 and 25 C. (After 5 more passages at 25 C, the strain now shows an 87 per cent inhibition at 37 C and a 97.3 per cent inhibition at 38 C.) (From N. Groman, A. and M. Lwoff, *unpublished data.*)

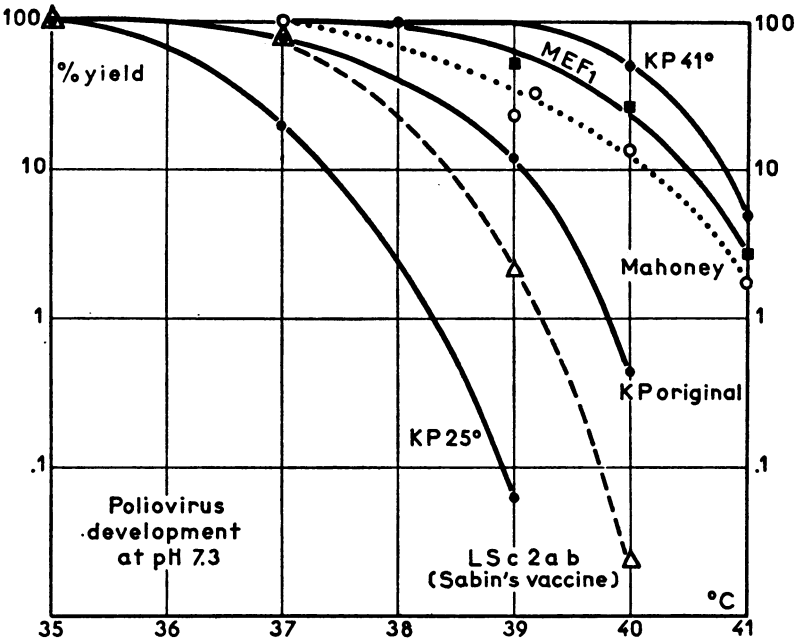

Figure 9. Comparative effect of temperature on the development of various strains of poliovirus and their mutants. KP 41 (Brunhilde-Enders-Chimpanzee) has undergone 9 passages at 41 C. KP 25 has been grown at 25 C (see legend of figure 8).

tures than the original strain. According to Dubes and Wenner, polioviruses grown at 23 C show a markedly diminished virulence by the intraspinal route. Our cold strain did not produce paralysis when doses of 10^6 pfu were placed directly in the gray matter of the lumbar spinal cord of monkeys, whereas the original strain was neurovirulent at low doses (Sabin and Lwoff (42)). It is evident that in order to produce a disease in an animal, a given viral species must be able to multiply at the temperature of this animal; this the cold strain can not do at the temperature of the monkey, which is around 39 C. We can therefore agree with Dubes and Wenner's conclusion: the relative avirulence of a cold adapted strain appears to be due to its reduced capacity to propagate at 37 C or above.

Let us now consider the hot strains. Two of our strains, Brunhilde-Enders and LSc2ab, were passaged on K.B. cells a few times at 39, 39.3, 39.6, 40, and 41 C. After 9 passages at 41 C, their development was markedly more resistant to high temperatures than that of the highly virulent Mahoney strain (figure 9). The curve corresponding to the hot LSc2ab is not represented on the figure: it is very close to that of the hot Brunhilde-Enders strain (labeled KP41) (A. and M. Lwoff (38)).

The neurovirulence of the two hot variants proved to be considerable. Paralysis and prostration were produced by intracerebral inoculation in the monkeys with 170 and even 4 pfu (Sabin and Lwoff (42)). The study of a number of poliovirus strains of types I, II, and III confirms the existence of a correlation between virulence and the possibility for the virulent strains to develop at 40 C. It should be noted that repeated passages of the two type I strains on K.B. cells at 37 C does not alter their neurovirulence (Sabin and Lwoff (42)).

The study of the growth rate (figure 10) has revealed that the hot strains develop more rapidly than the cold ones (A. and M. Lwoff (38)). Finally, it should be added that whereas the average yield per infected cell is generally around 2300 pfu for the hot strains, it is around 100 to 200 pfu for the cold. However, many more strains must be studied before a correlation between rate and yield can be considered as proved.

Several conclusions can be drawn from our data: (a) one is not allowed to state that the development of a virus is "blocked" at a given temperature until the critical point of the system has been determined; (b) the development of each viral strain exhibits a characteristic curve of temperature sensitivity; (c) each viral strain

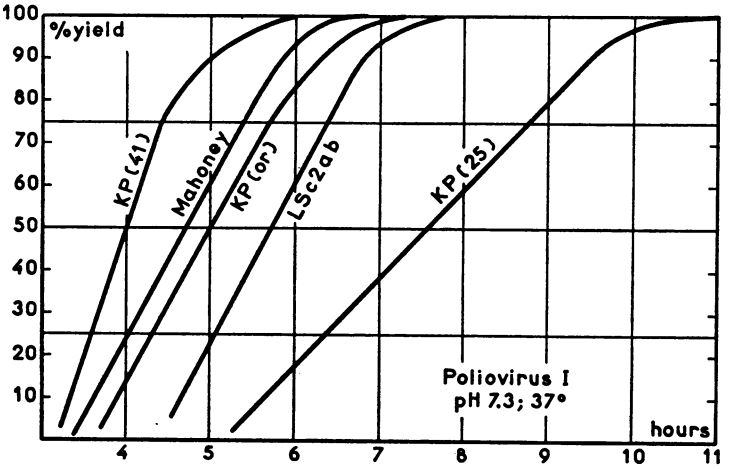

Figure 10. Rate of viral multiplication of various strains of poliovirus I. Note the considerable difference of rates and, by comparison with figure 9, the correlation between thermal sensitivity of viral reproduction and rate.

exhibits a specific rate of multiplication; (d) mutations can shift the curve in one direction or in another.

Viral development being remarkably influenced by temperature within the range of variation of the temperature of the animal body, sensitivity to temperature is necessarily a component of virulence. It is not the only one: sensitivity of the viral development to pH is another.

It was observed by Vogt *et al.* (48) that virulent strains of poliovirus have the same efficiency of plating, that is, give the same number of plaques, on normal media, at pH 7.4, and on media of lower pH. On the contrary, nonvirulent strains do not give plaques on acid media, or give only a very small number. Sabin (41) found that the yield of various strains of poliovirus was decreased at a low pH. The final concentration was measured after 2 to 3 days, when all the cells were destroyed. According to Sabin, the virulent strains are less inhibited than the avirulent ones. It is obvious (figure 5) that the effect of pH on viral development is important. The virulent strains are less affected than nonvirulent ones (A. and M. Lwoff (38)).

The correlation between neurovirulence and pH sensitivity as shown by Sabin (41) and by Hsiung and Melnick (24) is, however, not absolute. Both authors found that mutation toward acid resistance is not necessarily associated with a change of neurovirulence. This is quite natural if the possibility to develop at low pH is not the only component of virulence. In order that a strain be virulent, its development should not be too sensitive to acidity, for the pH of an inflammatory zone is low. But as already seen, its development should also be insensitive to high temperature, for an infected animal may develop fever. All the strains considered so far belong to the type I. A type II MEF_1 strain has been studied which is highly neurovirulent for the mouse. It is of little sensitivity to pH and is less sensitive to high temperature than the virulent Mahoney strain (figure 9). An extensive search of the temperature sensitivity of viral development in relation to virulence will be necessary in order to draw definite conclusions. From the available data, it already appears that the virulent strains are relatively insensitive to high temperatures and to low pH, and that they develop at a relatively high rate. We understand why a virus possessing such features is able to counteract victoriously some of the nonspecific responses of the host which tend to depress viral reproduction.

All this is very simple. But because it is simple, it is not necessarily wrong. Of course, a virus might be able to develop rapidly and abundantly at low pH and at high temperature without necessarily being virulent. The temperature of the chimpanzee is lower than that of the monkey and it is nevertheless more resistant to poliovirus. To produce a disease, a virus must be able to infect some cell species of its host. The sensitivity of the different cells of different tissues may be widely different. Virulence is

obviously a result of the interplay of a number of factors. Two only have been considered up to now, and everything has been unduly simplified.

VIII. GENERALIZED DISCUSSION

To ensure the development of his work, the scientist must make abstraction of many aspects of his problem and decide that his approach is the only important one. The scientist has also to be conscious of the fact that each single phenomenon, as isolated as it may look, is the particular expression of a general law. Generalization is among the most efficient heuristic methods. Is it possible to express our conclusions concerning pH and temperature in such a way that a general principle emerges out of them? We have to try.

Increase of temperature and drop of pH are the consequences of two nonspecific responses of the infected organism. If it is true that virulent strains necessarily possess a low sensitivity to these factors, then the general expression of the hypothesis would be as follows: virulent viral strains have a low sensitivity to the nonspecific responses of the organism controlling viral reproduction. How are things in reality?

The highly virulent strain of myxoma virus kills 99.5 per cent of the infected rabbits within 10 days. According to Fenner and Woodroofe (16), a titer of 10^7 LD_{50} per g is reached around the 4th day in the incubation site and in the local lymph nodes. Afterwards, the titer increases to 5×10^8 in various tissues. Antibodies able to neutralize infectious particles make their appearance on the 9th day. The neutralizing antibodies appear too late, when viral multiplication has been already too abundant and lesions already too developed. Antibodies can decide the fate of an infected animal only if their titer is high enough at a time when, as a consequence of viral multiplication, pathological alterations are not too developed.

According to a general rule any host-virus system undergoes an evolution which involves either one or both partners; the result being that the primary infection becomes rarely fatal, generally mild, and sometimes latent (Burnet (8)).

The original virulent strain of myxoma kills 99.5 per cent of the rabbits. A few years after its introduction in Australia and in France, strains of lower virulence were found in extensive areas which kill only 60 per cent of the infected animals (Fenner and Marshall (15), Fenner and Woodroofe (16), Fenner *et al.* (17), Jacotot *et al.* (26, 27)). The nature of the difference between the virulent and nonvirulent strains of myxoma has not yet been ascertained. It could be that the nonvirulent strain is more sensitive to the nonspecific factors and responses controlling viral multiplication than the virulent one. A support for this hypothesis is found in Kilham's experimental data (30). In cell culture, the virulent myxoma virus grows more rapidly and reaches a higher titer than the closely related avirulent fibroma virus. Moreover, the myxoma virus multiplies at 40 C, whereas the fibroma virus does not proliferate above 38 C.

If the multiplication rate of a given virus in a given host is high, the animal is likely to die before the level of antibodies is such that it can effectually depress or stop viral reproduction. It is clear that the fate of a host-virus system is controlled by a variety of factors: the genetic constitution of the host, which controls the phenotype and susceptibility of its cells; the physiological state of the host, as influenced by age, nutrition, hormonal balance, environmental factors, and the like; the genetic constitution of the virus which controls its ability to infect a given type of cell and the rate and intensity of its multiplication, as affected by the nonspecific reactions of the infected host. The nature and intensity of these nonspecific reactions in turn depend on the nature of the cells and tissues attacked, and on the nature of the virus, that is, on the interaction of the host-virus system. Thus, the "pathogenic activity" of a virus is governed by a number of independent and interdependent variables. However complex a host-virus system might be, one thing is certain: until antibodies have appeared, the nonspecific factors controlling viral multiplication necessarily play an important role in the fate of the infected organism.

In order to be virulent, a virus must multiply abundantly and/or destroy especially important groups of cells before its multiplication is first decreased and later on stopped by the specific responses of the host. Until the effective level of antibodies is reached, viral multiplication is under the potential control of the nonspecific responses. To be virulent, a virus has two solutions: either it does not elicit host responses at all or, if it elicits responses, it manages to be insensitive to them.

We have considered and discussed the possible

role of the resistance of viral multiplication to two of the nonspecific responses of the host. (A. and M. Lwoff (37)). This is certainly, as already stated, only one of the manifold aspects of virulence.

A number of virulent and avirulent viral strains have now to be studied comparatively with regard to the sensitivity of their development to nonspecific responses of the host. The problem of the correlation between rate of viral multiplication and viral yield must be investigated extensively, as must in turn the correlation between these two factors and sensitivity of viral reproduction to high temperature and, finally, the correlation between these three characters and neurovirulence and virulence in general.

Because we know for certain that an increase of temperature can drastically modify the course of a viral disease and because we know, or we think we know, how fever acts, pyretotherapy, ceasing to be a purely empirical procedure, could perhaps be reconsidered—cautiously.

A number of problems have been posed and this paper is, in a certain sense, premature. It is a tragedy of our time that scientists, under the pressure of circumstances, feel compelled to give so many lectures and are thus brought to discuss subjects which sometimes are not quite ripe. But the sour topics have also their charm which partly lies in their unpredictable future.

The conclusions which have been reached concerning virulence can be expressed in the form of a few *vérités de la Palice*.[4] A virus can multiply only if conditions are such that its reproduction is not stopped. In order to multiply and to produce a disease, the reproduction of a virus should not be blocked by the conditions prevailing in its host. The factors which decrease the rate of viral reproduction are likely to decrease also the severity of viral infections. The early nonspecific responses take place before the later specific responses. The resistance of a virus to the host's responses being among the components of virulence, its resistance to the nonspecific responses is necessarily among the components of virulence.

A truism is so evident a proposition that it does not need to be stated. Most of the above statements seem to belong in this category. Under the aspect of truisms, however, a hard truth is hidden which is difficult to bring to light. Once an intricate problem has been solved, its solution appears to be obvious. It is sometimes really so obvious that a long time escapes before it is uncovered. So obvious also that the impression dominates, once the solution has been expressed, that it was known for a long time. Even when the search in textbooks is negative, the scientist necessarily feels that he breaks open an open door and he is afraid of being compromised by producing truisms. Yet truisms must from time to time be expressed.

IX. RETRODUCTION

A review should start with an historical survey, whereas the problem is introduced here as if it were a personal one. This was of course not an absolute necessity but happened to correspond to the lecturer's idiosyncrasy. For this article is not a review but a lecture. As it is here, the lecture will appear in the form of a book together with fifteen other Squibb Contennial Lectures. But a certain number of colleagues have kindly suggested that it should be made available to the bulk of microbiologists, and I am grateful to the Editors of *Bacteriological Reviews* for having once more accepted an unorthodox (at least in form) piece of writing.

The problem of the temperature of the host, normal and pathological, as affecting invasiveness of bacteria has been already discussed by Dubos (11) but, so far as viruses are concerned, this is probably the first attempt at a synthesis. It would have been interesting to compare the data and ideas concerning viruses with those dealing with bacteria; a really general discussion of this important question should certainly see light in the near future. Moreover, although we have dealt with animal viruses, the problem of the action of high temperature on viral diseases of plants (reviewed by Kassanis (29)) has been left aside, as has also the question of the

[4] In 1525, at the battle of Pavia, a French captain of the army of Francis the First was killed in action. His name was Jacques de Chabannes de la Palice. The soldiers composed a song in his honor in which the following verses were to be found: "Monsieur de la Palice is dead. He died by losing his life. A quarter of an hour before his death, he was still alive." This probably meant that la Palice had fought gallantly until the last moment. But only the naive meaning of the verses was retained and the gallant captain was made responsible for a number of very naive statements. This type of truth is called in France "*vérité de la Palice*."

action of temperature on the virus sensitizing *Drosophila* to CO_2 (reviewed by L'Héritier (33)), which has not even been mentioned. The data concerning the action of high and low temperature on the development of bacteriophage have not been mentioned either and this is certainly regrettable, for they are reviewed nowhere. Finally, data concerning temperature as affecting the relative rate of multiplication of organelles such as chloroplasts, or of parasites such as the *kappa* particle, could have been discussed. This would, however, have led to a monograph on biological phenomena as affected by temperature. Until more is known about the mechanisms involved, such a review would not have been very exciting. Considering, however, rapid development of research in this field and the malignant multiplication of symposia, it seems probable that the topic will be selected in the not too distant future.

X. ADDENDUM (JUNE 1959)

I had recently the privilege of discussing the problem of virulence as related to temperature with Sir MacFarlane Burnet, who called my attention to C. Armstrong's work on vaccinia virus (The selection of a heat-resistant strain of vaccine virus (rabbit testicular), Public Health Repts. (U. S.), **44**, 1183–1191, 1929). Armstrong selected viral particles resistant to heating at 37.5 C and passed the selected virus in rabbit testis. A strain was thus obtained exhibiting a much higher thermal resistance than the original one. It was also more pathogenic for the testis, where it produced severe hemorrhagic lesions, and also more invasive, as shown by production of lesions in organs far away from the site of inoculation. Moreover, the heat-resistant strain, as shown by C. Armstrong and R. D. Lillie (Vaccine virus pneumonia in rabbits, Public Health Repts. (U. S.), **44**, 2635–2647, 1929) was able to produce a fatal lobar pneumonia in the rabbit by respiratory infection, a property absent in the original strain.

It would be interesting to repeat these experiments and to compare the effects of growing vaccinia strains at high temperature in cell culture with those of mere passages in the testis of the rabbit. Moreover, it seems now worthwhile to select systematically cold and hot variants of a large number of viruses and assess their properties. These experiments should provide valuable data, especially to test the validity and generality of the hypotheses relating virulence to the ability to grow at high temperature.

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