

ANALYSES OF THE EFFECTS OF FOUR COMPONENTS IN CIGARETTES ON *PARAMECIUM AURELIA*

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ABSTRACT.—Methods were described for testing the toxicity of aqueous solutions of cigarette tobacco, cigarette smoke, cigarette ash, and ash of cigarette paper, utilizing a Kappaless *Paramecium aurelia* as the assay organism. Observations were made on populations of this organism in 1 ml of each of these solutions from 1 non-filter cigarette. Overall toxicity, based on survival time, of these cigarette components was found to increase in this order: paper ash, whole ash, smoke and tobacco. In reality, however, the smoke is most toxic since only a small fraction of the smoke from one cigarette was used in the experiments. Solutions of tobacco and smoke both were completely lethal to the protozoon in that they brought quick death to every organism in a population. There was neither body deformation prior to, nor disintegration after, death. A unique "polar blistering" was observed in case of the tobacco solution. Solutions of whole cigarette ash and paper ash both brought erratic changes in the organism's motility pattern; they also imparted an incomplete lethality to the population. But while the paper ash solutions caused pronounced body deformation and severe blistering followed by disintegration, solutions of whole cigarette ash characteristically elicited a dislocating clumping of endoplasmic granules, leading to death. The sensitivity of *Paramecium aurelia* to cigarette toxicity was further evidenced by its "ability" to distinguish, by virtue of clear-cut differential reaction patterns, solutions of whole cigarette ash from those of tobacco ash alone. These results did not vary significantly from one brand of cigarette to another. It is suggested that the organism's different reaction patterns to the cigarette components may possibly be related to cer-

tain specific chemicals in which the tested components differ from each other.

Two previous reports (Wang, 1959 and 1960), especially the latter, indicated that *Paramecium aurelia* is not just chemically sensitive, but that it is capable of differentiating various chemical stimuli in its environment by means of certain set patterns in both behavior and morphology. These observations, plus the consideration that the protozoon is a cell and an organism at the same time, prompted me to use it as a test organism for cigarette toxicities (Wang, 1963a). Since then, more observations on the reaction patterns of *Paramecium aurelia* elicited by each of the four cigarette components have been made. Part of these efforts has resulted in a cinematographical study: the death of *Paramecium aurelia* from cigarette paper toxicities (Wang, 1963b). The purpose of this report is two-fold: (a) to present a more detailed record of how *Paramecium aurelia* was affected by each of the four cigarette components in both quantitative and qualitative terms with special emphasis on its reaction pattern to the cigarette paper ash, and (b) to publicize once more that *Paramecium*

aurelia is a useful assay organism for tobacco and other toxicity studies.

MATERIAL AND METHODS

Six domestic and one imported (from Taiwan) brands of non-filter cigarettes were used in this work. Cigarettes were smoked with a simple artificial device. It utilized a conventional suction filter flask attached to a tap faucet and with a two-hole rubber stopper replacing the filter funnel. To one end of the tube leading into the flask was fitted a cigarette holder while the other (inside the flask), was dipped into a test-tube holding 20 ml water. A cigarette was inserted into the holder and lit; water was turned on with its pressure so adjusted that a cigarette was steadily consumed within three and a half minutes, leaving a butt of approximately 10 mm. During this process the mainstream smoke bubbled through the water. Much of it escaped into the flask; only a fraction was dissolved in the water. In view of the low solubility of smoke and the particular method here used to attain its solution, this amount was estimated to be under 5 per cent of the total smoke produced. The resulting cigarette ash was collected in a beaker, 20 ml water was added to it, and was filtered after one hour.

For preparation of tobacco and cigarette paper solutions, a cigarette was separated into tobacco and the wrapper. The former was placed in 20 ml water and was likewise filtered after one hour. The cigarette paper was burned in a porcelain tray with a Bunsen flame directed at it for one minute. The resulting ash (white) was placed in 20 ml water, and similarly filtered.

A paperless ash was prepared for comparison with regular ash. It was made by carefully stripping the paper off a well burning cigarette, allowing the tobacco to burn to completion undisturbed. The resulting ash (of tobacco only) was processed exactly as in the case of regular cigarette ash.

Twenty ml solutions were thus prepared from each of the four cigarette components: tobacco, smoke, whole, and paper ash. They constitute our standard stock solutions. For each experiment, 1 ml of a standard solution, arbitrarily designated as having a concentration of 1.0, was utilized. This concentration was found through repeated testing to be the most useful for our experiments.

An experiment was run in the following manner. The 1 ml test solution was

transferred to a depression slide (3" x 1½") with a central 1½" concavity. To it was added 2 drops of paramecia from a flourishing culture. The fine micropipette used for this purpose had a caliber (at mouth) that 40 drops are equal to 1 ml. Samples were only taken from sufficiently dense (luxuriant) populations, e.g., one pipette drop contained several hundred paramecia.

Raw rice was used for culturing. It proved to be a better source of bacteria than oat grains used previously by us. Ten grains of rice were added to 25 ml water in a sterilized Petri dish, and seeded with a dropful of paramecia. It took, on the average, 10 to 14 days for a culture to reach maximum density and can be maintained in that condition for two weeks or longer, depending on the temperature. New cultures were periodically grown in order to have a continuous supply on hand.

Observations using an AO-Spencer cycloptic stereo-dissecting microscope were made beginning from the time paramecia were introduced into a test solution until death resulted. The duration was recorded as survival time which was used as a basis for rating the overall lethality of a test solution. Criteria of death were cessation of all movements (cilia, contractile vacuoles, and endoplasmic streaming) and stoppage of all signs of life.

Close attention was given to changes in motility and morphology of the organisms. Permanent records of the morphological manifestations were made with both light and phase microscopy. While most changes could be readily photographed, certain types of deformities, because of the incessant motion of the affected organisms, were rather difficult, if not impossible to photograph. Generally, light photomicrography was used for groups of paramecia under low magnifications (Figs. 1, 2, 3, 4, 6, 7, 8) while individual organisms of interest were photographed with phase microscopy at much higher magnifications on a ZEISS-WI research microscope with Neofluar 25X and 40X objectives (Figs. 5, 9, 10, 11, 12, 14, 15 and 16 through 28). Figures 16 through 29 are prints made from negatives of 35 mm. Kodachrome transparencies.

A procedure for making preparations for photomicrography was adopted. Under the binocular dissecting microscope and with a micropipette of even a smaller caliber than the one used for transferring paramecia to experimental solutions, the affected organisms were

removed to a clean slide. In order to avoid crushing the organisms, four pieces of glass chips from a broken coverslip were carefully placed along the edge of the drop before a coverslip was placed over it. With glass chips thus serving as support for the coverslip, the preparation was insured against artifacts, which might result from mechanical pressure.

Experiments were carried out at room temperature, 23 to 31° C. This is a safe range within which the organisms' responses may vary, but only in degree of intensities. Usually, an increase in either temperature or concentration of the solution shortens the survival time, accelerates the movement and increases the extent and/or speed of disintegration following death. It has been ascertained that temperature variations within this range would not change the nature of the reaction patterns of *Paramecium aurelia* to any appreciable degree. In the experiments, pH of all solutions including distilled water and culture media were determined on a pH meter (Beckman, Model H2). The range used was pH 5.0 to 8.0, which has proven safe, i.e., harmless for this organism. Water used for all purposes in this work was chemically pure, obtained from an automatic Pyrex distiller (Stokes, Model 171-E). For double safety this water was periodically checked to make sure that it was not the least toxic to the experimental organism used.

RESULTS

The results show that the seven brands of cigarettes used presented a range in survival time, which is not widely spread. The observed narrow range could mean only one thing, namely that there are no significant differences between the brands as far as toxicity to paramecia is concerned. On the other hand, the overall toxicity of the cigarette components as measured by survival time was statistically significant. It was 8, 14, 21 and 27 minutes at 23° C and 4, 5, 10, and 12 minutes at 31° C for, respectively, tobacco, smoke, whole ash and paper ash.

One observation deserves special mention: the paramecia were killed in tobacco and smoke solutions significantly faster than in solutions of whole ash or paper ash. The difference may be taken as evidence of a greater overall toxicity of the former two cigarette components. In relation with this, another phenomenon was consistently observed. That is, the two weaker components (whole cigarette ash and cigarette paper ash) were not able to kill an entire population of experimental animals, but invariably a few paramecia survived. These survivors remained totally unaffected and healthy throughout the experiment. In fact, they were found to be able to give rise to new individuals by fission after the experiment. Using this as a criterion, the four cigarette components, at the concentration used in the experiments, may be divided into two groups, namely those which are completely lethal (tobacco and smoke) and those which are incompletely lethal (whole and paper ash) to *Paramecium aurelia*. From this, the importance of using a critical concentration for the test solutions is easily realized.

The remainder of our data concern with motility and morphology of the affected animals. The observations to be presently described were based on many more experiments than those from which survival time was obtained. The experiments were carried out at concentration 1.0, unless otherwise stated, and within a temperature range of 23 to 31° C.

OBSERVATION ON MOTILITY

Two general patterns of modified motility were observed. With tobacco or

smoke, the organisms became quickly quiesced, ending in complete cessation of motion within few minutes. Whole ash or paper ash, on the other hand, elicited an opposite effect. It was one of energization, excitation, and stimulation. The paramecia seemed to be activated on contact with the test solution and immediately began to move around at a high tempo in manners deviating from the normal. At first, the induced motility pattern took on an accelerated "avoiding reaction" (Jennings, 1906). The animals swiftly zig-zagged, changing directions repeatedly as if to avoid some adversity. Soon this subsided, and it was replaced by several peculiar types of motion. Some of them began to swim "pin wheel" fashion. Others moved forward spirally with the anterior end whirling a complete circle. Still others exhibited a vibrating or oscillating type of motion at the posterior end as the organism slowly propelled ahead. And all these patterns could be present simultaneously so that the composite motion of the population of affected paramecia was bizarre and really beyond description. What has been said can be applied equally well to the ash solution of a whole cigarette or of the paper only, generally to a greater degree in case of the whole ash. Just prior to waning of these induced movements, morphological changes set in, leading finally to death of the animal.

OBSERVATION ON MORPHOLOGY

Tobacco. The action of tobacco solution was lethal to paramecia. Death was not preceded by conspicuous morphological changes aside from an occasional mild shrinkage (Fig. 7). The occurrence of shrinkage was indicated by a flattening of the body, frequently into the thinness of a leaf. Such an effect could be clearly seen in a moving (rotating) paramecium, but is not so obvious from a still photograph. In addition, a good portion of the population extended an ectoplasmic blister (in most cases only one) from either end of the animal (hence the term, polar blistering, as seen in Fig. 7). The differences between this kind of blister and those characterizing the effect of paper ash can be learned by comparing Figure 7 with Figures 3, 11, 13 and 14. As a rule, the bodies of dead organisms remained fairly intact, undergoing little disintegration if at all.

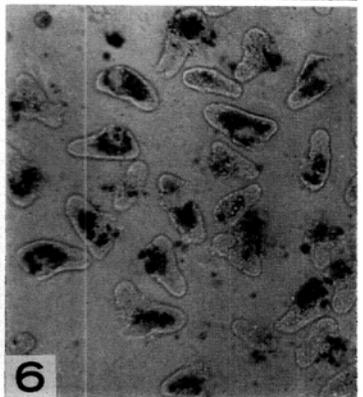
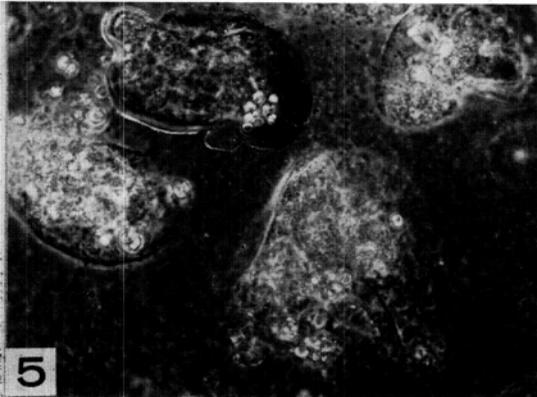
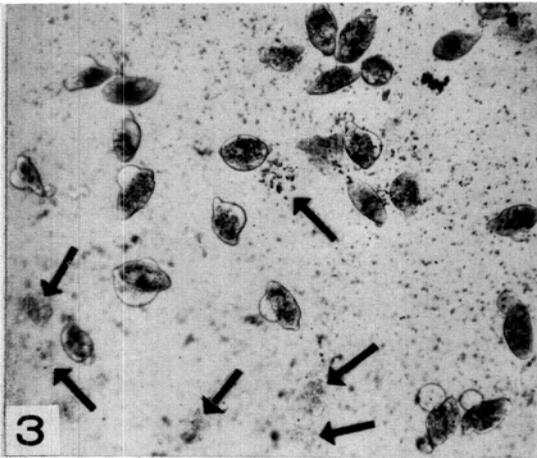
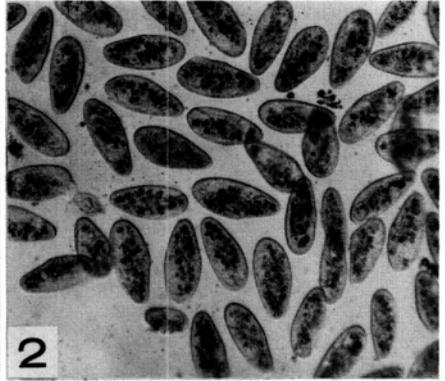
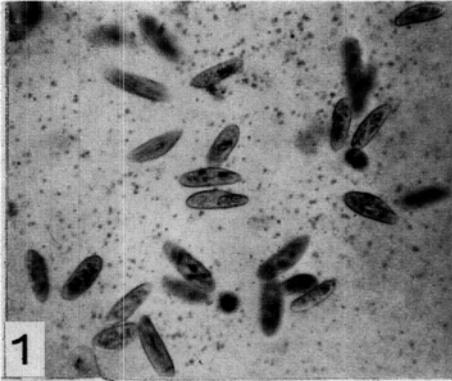
Smoke. The action of this cigarette component was also lethal. Death came

about quietly and quickly to the entire population like a sweeping lethal wave (Fig. 2). The dead organisms appeared somewhat swollen (cf. Figs. 1 and 2, same magnification) with some post-mortem disintegration, the amount of which seldom exceeded the extent indicated in Figure 4. At higher magnifications, however, certain damage done to the endoplasmic granules and the arrangement of cilia were visible (Figs. 9 and 10). A conspicuous feature was the translocation of endoplasmic granules toward one end of the cell. These were relatively mild deformities compared to what will be described below for the toxic effect of cigarette paper ash.

Ash. As described above, its effect on motility lasted only a few minutes. The activated organisms finally slowed down, came to a standstill and started to succumb. Their endoplasmic granules at this time exhibited a conspicuous aggregation, most of them being localized around the macronucleus (dark granules in Fig. 6 and Fig. 29).

This is a characteristic reaction pattern seen in solutions of whole cigarette ash or the ash of paperless cigarette (i.e., tobacco ash alone). The noteworthy thing is that we have been able to distinguish the reaction of *Paramecium aurelia* to these two kinds of ash solutions. Thus, in the former the dead organisms underwent body swelling and disintegration (Fig. 6) to a noticeably greater extent than seen in the latter (Fig. 8). Another difference was in connection with a mild form of trichocyst exposure occasionally seen from dead or dying paramecia. The released trichocysts seen as extremely fine dark fibrils with phase microscopy appeared always associated with the cytoplasmic debris resulting from the disintegrated organisms. The trichocysts and this debris seemed to have an affinity for each other, and the resulting products of mingling made up a gluey mass. The latter was distinctly thicker in the solution of a whole cigarette ash than in the solution of tobacco ash alone. This ability of *Paramecium aurelia* to differentiate the toxic action of these two kinds of ash solutions probably has a chemical basis (see discussion).

Paper. The most unique feature of the action of cigarette paper ash is that it produces the most severe and extensive deformations (Figs. 3 and 11 through 28). A small proportion of the population was killed in the first few minutes following exposure. These ani-



mals virtually exploded into a mass of cytoplasmic debris (Figs. 5, 15). About half of the population, being the next weakest, became severely affected within the next few minutes. There appeared, as though, a wave of destruction had suddenly struck them, and that the wave quickly spread and overtook the individuals with bursting disintegration. Some of their remains ("ghosts") are shown in Figure 3 (arrows). In this photograph taken 12 minutes after immersion, there were two groups of paramecia: those that have had long since succumbed and undergone advanced disintegration and those which displayed body deformation of one kind or another coupled with extensive blister formation.

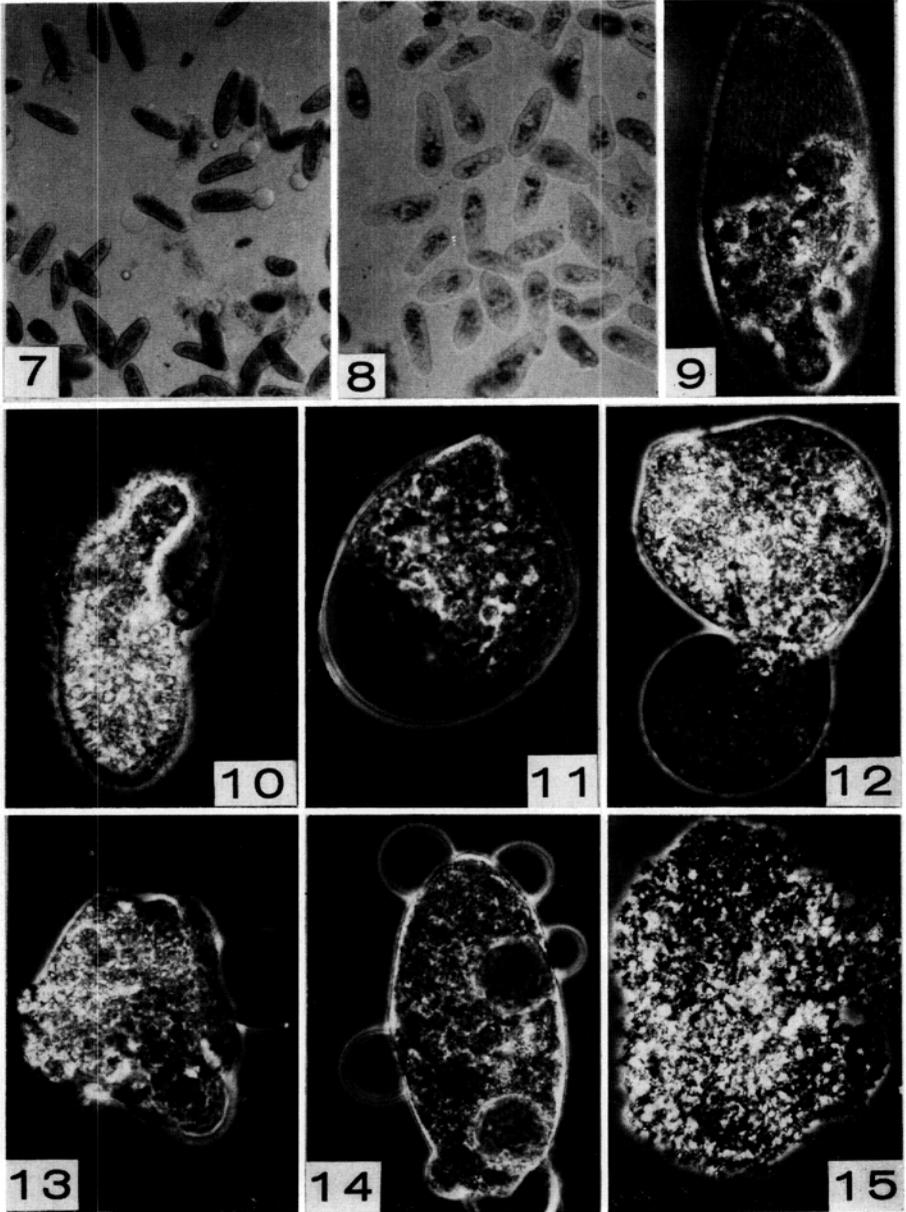
The blisters, when first formed, were of the ectoplasmic type filled with a gel of the same consistency as the cortex. One organism may bear more than one blister, and the blisters once formed would proceed to undergo changes in size and composition. In most cases blistering paramecia remained active for some time, moving around in strange erratic fashions that defy adequate description. The best one can hope to do (next to seeing the motion picture; Wang, 1963b) is to record a variety of their morphology with phase micrographs (Figs. 11 through 14; 16 through 28). The blisters shown in most of these illustrations are of the ectoplasmic kind. A few endoplasmic blisters are shown in Figures 12, 20, 24, 27, and one in Figure 19, close to the right-hand edge. I am reasonably certain that all blisters started out as ectoplas-

mic blisters. Most of them, however, underwent a sequence of changes, eventually transformed into endoplasmic blisters before they finally burst open and collapsed. The events of this transformation can be seen in a single organism, which bore two blisters shown in the process of this transformation (Figs. 16, 17, 18). See also Figures 12, 19 and 20.

One extreme deformity occurring only in solutions of cigarette paper ash was a combination of blistering and body protrusions (Figs. 14, 21, 23, 25, 27, 28). The latter wore cytoplasmic evaginations of various size, shape, and loci. One such protrusion was formed on a banana-shaped body (Fig. 23), another, on a sausage-shaped body (Fig. 24). Paramecia of this monstrous appearance with one or more blisters characteristically moved around not only in most strange ways, but with a rhythmic beat. The latter is probably attributable to the greatly disturbed weight distribution (Figs. 20, 22, 25, 27) with the apparent result that gravity takes over the control of body movement, a function which is normally executed by the cilia.

Distinct differences between body protrusions and blisters can be recognized. The former, from the time of inception and throughout their existence, contain no appreciable amount of ectoplasm, and may be of any size and shape (Figs. 19, 20, 25, 27, 28). The shape may change from time to time (cf. Figs. 19 and 20), but never assume a spherical contour. On the other hand, a blister, regardless of its content (ectoplasmic, Fig. 19; endoplasmic, Fig. 22) or with

Figures 1-8.—Illustrations of paramecia. 1. Control: live paramecia taken with 1/50 sec. exposure (not fast enough to catch them all in sharp focus). Note variation in body size and other features. (X85). 2. Dead intact paramecia in smoke solution, 20 minutes after immersion. The plumpness (approximately twice the width and 30% longer than controls in Fig. 1) was the result of increased permeability. (X85). 3. Paramecia in cigarette paper ash solution, 12 minutes after immersion: those that still had intact bodies with or without blisters and those which had already been dead, leaving "ghosts" and cytoplasmic debris at spots (arrows). Most of the blisters born on intact organisms were of the ectoplasmic type. (X85). 4. Portion of the preparation shown in Figure 2, taken 13 minutes later, showing still intact but considerably flattened-out bodies. Note: magnification of this figure is X120 as compared to Figure 2, X85. 5. Phase micrograph of 4 paramecia from the same preparation shown in Figure 3, 25 minutes post immersion (13 minutes after Fig. 3 was taken). The 4 organisms could be any of the blistering organisms shown in Figure 3: the lower two already burst open, the upper two, about to disintegrate. The thick granular material filling the background constituted a debris from disintegrated organisms. (X260). 6. Dead paramecia in cigarette ash solution, 10 minutes after immersion: 1 or 2 organisms still intact; most others already burst through at one point. Note the aggregated dark cytoplasmic granules in the cells, some of which had been liberated from the burst cells. (X85).



both, Figs. 25 and 27) had the unique feature of possessing a round contour. The contrast between the endoplasmic blisters as shown on top of both FIGURES 22 and 23 and a body protrusion as shown in FIGURE 25 seems quite conceivable. Yet another case is illustrated by FIGURE 27, which shows a small limb-bud-like protrusion next to a half-circle endoplasmic blister and two distinct ectoplasmic blisters (one large and one small) in addition.

It was not always possible to follow the fate of the deformed blister-bearing paramecia. In most cases the terminal events were characterized by an impeded ciliary motion. Such organisms lacked the normal wavy unison beat of cilia. Soon a rupture occurred at some spot or point (not necessarily on either end of the animal), and this was quickly followed by disintegration. The cell was virtually blown up to pieces, ending in complete cytolysis. All this happened very fast and the preparation became clogged up with thick cytoplasmic debris (FIG. 5). Concomitantly, the blisters yielded and vanished without leaving a trace (FIG. 18). It appears as though blisters can be maintained only on an intact body. A break-through anywhere on the surface of the body brought instant collapse to them. Meanwhile, as mentioned above, a small portion of the population remained totally unaffected. These resistant organisms, like those encountered in the solution of whole cigarette ash, survived unharmed. Moreover, these survivors displayed the remarkable resistance to the action of the

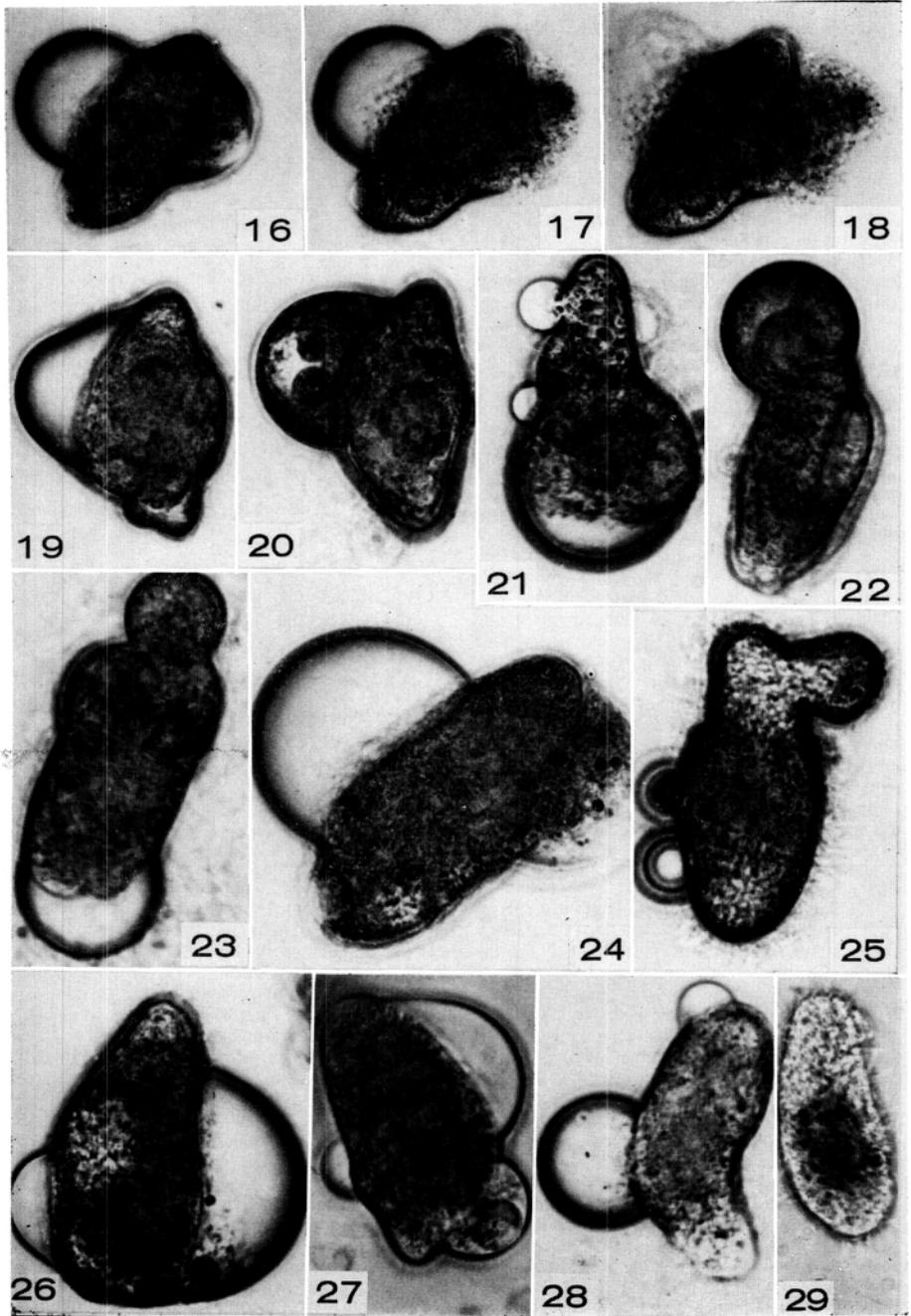
ash solutions from the start of the experiment. Once a paramecium came under the sway of the toxic action, it would proceed all way to death. Acclimatization to or recovery from the deleterious effects of cigarette toxicities has not been observed.

DISCUSSION

Paramecium, an ideal assay organism. If this study has contributed anything to our understanding of cigarette toxicities, it was made possible by the fact that *Paramecium aurelia* happens to be a cell and organism at the same time. As such, it permits the worker to obtain data of both quantitative and qualitative nature from the same experimental setup. This is a condition which constitutes a unique advantage in using protozoa as assay organisms. The same cannot be said of metazoa employed similarly in toxicity studies.

The results have shown that *Paramecium aurelia* is not killed alike by all cigarette components rather, it succumbs to the toxicities at different rates and with different manners. This denotes that both quantitative

Figures 7-15. — Illustrations of paramecia. 7. Dead paramecia in tobacco solution, 25 minutes after immersion: most of them, different from normal controls (FIG. 1) about a third of them bore an ectoplasmic blister on either end of the animal. Compare with FIGURE 3 for difference in the nature of blistering. (X85). 8. Dead paramecia in paperless ash solution, 10 minutes after immersion. Note body deformed to a milder degree than those in FIGURE 6, however, with the same kind of aggregation of endoplasmic granules. Some of them would have subsequently disintegrated following the same manner indicated in FIGURE 6, but to a lesser degree. (X85). 9. Dead paramecium in smoke solution, 30 minutes after immersion, portraying typical morphological deformation pattern effected by smoke. The animal remained intact with endoplasmic mass translocated toward one end, and revealing rows of "pits" on the pellicle from which the cilia (deranged) extended. (X450). 10. Another paramecium of the kind shown in FIGURE 9, showing again deranged cilia and translocated endoplasm, and the longitudinal rows of cilia pits. (X450). 11, 12, 13 and 14. Paramecia from cigarette paper solution showing different types of blistering and body deformation photographed just prior to onset of disintegration. The blisters shown in FIGURES 11 and 13 were of ectoplasmic type while that in FIGURE 12 was endoplasmic. The latter appeared to have just been transformed from an ectoplasmic blister. The paramecium in FIGURE 13 assumed the shape of an amoeba. The one in FIGURE 14 had blisters of both types, though predominantly ectoplasmic. (X450). 15. A disintegrating paramecium from cigarette solution, 14 minutes after immersion. (X450).



and qualitative factors were at work. These factors were assessed, respectively, as survival time and manifestation patterns. The former served as an index of lethality while the latter portrayed the exact ways in which death of the animal came about. Evidently, both kinds of data are useful; but, only the latter kind qualify as diagnostic criteria pertinent to investigations of this kind.

Other examples of quantitative factors were temperature and concentration of the test solutions. They affect only the rate or intensities of chemical processes (rate of induced motion, extent of postmortem disintegration, extensiveness of blistering, etc.), definitely not the nature of reaction patterns. But when temperature and/or concentration are raised to such a level that death of the organism becomes almost instant, the animal would have no chance to react. This is exactly what happened in solutions of cigarette paper

ash at a 3.0 instead of 1.0 concentration, and at 32° C. or higher. Under these circumstances paramecia simply burst to pieces upon immersion. The adoption of the standard 1.0 concentration throughout the work was thus a necessary prerequisite for making sure that the ensuing results would be the most meaningful.

Basis for interpretation of results.

Cigarette toxicity stems from a multitude of chemicals and certain extrinsic factors peculiarly inherent in cigarette smoking (Ermala and Holsti, 1956; Green, 1955; Lam, 1955). Together, they constitute a material basis for a causal relationship between cigarette smoking and lung cancer (Carruthers, 1960; Boyland, 1964; Flory, 1941; Ochsner, 1955; Watson and Conte, 1954, 1955; Wynder et al., 1953, 1955). One is impressed with the long list of chemical constituents identified from each of the cigarette components (Kenna-

Figures 16-29. — Illustrations of paramecia. 16, 17 and 18. One and the same paramecium, respectively, 2, 2½, and 3 minutes after immersion in a 2.0 conc. cigarette paper ash solution at 24° C., showing the collapse of two blisters, one following the other. The sequence of events can be readily traced. (X400). 19 and 20. One and the same paramecium, taken 7 minutes apart (cigarette paper ash solution at 27° C) during which time an ectoplasmic blister was converted into an endoplasmic one. (X400). 21. A monster paramecium, 12 minutes after immersion in paper ash solution, illustrating combination of body deformation and blistering. (X400). 22. Another paramecium from the above preparation showing a large polar endoplasmic blister. (X400). 23. A deformed paramecium 9 minutes after immersion in cigarette paper solution, bearing an ectoplasmic and an endoplasmic blister at opposite poles. (X400). 24. Another organism from the above preparation, showing a huge ectoplasmic blister and another in the process of being converted into an endoplasmic blister. (X400). 25. A deformed paramecium with a handle-like protrusion at one side; the two spherical things at left are not blisters, but were an air bubble and a small unidentified protozoon. (X400). 26. A sausage-shaped paramecium bearing two blisters of different sizes, the larger of them having already incorporated some endoplasmic granules (10 minutes in cigarette paper ash solution). (X400). 27. A paramecium with irregularly deformed body showing two blisters of contrasting sizes, one endoplasmic blister and one tiny limb-bud-like body protrusion (12 minutes in cigarette paper ash solution). (X400). 28. Another organism from the above preparation with a banana-shaped body and two ectoplasmic blisters, showing one end of body (lower) already disintegrating. (X400). 29. A dead yet intact paramecium in whole cigarette ash solution (6 minutes at 23° C) showing central aggregation of endoplasmic granules and deranged cilia. (X375).

way and Lindsey, 1958; Cummins et al., 1954; Cooper and Lindsey, 1955) and especially the fact that no two components have exactly the same chemical composition. In the light of this knowledge it would seem reasonable that the present results be interpreted as reflecting this tremendous chemical complexity. That is to say, each of the reaction patterns given by *Paramecium aurelia* possibly could be caused by a different yet specific array of chemical compounds. Certain of them bring quick death, others induce strange motility patterns, yet others alter body form and cause blistering, and so on. Somehow we must recognize that this animal is endowed with the incredible ability to detect the presence of various chemicals and respond to them accordingly. The sensitivity of such responses is best illustrated by its ability to distinguish whole cigarette ash from the ash of tobacco alone.

Causation of death. Pursuing this line of reasoning further, a line may be drawn between factors which are directly lethal to paramecia and those which cause death of the animal only indirectly. The best examples of the former were furnished by the action of tobacco and smoke, both of which inflict death quickly and completely (with no survivors) showing little or no accompanying damage. There must be present in these solutions some very toxic substances so that the animals were killed before they had a chance to react otherwise. On the other hand, chemicals present in the ash solutions (whole or paper) at the concentration used are apparently not lethal in the strict sense, but that they primarily cause certain structural

changes and that death finally comes as a result of these changes, e.g., clumping and dislocation of endoplasmic granules in whole ash solution and the bursting of deformed blistering bodies in paper ash solution. The fact that both of the ash solutions induced changes in the organism's movement suggest that the same chemicals responsible for this are present in both. It is thus evident that death in these latter cases resulted from an impairment or drastic disruption of some vital function. One recalls also that the survivors (resistant paramecia) were present only in these ash solutions, suggesting that the directly lethal factors are the more toxic agents as compared to those which cause death indirectly. There is no apparent correlation between how quickly an organism succumbs and in what manner its death comes about.

Permeability changes, an important factor. Many chemicals are known to affect the permeability of plasma membrane one way or the other (Heilbrunn, 1952, 1956). These effects were indeed frequently seen in our experiments: a mild shrinkage in tobacco solution (Fig. 7), moderate swelling in smoke solution (Fig. 2 and 4), and in whole ash solution (Fig. 6). It is reasonable to suppose that in all these instances the effect (whether due to an increased or decreased permeability) could be lethal. In the final analysis, a change in permeability may act as a common denominator also for body deformation and blistering. Most blisters such as those occurring in paper ash solutions begin as balloons of pure ectoplasm (gel). Haas (1953) in his work with the slime mold called them

"hyaline extrusions" for this reason. In this paper I preferred a more neutral term, "blister", because there were blisters which contain endoplasm (Figs. 12, 22, 24, 27). In details, the blisters caused by metallic ions (Wang, 1959); by the proteolytic enzyme, ficin (Wang, 1960) and those involved in the present investigation are not exactly the same. They also differ from those of "blebbing" described by Cooper et al., 1962) and "bubbling" of cells in tissue cultures (Buchsbbaum and Kontz, 1944). In the latter, relative fluidity of a surface protoplasm featured as a dominant factor. Presumably, therefore, blistering and similarly related phenomena could be brought about by a variety of factors, chemical as well as physical. They may be ions of many kinds acting at any point of the cortex, particulate matters of the dimension of a colloid, or certain physical stimulus or condition such as gas-membrane interface (Cooper et al., loc. cit.) to name just a few. In all these instances the common denominator for the phenomenon seems to be one which is intricately associated with sol-gel relationship of the cell surface (cf. Landau, 1960).

Practical significance of the results. Aside from the fact that *Paramecium aurelia* has proven itself a sensitive indicator for cigarette toxicities, the mainstream smoke was found to be the most toxic of the cigarette components analyzed. This finding is of significance because it agrees with the consensus of opinion on this subject, and that it is the smoke which comes into direct contact with cells lining the entire respiratory tract. Various deleterious effects of

smoke on the epithelial cells (ciliated) of the tracheobronchial tree are known (Hilding, 1956; Chang, 1957; Ide et al., 1958). More specifically, the cilia of these cells are most directly affected, leading to complete cessation of their activity followed by disintegration (Ballenger, 1960; Tremmer et al., 1959; Falk et al., 1959; Kotin and Falk, 1960). The use of a ciliate protozoon, and other animals (Guillerm, 1961) will aid us in our understanding of this vital subject without necessarily implying that their findings may be directly compared to those from human. Neither this work nor any other of this series (Wang, 1965) intends in the least to relate the results obtained from the protozoon, *Paramecium aurelia*, directly for considerations of similar studies on the human. Yet one finds it difficult to resist the reminder that all cilia, regardless of origin and kind, do have a basic similarity in structure and function (Gibbons, 1961; Sotelo and Trujello-Cenoz, 1959). The adverse effects of cigarette smoke on the cilia of paramecia (Figs. 9 and 10) has been recently confirmed by Weiss and Weiss (1964). In this connection, Thayer and Kensler (1964) reported the effectiveness of filter in reducing the deleterious effect of smoke.

A basic biological problem: resistance of some paramecia to cigarette ash and paper ash solutions. The phenomenon of resistance offered by certain individuals of a population to the action of cigarette and paper ash solutions is interesting, but so far no satisfactory explanation can be given except that it is regarded as an indication that the overall toxicity

of these solutions is below that of tobacco and smoke solutions. Protozoologists are generally aware of the fact that the behavior of *Paramecium* and other unicellular organisms is conditioned by the physiological state of the organism at any given moment. This situation may well apply to the present case. It is possible that the phenomenon of resistance to a toxicity simply reflects the degree of "vigor" or "state of general health" of the animal at the time. There are many factors which presumably determine this vigor. They are size, and age of the animal, nutritional state (food availability), and others. These are all somatic factors, which may vary a great deal. There is even the possibility that the phenomenon is of germinal origin, i.e., mutation. And the analysis of a problem in which so many possible variables are apparently involved is, of course, difficult. It certainly is a challenge to all who are interested in fundamental biology.

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