of identification, powder diffraction interferences are given in Table 1.

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OBSERVATION OF BACTERIOPHAGE THROUGH A LIGHT MICROSCOPE[1]

ELECTRON micrographs of bacteriophage attacking *Escherichia coli*,[2] *Salmonella pullorum*[3] and *Rhizobium leguminosarum*[4] show that the particles have a diameter greater than that of bacterial flagella; with the latter organism, the particles have a diameter 5 to 10 times greater than that of flagella. Since the bacterial flagella can be stained and made visible under an ordinary light microscope, it seemed that the same should be possible with bacteriophage particles (sometimes called bacterial viruses).[2]

The discovery was made during the winter of 1943–44 that bacteriophage preparations treated with auramin and observed under the microscope when radiated with ultraviolet rays from an H-4 mercury arc show the presence of many bright yellow pinpoints of light in an otherwise dark field. Bacteria failed to show anything other than a very pale and weak yellow color, and the granules were not observed unless bacteriophage particles were known to be present. The past winter this work was repeated with quite similar results, and two additional methods for revealing bacteriophage particles were developed. These involved the use of stains (one, a modification of the acid-fast stain) and ordinary light; and by these the bacteriophage particles were seen to have the same shape as under the electron microscope.

Since at that time the bacteriophage had not been studied in active condition, there was no way of being absolutely certain as to the sequence in the process of lysis of the various structures. The drawings presented in Fig. 1, however, show the apparent order of events. Beginning with bacterial cells of the pea nodule organism (*a*), which show the refractile bodies usually seen following light staining with methylene blue or rose bengal, and the bacteriophage (*b*), which always stained heavily, it seemed that the first contact of the bacteriophage with the cell resulted in a delicate connection between the two, as shown in the first drawing in (*c*), the particle appearing at a greater distance from the cell than would ordinarily be expected. Other cells were seen in which the bacteriophage particle had developed to larger size and in which there had been a corresponding shortening of the connection to the bacterium, and still other cells to which relatively large particles of bacteriophage were attached.

Still other cells were observed in which these phenomena were absent (*d*), but in which the usual refractile bodies were replaced by other particles which stained heavily and had a quite different position in the cell. Other cells had apparently broken down, leaving a faintly colored material which stained in a manner characteristic of cell protoplasm, but the material was quite different in outline from the original cell and included varying numbers of deeply stained bodies. In some such partially destroyed cells, the latter bodies were small, and in others, relatively large. Next in the process of lysis it seemed that the typical structures consisted of faintly staining rem-

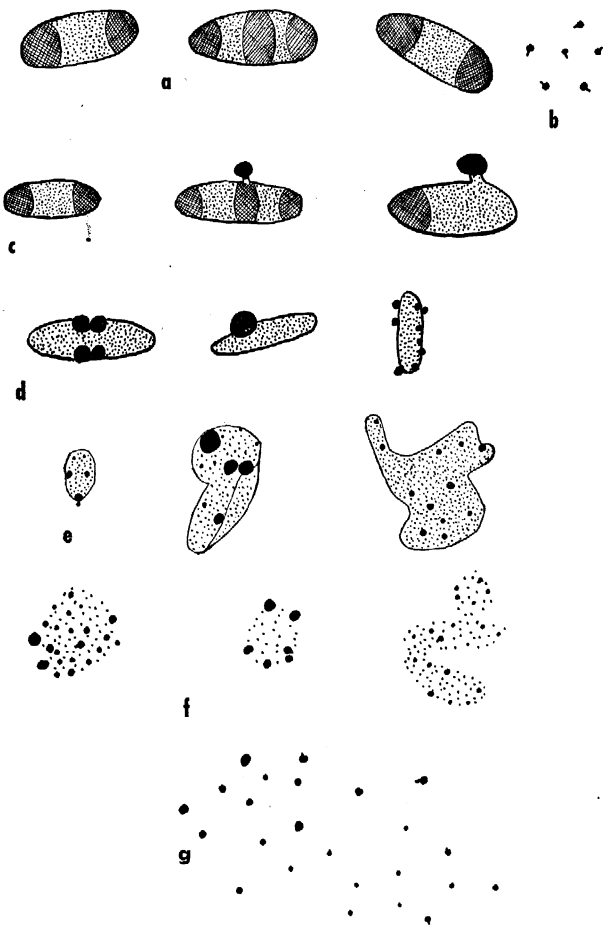

FIG. 1. Progressive stages in the lysis of bacterial cells as indicated by stained material examined under the ordinary microscope. a. Cells of the pea nodule organism. b. Bacteriophage particles. c. Cells in early stages of bacteriophage action. d. Cells in which bacteriophage action was well under way. e. Partially lysed cells showing protoplasm in which the development of densely staining bodies is evident. f. Remnants of cell protoplasm among which are numerous bacteriophage particles that have developed from the cell protoplasm. g. A concentration of densely staining bodies around an area that was probably one or more cells, but in which no cell material is evident.

[1] Jour. Paper No. 623, N. Y. S. Agric. Exp. Station, Geneva, N. Y., March 28, 1945.

[2] S. E. Luria, M. Delbrück and T. F. Anderson, *Jour. Bact.*, 46: 57–76, 1943.

[3] M. R. B. Baylor, J. M. Severens and G. L. Clark, *Jour. Bact.*, 47: 277–284, 1944.

[4] A. W. Hofer. Unpublished data.

nants of cells (*f*), among which were numerous minute bodies with an intense color. In some areas (*g*) only the latter bodies were seen, the cell material apparently having disappeared. In general, the material in which the process of lysis was near completion stained easily and gave clear-cut results, while particles stained during the early stages of lysis were hard to detect; it is possible that the bacteriophage particles lost their usual staining qualities when in contact with the cell.

The difficulty involved in deciphering the exact sequence of events was largely overcome by use of the new phase difference microscope.[5] Under this, not only were structures noted as above, but the process of lysis was observed, making the details sufficiently certain that the above sequence could be completed from the stained material. The first steps in the process of lysis were followed most easily, difficulty occurring only in the observation of the last phases (due to the difficulty of maintaining the preparation on a slide). Yet, enough was seen to indicate that the process of lysis by bacteriophage may not be a well-defined, simple series of steps, but rather a group of phenomena of variable nature. Even in the study of two strains of the pea nodule organism and two races of bacteriophage specific for them, a number of unexpected developments were observed.

Beginning with the cells, which were motile and uniformly colorless, and the bacteriophage, which occurred as minute, highly refractile bluish particles, the placing of both in the same suspension bring about one or more of the following results, though so far not all have occurred in any one suspension:

(1) Loss of motility of the cells within 1 to 2 minutes after addition of the bacteriophage.

(2) Clumping of the cells, as by agglutination.

(3) Development of a blue color in the cells, apparently due to a change in the refractive index.

(4) A tendency for the cells to assume a vertical instead of a horizontal position, as if one end of the cell were heavier than the other.

(5) The uniting of a bacteriophage particle with a cell, the anchoring of the tail of the particle to the cell, the lengthening of the tail as shown by the first drawing in (c), the movement of the head of the particle with Brownian movement while the tail remained solidly anchored to the cell, the enlargement of the entire particle and the eventual rupture of the cell, with loss of cell material to such an extent that it eventually surrounded and concealed the original bacteriophage particle.

(6) The development within a cell of relatively large refractile bodies as in (d).

(7) The appearance in suspensions of lysed bacteria of many pearly white masses of cell protoplasm, each containing highly refractile bodies as in (e). Under the phase difference microscope the unstained cell material had a pearly white color, amid which developed blue bodies of two or three different sizes.

(8) Cells that were almost completely lyzed as in (f) (groups of single bodies as in (g) were not seen, as these would tend to become scattered in the suspension, even though no motility of the bacteriophage was noted).

(9) Many cells that had developed differently from what was described above, there being little or no evidence of the presence of bacteriophage of the shape indicated by the electron microscope. Such cells assume grotesque shapes, develop greatly in size, and it is believed that eventually they disappear quite suddenly.

(10) Occasional large bodies of what appears to be cell protoplasm containing small blue bodies so arranged as to be suggestive of chromatin-like material.

Thus, the evidence from the electron microscope, the stained preparations and the phase difference microscope is similar as regards the first and last stages of lysis, and the two latter agree quite well in what they show of the phenomena occurring between these points. Further evidence to the same effect is furnished by the dark-field motion picture made by Dr. A. J. Pijper, of Pretoria, South Africa, and just made available through the courtesy of Dr. Harry E. Morton, of Philadelphia.

This shows lysis by relatively large particles of a bacteriophage specific for and acting upon a non-motile strain of *Eberthella typhosa*. While the attachment of the particles to the cells does not show as clearly as some other parts of the process, the development of the bacteriophage bodies within the cell is striking. Instead of oozing cell material, however, these cells collapsed or produced "bubbles" in the motion pictures. Thus, the interpretation of the process developed by the present authors, as it occurs with *Rhizobium leguminosarum*, is essentially similar to what was later seen in the film of *Eberthella typhosa*. Such differences as occur between the two observations are possibly due to the fact that the bacteria and bacteriophage used in the two cases were different.

Because the results of the four different methods of observation are so similar, it seems well to present this preliminary report. It is also felt that special mention should be made of the advantages of phase difference microscopy in the study of such minute bodies in active condition. Photographs taken of such material under the light microscope, procedures used in the staining of bacteriophage, description of some of the pitfalls awaiting the newcomer in the field and

[5] A. H. Bennett, *Anat. Rec.*, 89s: 19, 1944; O. W. Richards, *Nature*, 154: 672, 1944.

other detailed information will be published as soon as conditions permit.[6]

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EFFECT OF ALLOXAN DIABETES ON THE GROWING RAT

THE purpose of this present investigation was to study alloxan diabetes in the immature animal and to observe the effect of early insulin deprivation on somatic growth. The effect of alloxan in destroying the β-cells of the Langerhans islets[1-5] provides a convenient method of producing diabetes experimentally. It is difficult to ascertain to what extent this metabolic disturbance affects growth in man, since most juvenile diabetics have already attained an excessive height for their age at the time the diabetes becomes apparent.

The animals used for this experiment were 26-day-old Sprague Dawley rats on a diet of Wayne Fox Chow Blox. They were divided into four groups, each consisting of four males and four females. One group was not injected and served as control for the other three groups, which were injected subcutaneously with a 1 per cent. solution of alloxan monohydrate in doses of 25, 30 and 40 mg per gm of body weight, respectively. Within half an hour after injection, alloxan appeared in the urine. It was accompanied by a fall in pH and by the red color which alloxan produces in the presence of amino acids. To control the hypoglycemia[6] which develops after the initial hyperglycemia, the rats were given a 5 per cent. glucose solution to drink for the first day after injection. Insulin was at no time administered to these animals. The alloxan treated rats except one of the females injected with the lowest dose developed diabetes. Glycosuria was more severe in the group with the largest dose and the diabetes persisted in this group, although it disappeared in several of the animals with smaller doses. All the animals in this experiment were lively after the first day and survived until sacrificed at 61 days of age or at some later date.

The effect of alloxan diabetes in stunting the growth of the rat is apparent from the figures in Table 1.

TABLE 1

Dose of alloxan mg/100 g	Sex	Body weight in grams at the age of: 26 days	35 days	42 days	48 days	55 days	61 days
Control	F	46	74	104	121	144	158 (144–168)*
25	F	45	64	94	112	131	144 (136–153)
30	F	45	67	98	115	124	149 (142–155)
40	F	47	57	70	78	95	106 (55–138)
Control	M	46	74	103	126	163	191 (180–213)
25	M	50	72	100	116	143	164 (131–192)
30	M	46	74	109	124	147	179 (169–195)
40	M	47	56	64	70	76	87 (58–111)

* Figures in parenthesis indicate range of values.

Certain other differences were observed in the diabetic rats. One out of eight animals, 0/8, and 3/8 of the 25 mg, 30 mg and 40 mg groups respectively developed cataracts of the lenses.[7] The dwarfed animals had distended abdomens, and at autopsy the stomach and intestines of these animals measured longer, relative to their body weight, than those of the controls. Some of these rats also showed infantile primary and secondary sex organs.

Summary: Severe alloxan diabetes dwarfed the immature rat.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE, RAPID TECHNIC OF PREPARING WATER-IN-OIL EMULSIONS OF PENICILLIN, DRUGS AND BIOLOGICS[1]

WATER-IN-OIL emulsions have proved of value in experimental immunization procedures[2] and may become applicable to the immunization of man and domestic animals. Strauch[3] described the use of

[6] The authors wish to acknowledge helpful suggestions in regard to this work by H. J. Conn and Mary A. Darrow; the senior author wishes to express his gratitude to A. H. Bennett for the use of experimental equipment.

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[6] C. C. Bailey and O. T. Bailey, *Jour. Am. Med. Asn.*, 122: 1165, 1943.

[7] C. C. Bailey, O. T. Bailey and R. S. Leech, *New Eng. Jour. Med.*, 230: 533, 1944.

[1] From the Public Health Research Institute of the City of New York, Inc.

[2] (a) J. Freund and K. McDermott, *Proc. Soc. Exp. Biol. and Med.*, 49: 548, 1942. (b) J. Freund and M. V. Bonanto, *Jour. Immunol.*, 48: 325, 1944. (c) M. W. Chase, *Proc. Soc. Exp. Biol. and Med.*, 52: 238, 1943. (d) L. M. Kopeloff and N. Kopeloff, *Fed. Proc.* (*Am.*