These changes were present in varying degrees in all the growth hormone–treated, adrenalectomized-ovariectomized rats, in but one of the growth hormone–treated normal controls, and in none of the untreated controls. In addition, both groups of growth hormone-treated animals exhibited distinct extra-articular calcifications at the ankle joint and in the neighborhood of the Achilles tendon and in adjoining fascial planes.

The importance of these observations is provisionally thought to be related to the well-known antagonism existing between the pituitary growth hormone and certain adrenal steroids (as well as ACTH acting indirectly) on the various manifestations of growth. It should be noted that the demonstrated arthropathic effects of purified pituitary growth hormone are *not* mediated by the adrenal gland (or gonads).

If the above observations are confirmed, the groundwork can be laid for the verification of a hypothesis which holds (1) that the pituitary growth hormone may be of direct etiological importance in the chronic arthritides and in related conditions; and (2) that the ameliorative antiarthritic effects of ACTH, cortisone, and hydrocortisone may be considered to represent either suppression of pituitary growth hormone secretion, or antagonism to growth hormone (or to its local effects) at the tissue level, or both. It should be noted, however, that the experimental evidence described herein does not preclude the possible existence of sensitization to growth hormone (endogenous or exogenous) or of production of hypersensitivity to other allergenic factors or agents.

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The Relation of Bacteriophage to the Change of *Corynebacterium diphtheriae* from Avirulence to Virulence[1]

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Freeman (*1, 2*) has reported that exposure of an avirulent strain of *Corynebacterium diphtheriae* to a specific bacteriophage results in the production of virulent *C. diphtheriae*.[2] In addition he has observed that all virulent strains derived in this manner exhibit lysogenicity when tested against the parent avirulent strain. These results, confirmed in part by Parsons and Frobisher (*3*), have been fully confirmed in our own laboratory (*4*). Two possible explanations for the origin of these virulent organisms have been advanced (*1, 2*). They are (*a*) that virulent mutants develop in the avirulent culture and are subsequently selected for by bacteriophage action, and (*b*) that infection and the establishment of the lysogenic state alter the metabolism of the infected cells, with resultant production of toxin. The present report provides evidence compatible with the hypothesis linking lysogenicity and virulence and inconsistent with the hypothesis of mutation and selection.

Strain 444 of avirulent *C. diphtheriae*[3] as designated by Freeman (*1*) was used in the work to be described. This parent avirulent strain and the derived virulent strains will be referred to as 444A and 444V, respectively. The bacteriophage employed throughout has been designated 444V/A. It was isolated from strain 444V, produced by exposing 444A to bacteriophage B, described by Freeman (*1*), and was propagated on 444A. In all probability it is identical with bacteriophage B. Investigation of this phage-host system (*4*) indicates that, although it is strongly lytic, lysogenic cells are produced with extraordinary facility. It is similar in this respect to systems described by Burnet and Lush (*5*) for a *Staphylococcus* and Boyd (*6*) for *Salmonella typhimurium.*

The correlation between virulence and lysogenicity observed by Freeman (*1*) in the derived strain 444V, and repeatedly confirmed in the course of the present work, is highly suggestive of a causal relationship between the two changes in character. Nevertheless, it can be argued that a virulent mutant arising independently of bacteriophage action might simultaneously become receptive to a state of lysogenicity. If this occurred the establishment of the lysogenic state would be a result of the change to virulence rather than its cause. Thus, other evidence is required before any significance can be attached to this correlation.

Strong evidence against the mutation-selection hypothesis was obtained in the following manner. Samples were removed periodically from a mixture of *C. diphtheriae* 444A and bacteriophage 444V/A. Each sample was plated for total bacterial count and analyzed for the relative numbers of virulent and avirulent cells present. A differential medium exploiting the visibility of toxin-antitoxin precipitates in an *in vitro* system was used to distinguish between virulent and avirulent colonies. Plate differentiation was confirmed by guinea pig intracutaneous tests and *in vitro* virulence tests (*7*). Lysogenicity was demonstrated using parent strain 444A as the indicator strain. Because of the clumping exhibited in the normal growth of *C. diphtheriae* the counts obtained represent "clump" counts. The data are presented in Table 1.

[1] Supported in part by State of Washington funds for medical and biological research.

[2] The terms "avirulent" and "virulent" are used synonymously with "nontoxigenic" and "toxigenic."

[3] Kindly supplied by V. J. Freeman.

TABLE 1

NUMBERS OF VIRULENT *C. diphtheriae* PRESENT ON INCUBATING AVIRULENT STRAIN 444A WITH BACTERIOPHAGE 444V/A

Expt. no.	Time elapsed (hr)	Bacteria	
		Total no./ml	No. virulent/ml
1	0	2.4×10^5	< 1*
	3¾	1.4×10^5	640
	5¾	5.0×10^4	23,000
2	0	1.9×10^5	< 1*
	3½	9.6×10^4	2,000
	5	6.4×10^4	37,000

* None detected in the sample.

Examination of the increase in the numbers of virulent cells (Table 1) reveals some important facts. It has been determined in independent experiments that the shortest generation time of virulent strain 444V is 51 min under the conditions of the experiments. It can be calculated that in the period of 3¾ to 5¾ hr (Expt. 1) the virulent population of 640 cells/ml, irrespective of its origin, would have reached a density of 3300 cells/ml had it multiplied at the maximum rate. The determined population of 23,000 virulent cells/ml is far in excess of this. Similarly during the 3½–5-hr period of Expt. 2 the population density would have increased from 2000 to 6800 virulent cells/ml had multiplication occurred at the optimal rate. Again the determined population of 37,000 cells/ml is far in excess of this figure. Thus, in both instances many more virulent cells were present at the end of the experimental interval than could be accounted for by division alone even if multiplication had occurred at the optimal rate. It is apparent from these facts that large numbers of additional virulent cells appeared during the experimental interval. It can also be calculated that it would have required mutation to virulence during the first division of 2.7% (Expt. 1) and 9.2% (Expt. 2) of the avirulent population present at the beginning of the cited experimental intervals to achieve the virulent populations present at their termination. This far exceeds any expected rate of spontaneous mutation. Furthermore, the decrease in total bacterial count, resulting from continued lysis of large numbers of avirulent cells by bacteriophage, would tend to increase these theoretical mutation rate requirements.

TABLE 2

NUMBERS OF VIRULENT COLONIES OF *C. diphtheriae* DEVELOPING AFTER A 30-MIN CONTACT BETWEEN AVIRULENT STRAIN 444A AND BACTERIOPHAGE 444V/A

Expt. no.	No. phage-infected cells deposited on the plate	No. of virulent colonies developed	Percentage cells converted to virulence
1	142	7	4.9
2	108	3	2.8
3	99	1	1.0
4	268	4	1.5
5	701	1	0.1

Further evidence was obtained which fails to support the hypothesis of mutant selection by bacteriophage. In a series of experiments, strain 444A was mixed with bacteriophage 444V/A in heart infusion broth (Difco) and incubated in a 37°C water bath for 30 min. The mixture was then diluted and aliquots were spread directly on the differential medium. In all cases the concentration of bacteriophage on the plate was low enough to rule out any reasonable possibility of contact between a phage particle and a developing colony.

The data presented in Table 2 show that 0.1–4.9% of the phage-infected cells deposited on the plates developed into virulent colonies. The percentage of virulent clones produced far exceeds any anticipated mutation rate. Furthermore, controls of avirulent cells alone plated in these and in numerous experiments performed during the past year have never given evidence of a single virulent colony. Thus again, new, virulent cells were produced by exposure of avirulent cells to bacteriophage lysates and at a rate far in excess of any expected spontaneous mutation.

The induction of virulence, as evidenced above, could conceivably be due to the presence of a soluble transforming principle released during the preparation of the bacteriophage stocks. Preliminary experiments were performed in which the stock solution of bacteriophage 444V/A was treated with crystalline desoxyribonuclease (Worthington) under conditions of activity described by McCarty and Avery (*8*). The enzyme concentration was 200 times the maximum used by these investigators to completely destroy the activity of pneumococcal transforming factor. The enzyme-treated bacteriophage suspension retained its ability to produce virulent *C. diphtheriae* from strain 444A. This strongly indicates the absence of a soluble transforming principle of the desoxyribonucleic acid type. However, other soluble transforming agents may exist which would be unaffected by this treatment.

In summary then, the theory that virulent mutants arise in strain 444A of *C. diphtheriae* and are selected for by bacteriophage is not supported by the facts presented here. Thus it appears that the change to virulence is an induced phenomenon. The evidence presented tends to rule out transformation by a soluble principle of the DNA type. On the other hand, the striking correlation between lysogenicity and virulence makes a causal relationship between these two changes seem probable. The high concentration of desoxyribonucleic acid present in the bacterial viruses that have been studied (*9*), and the intimate relationship that must exist between a bacterial cell and its symbiotic phage, give strong support to the concept that bacteriophage may indeed act as a particulate transforming principle. It is proposed to retain as a working hypothesis that the establishment of the lyso-

genic state results in an altered metabolism of the avirulent cell, an alteration manifest as toxin production.

A complete report of this work will be published elsewhere.

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Technical Modifications of Radiocardiography[1]

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Radiocardiography was described in 1948 by Prinzmetal *et al.* (*1*), and its clinical applications were presented a year later (*2*). In this method, a Geiger-Mueller counter with auxiliary equipment records graphically the passing of radioactive blood through the cardiac chambers. Several disadvantages found in the application of the original method led to modifications which are reported below.

Original method (*1, 2*). A shielded Geiger-Mueller (GM) tube is placed in front of the precordium of the sitting patient, and 0.1–0.2 mc radiosodium (Na^{24}) is injected into one of the antecubital veins. The counts are graphically recorded from right to left by means of a specially constructed ink-writing device. The curve is corrected by taking the means of counts, and the reconstructed tracing consists of two main waves (*R* and *L*) representing the passage of the isotope through the right and left ventricles, respectively. The two waves are connected by a transitional plateau. The end of the second wave is usually at a much higher level than the baseline.

Disadvantages of the method. The following disadvantages were found in preliminary experiments:

a) Radioactive sodium has a short half-life (14.8 hr) and may not be available when necessary. Shipment from production centers to the laboratory is by air freight and is very expensive.

b) The poor sensitivity of the GM counter originally used required a large dose of isotope for injection.

c) The ink-writing device is not very accurate. Writing from right to left is in contrast with accepted techniques and makes the reading awkward. The actual graph consists of several irregular oscillations; the means of counts is a somewhat arbitrary and subjective procedure which lacks accuracy.

d) There is no possibility of simultaneously recording radiocardiograms and other tracings for physiological or clinical correlation.

Modifications of technique. The isotope used in our study was I^{131} in the form of diiodofluorescein;[2] a dose of 20 mc in 0.5–1 ml was injected. As the half-life of I^{131} is about one week, several clinical experiments were carried out with the material of each shipment.

The detector was a bismuth gamma GM tube[3] contained in a directional lead shield. The tube was suspended from a vertical stand, and the opening of the shield was placed about 1 in. from the center of the precordium (Erb's point) of the supine patient. The tube was connected to a count rate meter[4] and the outlet of the latter, ending in a telephone plug, was connected to a direct-writing electrocardiograph[5] with 4 channels. This permitted simultaneous recording of the radiocardiogram and of any other clinical tracing. Film speed generally used was 10 mm/sec. In some experiments, however, film speeds of 25 or 50 mm/sec were used. A signal marked the time of injection.

In our experiments, an electrocardiogram and a carotid or brachial tracing, or a respiratory tracing, were recorded with the radiocardiogram. This permitted us to ascertain the number of cardiac cycles necessary for the isotope to go through the right or the left side of the heart.

Several technical difficulties were still encountered, and some of them are not yet solved. The record of the Poly-Viso, like that of most amplifier-type galvanometers, is a plot of logarithmic intensity vs. time. There is therefore no linear proportion between the height of a deflection and the amount of isotope in the GM tube field. This tends to increase the smaller deflections and, therefore, the background effects. The use of a specially built amplifier is contemplated in future experiments.

With suitable degree of amplification, the special characteristics of the amplifier-type galvanometers automatically transform the multiple and irregular discharges of the counter into slower and more regular waves. This transformation is equivalent to, but more accurate than, the arbitrary means of discharges previously drawn over the graphs. It should be kept in mind that, following a large and slow positive deflection, the graph sometimes presents a negative deflection. This is an artifact that is due to the technical characteristics of the amplifier-type of galvanometers and should be disregarded.

[1] This study was performed under the tenure of a teaching grant of the National Heart Institute, USPHS, held by A. A. Luisada.

[2] The isotope was obtained from the Abbott Laboratories, North Chicago, Ill., on allocation from the U. S. Atomic Energy Commission.

[3] The tube was Mark 1, model 13, of Radiation Counter Laboratories.

[4] The meter was No. 1615, supplied by the Nuclear Instrument and Chemical Co., to whom we are indebted for their cooperation.

[5] The electrocardiograph used was a Sanborn Poly-Viso.