government issued an air-pollution alert for the New Jersey area. The average hourly aldehyde content during this time was greater than the average for normal days, but it did not become large enough to damage plants (Fig. 3). The oxidant content of the air averaged 0.125 parts per million for the hours between 11:00 A.M. and 3:00 P.M. and caused considerable damage to oxidant-sensitive plants. Both pollutants were in excess of normal levels and the inverse relationship between aldehyde and oxidant no longer held. Such interrelationships between contaminants may be of significance in explaining the total pollution complex.

Thus, it has been shown that the aldehyde content of ambient air fluctuates widely from day to day and from hour to hour depending on atmospheric conditions and other factors. Furthermore, each time the concentration exceeded 0.20 parts per million for 2 hours or 0.30 parts per million for a single hour, damage to petunia foliage could be observed in a day or two. Experimentally controlled fumigations are planned to verify the role of aldehydes in causing plant damage.

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References and Notes

1. O. C. Taylor, E. R. Stephens, E. F. Darley, E. A. Cardiff, *Am. Soc. Hort. Sci.* **75**, 435 (1960).
2. E. R. Stephens, E. F. Darley, O. C. Taylor, W. E. Scott, *Intern. J. Air Water Pollution* **4**, 79 (1961).
3. M. Jacobs, *The Chemical Analysis of Air Pollutants* (Interstate, New York, 1960), p. 223.
4. F. H. Goldman and H. Yagoda, *Anal. Chem.* **15**, 377 (1943).
5. A. P. Altshuller, I. R. Cohen, M. E. Meyere, A. F. Wartburg, Jr., *Anal. Chim. Acta* **25**, 101 (1961).

9 January 1964

Cell Wall Replication in Salmonella typhosa

Abstract. *Changes in the fluorescence of the cell wall of* Salmonella typhosa *(TY2W) were studied during growth after direct labeling with fluorescein conjugated homologous or "anti-O" globulins. Fluorescence decreased evenly with culture growth and cell division, but the addition of chloramphenicol resulted in large, nondividing cells that showed increasing interruption of fluorescence of the wall marker. The process thus differs from the equatorial origin and discrete hemispherical addition of new wall previously described in* Streptococcus pyogenes. *These findings, in addition to demonstrating the formation of new wall in the presence of chloramphenicol, appear consistent only with the concept that wall replication in the salmonellas occurs by means of diffuse intercalation of new materials among old.*

By direct immunofluorescent labeling of cell wall antigens of growing organisms, it has been shown that new wall in *Streptococcus pyogenes* is initiated in the equatorial region, from which it extends both peripherally and centrally (*1*). A similar discrete origin of new wall, though bilaterally apical instead of equatorial, was subsequently reported in *Schizosaccharomyces pombe* by May who used indirect immunofluorescence (*2*). We have now applied the direct technique to a study of wall replication in *Salmonella typhosa* (*3*).

The strain of *S. typhosa* used was TY2W, which lacks Vi antigen (*4*). Globulin fractions of rabbit antisera to whole TY2W organisms and to strain O-901 (O antigens, 9 and 12 only) were prepared as usual by 50 percent saturation with ammonium sulfate followed by dialysis. The globulins were labeled with fluorescein isothiocyanate and passed through Sephadex G-25 as previously described (*5*).

In a typical experiment, TY2W was grown in Penassay broth (*6*) for 8 hours; 0.5 ml of the culture was then washed and resuspended to an optical density of 0.05 at 530 mμ. This suspension was incubated for ½ hour with 1.0 ml of labeled homologous or O-901 antibody. (Results were the same with both antibodies). The cells were then removed by centrifugation, washed twice with fresh broth, and resuspended in 4.0 ml of fresh Penassay broth with or without chloramphenicol (10 μg/ml). Samples were taken immediately and the culture was again incubated at 37°C with shaking. More samples were taken at 30, 60, 120, 180, 240, and 300 minutes. From each sample, 0.3 ml was immediately removed, cooled in an ice bath, and centrifuged at 4°C. The cells obtained were washed twice with cold phosphate-buffered saline at *p*H 7.4, and smears were made on clean glass slides and dried in air. Another 0.1 ml of each sample was diluted appropriately from 1×10^{-3} through 1×10^{-7}, and pour plates were made in trypticase soy agar from the samples at 0, 60, 180, and 300 minutes. Colony counts were made after incubation of the plates at 37°C for 18 hours.

Another set of smears was made as described, except that the cells were exposed initially to unlabeled antibody globulin. These smears were subsequently stained on the slide by applying a portion of the same globulin which was fluorescein labeled. This procedure constitutes a reverse technique, as previously noted; methods of mounting, microscopic examination, and photography were also reported (*1*). All photographs represent the same conditions of exposure, development, and printing.

With increasing time of incubation in broth free of both globulin and chloramphenicol the fluorescence of the antibody marker diminished diffusely after direct labeling (Fig. 1, *a–c*). Correspondingly, the reverse method demonstrated increasing immunofluorescent stainability of the cell wall as time of incubation increased, although it changed little after 60 minutes. (Fig. 1, *d–f*). Samples at 300 minutes, after direct labeling, were too faint to reproduce photographically. Colony counts, $\times 10^6$ per 0.1 ml, were 12, 25, 120, and 365 at, respectively, 0, 60, 180, and 300 minutes. Growth was therefore exponential with a generation time of approximately 60 minutes under these conditions.

If the cells were killed by heat or formalin immediately after initial labeling, they remained brilliantly fluorescent throughout similar periods of incubation. After 300 minutes of incubation, amounts of supernatant from killed labeled cells, from living labeled cells, and from living cells binding unlabeled antibody were examined in an Aminco-Bowman spectrophotofluorometer at an excitation wavelength of 450 mμ and fluorescence emission at maximum intensity of 525 mμ (*7*). In each instance the emissions were the same within limits of error of dilution and reading, and did not therefore indicate detectable loss of the fluorescein label from either killed or living cells into the medium. In another control experiment to ascertain if loss of label from the living cell surface occurred, chloramphenicol was added to a series of replicate tubes containing

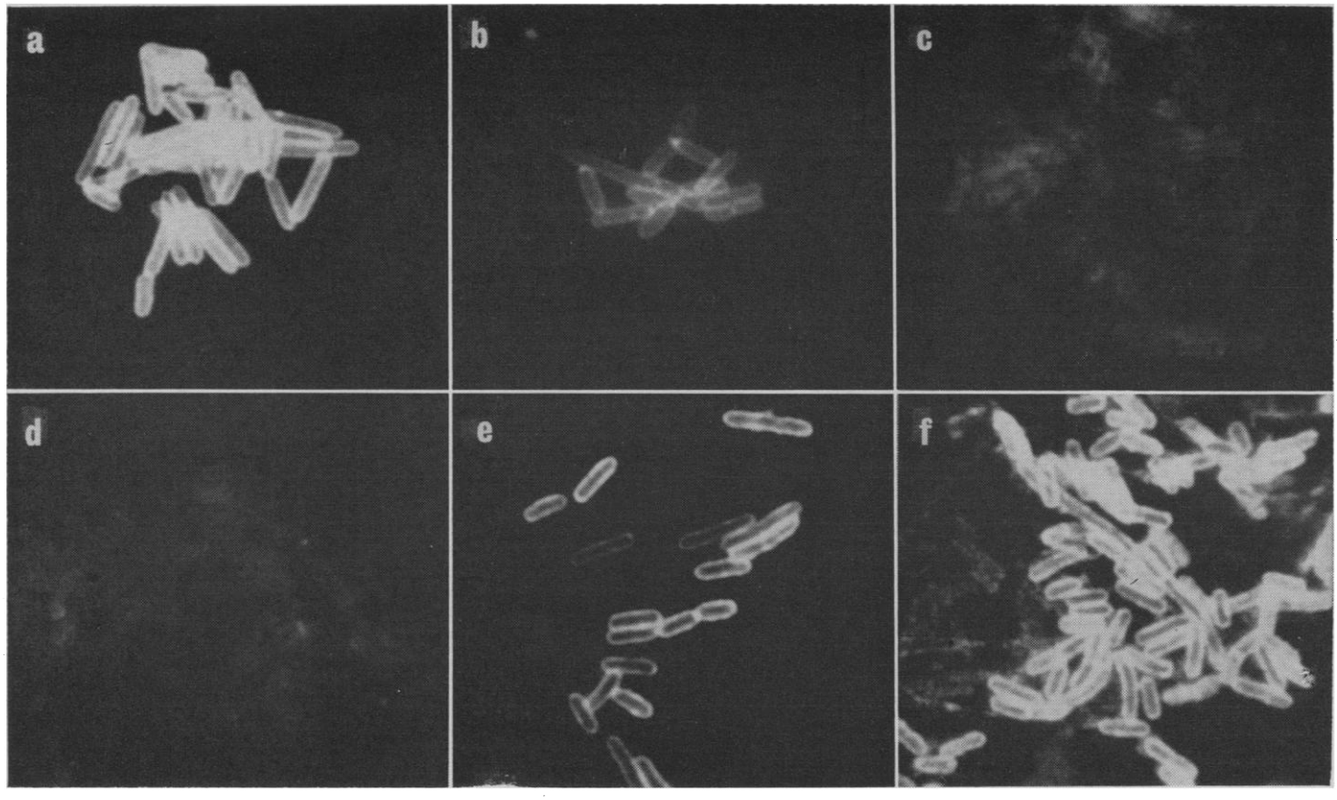

Fig. 1. *Salmonella typhosa*, TY2W, incubated ½ hour in fluorescein-labeled anti-O-901 globulin, washed, and examined (*a*) immediately, (*b*) after incubation for 60 minutes, and (*c*) after incubation for 120 minutes. *Salmonella typhosa*, incubated ½ hour in unlabeled anti-O-901 globulin, washed, and stained on slide with fluorescein-labeled anti-O-901 globulin (*d*) immediately, (*e*) after incubation for 60 minutes, and (*f*) after incubation for 120 minutes (× 2100).

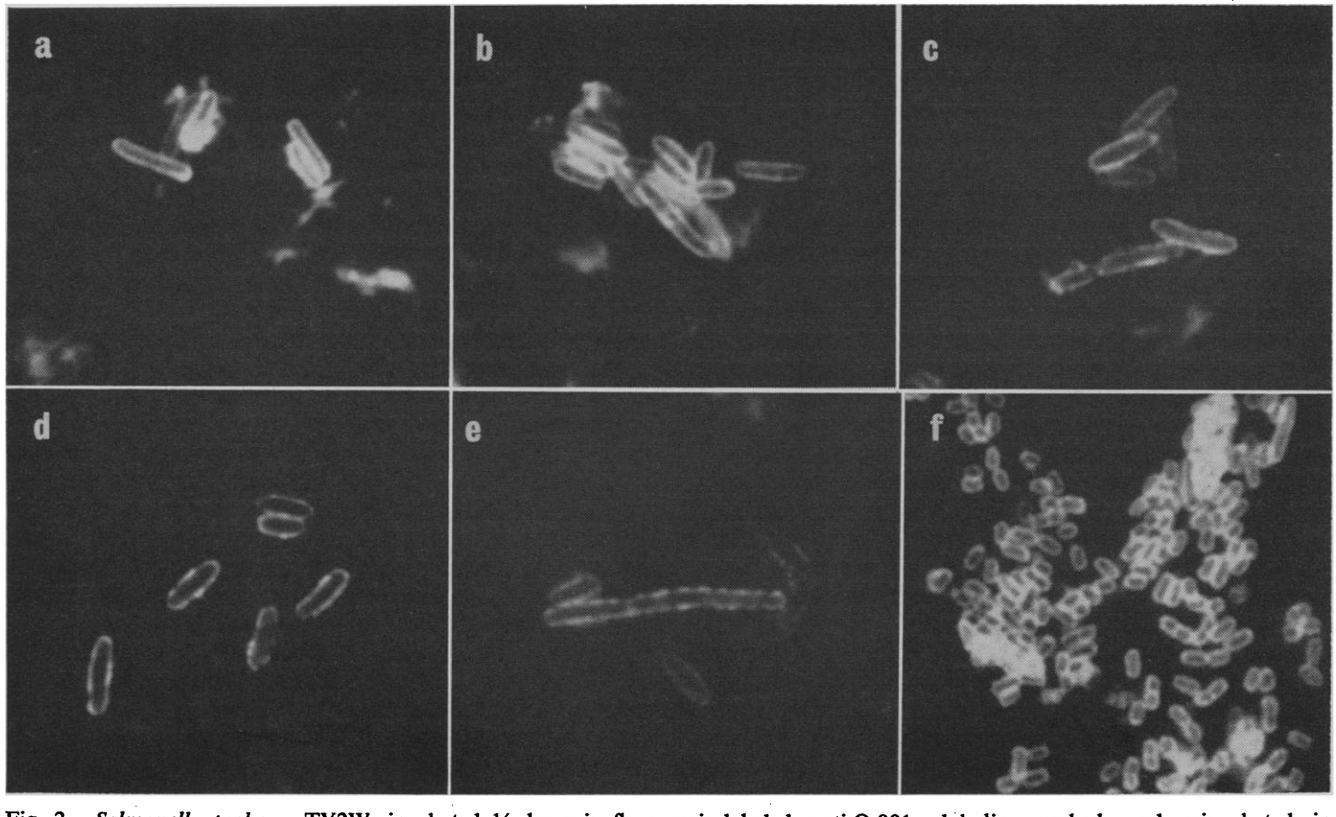

Fig. 2. *Salmonella typhosa*, TY2W, incubated ½ hour in fluorescein-labeled anti-O-901 globulin, washed, and reincubated in chloramphenicol broth for (*a*) 0 minutes, (*b*) 60 minutes, (*c*) 180 minutes, and (*d* and *e*) 300 minutes. (*f*) A 300-minute sample, grown without chloramphenicol for comparison, and stained with fluorescent antibody on slide. Note cell size (× 2100).

washed cells in Penassay broth. The antibiotic in the concentration used (10 μg/ml) suppressed cell division. The number of colonies, $\times 10^6$ per 0.1 ml, was 16, 21, 6, and 10, respectively, after incubation of 0, 60, 180, and 300 minutes. Cells from the chloramphenicol broth became much larger than cells from plain broth, particularly by 300 minutes (compare Fig. 2, *d* and *e*, with Fig. 2f). These antibiotic-treated cells, which had been labeled by the direct method, showed no change at zero time (Fig. 2*a*). In the interval between 60 to 180 minutes, some irregularity of the fluorescent outline became increasingly apparent (Fig. 2, *b* and *c*). At 300 minutes of incubation, there was definite nonfluorescent, irregular interruption of the still-brilliant fluorescence on the cell wall (Fig. 2, *d* and *e*).

By the methods used, observation is restricted to behavior during replication of those cell wall components that are antigenic and accessible. If the described behavior of the labeled antigens is considered representative of the sequence of events occurring in the wall as a whole (including the mucopeptide), then it is apparent that the mode of cell wall replication in *Salmonella typhosa* is quite different from that in *Streptococcus pyogenes* (*1*). My observations indicate that for immunofluorescence to decrease with time of incubation, the bacteria so labeled must be living and dividing; the gradual decrease in fluorescence is attributed to wall replication by a process of continuous diffuse interaction of new materials into old wall. Chloramphenicol, though not preventing replication at the concentration used, slows or limits such a process, resulting in the appearance of unlabeled portions in multiple sites between regions of old and still-labeled wall of the enlarged but nondividing cells. This observation also appears to demonstrate visually that cell wall synthesis continues in *Salmonella typhosa* in the presence of chloramphenicol, although we have no concomitant evidence of the lack of protein synthesis such as that shown in prior biochemical studies of continued wall synthesis by *Staphylococcus aureus* grown with added chloramphenicol (*8*).

These findings are inconsistent with the "growing-point" hypothesis of wall replication of flagellated bacilli, as expressed by Bisset *et al.* (*9*), by which one "daughter" cell would appear with entirely new wall. Instead, the results support the ideas of Quadling, Stocker, and Kerridge (*10*), based on the study of the unilinear transmission of motility and of the sharing of parental flagella at division, that replication of the wall of the salmonellas must be by diffuse intercalation. Additional evidence for their view appeared during preparation of this report. By indirect immunofluorescence, May (*11*) has shown that the adsorbed antibody marker on the cell wall (of *Salmonella typhimurium*) became "uniformly dispersed" with time of incubation.

It therefore appears that the mode of cell wall replication may differ with the organism, although the result in all instances studied thus far appears to be (*11*) that each of the progeny after a cell division receives essentially half old wall and half new. It remains for similar methods to be applied to study of other microorganisms.

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References and Notes

1. R. M. Cole and J. J. Hahn, *Science* **135**, 722 (1962).
2. J. W. May, *Exptl. Cell Res.* **27**, 170 (1962).
3. Reported in part at 63rd annual meeting, American Society for Microbiology, Cleveland, Ohio, 5–9 May, 1963; *Bacteriol. Proc.*, Abstr. G13, p. 26 (1963).
4. Some strains and antiserums were kindly furnished by L. S. Baron and E. H. LaBrec, Walter Reed Army Institute of Research, and J. P. Tully, National Institute of Allergy and Infectious Diseases.
5. J. D. Marshall, W. C. Eveland, C. W. Smith, *Proc. Soc. Exptl. Biol. Med.* **98**, 898 (1958); J. Killander, J. Ponten, L. Roden, *Nature*, **192**, 182 (1961).
6. Bacto Penassay broth (B243), dehydrated, Difco Laboratories, Detroit, Mich.
7. Courtesy of Joseph E. Hayes, Jr., National Heart Institute, Bethesda, Md.
8. J. Mandelstam and H. J. Rogers, *Nature*, **181**, 956 (1958); R. Hancock and J. T. Park, *ibid.*, **181**, 1050 (1958); J. Mandelstam and H. J. Rogers, *Biochem. J.* **72**, 654 (1959).
9. K. A. Bisset, *J. Gen. Microbiol.*, **5**, 155 (1951); ——— and P. Pease, *ibid.* **16**, 382 (1957); K. A. Bisset and C. M. F. Hale, *ibid.* **22**, 536 (1960).
10. C. Quadling, *ibid.* **18**, 227 (1958); ——— and B. A. D. Stocker, *ibid.* **28**, 257 (1962); D. Kerridge, in *Microbial Reaction to Environment*, 11th Symp. Soc. Gen. Microbiol. (Cambridge University Press, London, 1961), p. 41.
11. J. W. May, *Exptl. Cell Res.* **31**, 217 (1963).
12. The technical assistance of Richard S. Whitt is gratefully acknowledged.

27 September 1963

Multiple Authorship Trends in Scientific Papers

Abstract. *Since 1946 biomedical writers have shown no marked trend toward multiple authorship; the average number of authors per paper remains steady at about 2.3. This is in strong contrast to the conclusion of Price from a study of* Chemical Abstracts *that the chemists' trend toward four or more authors per paper has been during this period, and continues to be, steeply exponential.*

Price (*1*), apparently on the basis of a sampling of *Chemical Abstracts* for the period 1910–60, concludes that "a detailed examination of the incidence of collaborative work in science shows that this [the trend towards multiple authorship] is a phenomenon which has been increasing steadily and ever more rapidly since the beginning of the century."

I propose that, since this does not hold historically for biomedical papers, the generalization quoted, important as it is for students of changing patterns in scientific publication, is not valid for science as a whole. Authorship distributions in papers presented (*2*) at the annual meetings of the Federation of American Societies for Experimental Biology, from 1934 to 1963, were analyzed statistically for trend by using total counts or random samples. If a trend toward multiple authorship has existed since 1946, it is minute (Table 1 and Fig. 1).

In order to check the assumption that the papers in the data sources were randomly distributed with respect to different numbers of authors, random-sample counts (33 percent) as well as total counts were made of 2 years, 1942 and 1946. Random-sample percentage counts agreed in each authorship category within $\pm 2\sigma$ with the corresponding total count percentages; indeed, except in one instance, one-author papers for 1946, the agreement was within $\pm 1\sigma$.

Inspection of Fig. 1 leads immediately to a generalization: the factors in play during 1934–46 were different, in nature and quantitative effect or both, from those affecting authorship distributions from 1946 to date. Among the five curves after 1947 only that for one-author papers gives clear visual evidence of a trend. It was therefore decided to subject all the data to statistical tests for presence of trend.

Two tests were used: (i) The estimation of slope b and its standard deviation σ_b of the straight line fitted by least squares through the observed points (*3*); and (ii) the method of