numbers of cells analyzed and the obvious need for more experiments. Several lines of evidence, including cell cycle analyses and the gamma ray experiments, show that at least the majority of the cells were indeed in early G_1 at the time of irradiation. Scoring of aberration yields in serial samples over the first mitotic peak following irradiation from several other experiments with the A8W243 cell line in addition to that reported in Table 1, together with extensive mitotic index determinations, rules out the possibility that any substantial number of cells bearing UV-induced chromosome-type aberrations were missed because they came to mitosis earlier or later than those with chromatid-type aberrations. Because the synchrony of our irradiated populations was not perfect, it might be argued that the chromatid aberrations were actually coming from a small percentage of cells that could have been in the S phase of the cell cycle when irradiated. However, the mitotic index in our samples was never less than 0.93, and no more than 7 percent of the cells could then have been in S. Nevertheless, 80 percent of the A8W243 cells and over 60 percent of the V-79 cells treated with UV alone contained at least one chromatid aberration, so most of them must have been induced in G_1 cells. While it is possible to argue that perhaps some of the deletions listed as isochromatid type were actually of the chromosome type, this seems a trivial consideration in light of the large yields of clear-cut chromatid types and the lack of other chromosome types. We conclude that UV irradiation in the early G_1 phase of the cell cycle produces mainly chromatid-type aberrations and that most of the lesions giving rise to these aberrations can be photoreactivated.

While these results were unexpected in light of the reports of Humphrey *et al.* and of Chu (*3*), they are easily explained. The only UV-induced DNA lesion that is known to be reversible by photoreactivation is the cyclobutane dimer occurring between adjacent pyrimidines within one chain of the DNA double helix (*2*). Since at least the majority of the lesions leading to aberrations in the UV-irradiated A8W243 cells are photoreactivable, it follows that these lesions are probably in fact dimers. Because photoreactivable dimers occur within a single one of the two parallel chains of the DNA double helix, it is not surprising that chromatid- rather than chromosome-type aberrations result. Thus, although not conclusive, we take these results both as evidence that chromosomal aberrations may result from lesions (dimers) induced directly in the DNA molecule, and as evidence in support of a single-stranded or unineme structure of the eukaryote chromosome as well (*7*).

The induction of isochromatid-type deletions and the apparent photoreactivation of the lesions giving rise to them shows that the simple dimer mechanism is not adequate to explain all aberration production by UV, however. If the lesion responsible for isochromatid deletions is a simple intrachain dimer, then it must "spread" to the other chain of the double helix in some way, possibly through abortive operation of a normal repair mechanism. Alternatively, if the isochromatid deletions arise from some other type of initial lesion, then their photoreactivability indicates a previously unknown photorepair mechanism.

If the simple dimer model for the induction of at least most chromosomal aberrations by UV light is correct, then one would expect that UV irradiation of cells in the G_2 phase of the cell cycle would yield few if any visible aberrations in the cells' first postirradiation mitosis. The lesions would affect only one of the DNA chains, and little time would be available for repair mechanisms to convert them to gross aberrations. While the results are even less complete than our results for G_1-irradiated cells, G_2 irradiation experiments we are currently conducting appear to bear out this prediction.

Finally, although additional experiments will be necessary to resolve the question completely, it seems possible that the earlier reports of chromosome-type aberration induction (*3*) could be explained in terms of mitotic delay and "derived" chromosome-type aberrations. After a successful mitosis, at least one of the two daughters of a cell in which a chromatid-type aberration was induced will contain an aberrant chromosome, which at the next mitosis will appear to be of the chromosome type; a chromatid exchange, for example, can give rise to an apparent dicentric chromosome at the second division, while a chromatid ring becomes a chromosome-type ring. Thus variable division delay might cause "generation mixing" that might well account for both the apparent induction of chromatid-type aberrations in G_2-phase cells and the chromosome-type aberrations reported by Humphrey *et al.* and by Chu (*3*).

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8. Supported by PHS research grant CA 11953 and AEC contract AT(40-1)-4065. We thank Mrs. Pamela Walker for technical help.

18 October 1972

Membrane Recycling: Vesiculation of the Amoeba Contractile Vacuole at Systole

Abstract. *Ultrastructural data on the protozoan* Amoeba proteus *support a model of membrane recycling. At systole the amoeba contractile vacuole fuses with the cell surface and expels its contents. Observations by electron microscopy indicate that, as the vacuole empties, its bounding membrane transforms into tiny (35 nanometers in diameter) vesicles, identical to the vesicles that segregate fluid and contribute to the diastolic vacuole.*

Small membranous vesicles observed adjacent to the secretory surface in several different cell types immediately following a burst of secretory activity have suggested that the drastic increase in surface membrane due to secretory exocytosis is reversed by endocytosis of "empty" vesicles (*1*, *2*). As has been shown in a number of other situations (*3*), the processes that are carried out

by membrane systems in protozoans are often localized or hyperactive to such an extent that they are especially suitable for correlations of function and structure. In this regard, systole of the contractile vacuole in *Amoeba proteus* represents an exaggerated case of exocytosis, and ultrastructural examination of this process supports a model in which excess surface membrane is recycled by endocytosis of coated vesicles.

The amoeba contractile vacuole, which functions to segregate a dilute solution of water and electrolytes from the cytoplasm and finally to expel it from the cell (*4*), is observed in vivo as a sphere surrounded by a layer of mitochondria. It enlarges in diastole by fusion with smaller vacuoles. Previous electron microscope studies of the diastolic amoeba contractile vacuole have demonstrated that the vacuole is bounded by a trilaminar membrane exhibiting pitlike evaginations which suggest a dynamic relationship between the vacuole and the small vesicles situated in the adjacent cytoplasm (*5*). These vesicles, 35 to 85 nm in diameter, which, because of their position as the most proximal membranous elements of the vacuolar system, have been proposed to be the sites of fluid segregation, are composed of a uniquely modified "coated" membrane. Peg-shaped elements (6.5 by 15 nm) attached to the cytoplasmic dense lamina of the trilaminar membrane characterize this membrane modification, which has recently been demonstrated to be the homologous characteristic of the fluid segregation membranes of contractile vacuoles in ciliates, flagellates, amoebae, and sponges (*6*).

Upon reaching a diameter of 30 to 35 μm late in diastole, the contractile vacuole becomes embedded in the ectoplasmic gel, fuses with the cell surface, and expels its fluid contents. Cinemicrography of this process has demonstrated that the spherical layer of mitochondria surrounding the vacuole collapses at systole, the two hemispheres appearing to fuse into a single hemisphere convex to the cell surface (*7*). It also may be assumed from these observations that the vacuole itself collapses in systole; however, the fate of the vacuole membrane cannot be demonstrated with the light microscope. The vacuole simply disappears.

The fine structure of systole was investigated by fixing individual amoebae immediately after the onset of systole as observed under phase contrast illumination. Cytoplasmic movement ceased almost immediately (<2 seconds) when ice-cold osmium aldehyde was used as the fixative. Electron microscopy of the contractile vacuole region in such cells reveals that the mitochondrial sphere actually does collapse, but the two hemispheres do not merge (Fig. 1). They are separated by a thick layer of fluid segregation vesicles. Superficial consideration of this image might suggest that these vesicles represent the vesicles that surrounded the vacuole in diastole. Counts and calculations of the numbers of vesicles, however, indicate that fewer than 1.1×10^6 vesicles are present in the sphere surrounding a vacuole 30 μm in diameter prior to systole, while more than 2.0×10^6 vesicles are present in a postsystole hemisphere 26 μm in diameter (*8*). Since the membrane area of one vacuole 30 μm in diameter is equal to the surface area of 7.4×10^5 vesicles (35 nm in diameter), it is suggested that the additional vesicles observed immediately after systole arise from the vacuole membrane. This hypothesis is further supported by the preservation in some cells of a remnant of the systolic vacuole near the pore (Fig. 2). The contractile vacuole membrane in this situation exhibits a high density of the pitlike evaginations indicative of vesiculation. We conclude that the membrane of the amoeba contractile vacuole vesiculates at systole, recycling as fluid segregation, coated vesicles to participate in subsequent diastoles.

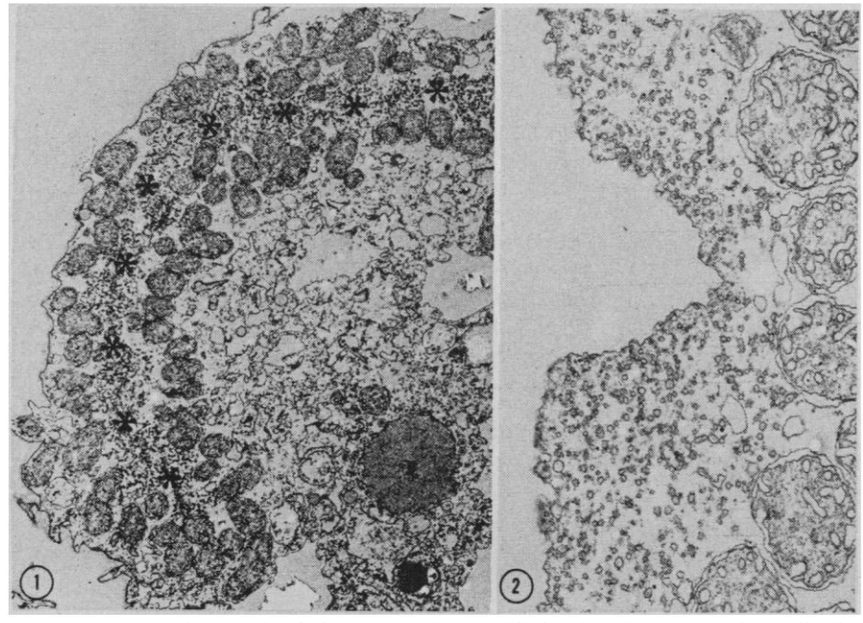

Fig. 1. Electron micrograph of the contractile vacuole in *Amoeba proteus* immediately after systole. The postsystole hemisphere is composed of two layers of mitochondria separated by a thick layer of vesicles (asterisks). Approximately one-half of the hemisphere is included in this figure (× 4000). Fig. 2. Membrane of the vacuole in the process of systole exhibits high density of pitlike evaginations (× 16,200).

Similar recycling of excess surface membrane has been indicated by studies of pancreatic and parotid acinar cells (*1*). Concomitant with the reduction in apical plasmalemma after secretory activity, vesicles appear in the apical cytoplasm; and although the membrane of these vesicles appears smooth, coated pits in the plasmalemma suggest that the vesicles may be coated at the time of endocytosis. In addition, coated pits and coated vesicles have been observed in axon terminals, and they are especially numerous after intense stimulation of the nerve (*2*). These data have been interpreted to indicate that the synaptic vesicles insert into the synaptic membrane at the time of transmitter release, and that the membrane subsequently recycles as coated vesicles. Although it is recognized that the two processes are not of necessity mutually exclusive, we feel that each additional bit of evidence for membrane recycling (as membrane) reduces the likelihood of membrane breakdown into molecules or micelles, except in digestive or autophagic vacuoles (*9*).

As has been suggested by previous investigations of coated vesicle endocytosis, it is probable that the membrane coat of the amoeba vesicles is important to vesicle formation. Although our morphological evidence is similar to that presented in other studies, we find no reason to support pre-

vious proposals (*10*) that vesiculation is facilitated by "contractile" properties of the coat elements. Comparative studies have documented the presence of similar membrane modifications (coats) on tubular and planar organelles which do not vesiculate, but which are active in the segregation and passage of fluid (*11*). These data implicate the coat in modulation of membrane permeability. Especially in models of membrane permeation which assume important roles for unstirred layers or vicinal water (*12*), the peg-shaped elements projecting into the unstirred layer are ideally situated to modulate its properties (*13*). Vesiculation, as well, could procede impelled by local changes in permeability and surface tension effected by the coat elements.

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8. Vesicles were counted in representative micrographs to give the number of vesicles per micrometer in the plane of the section. This figure was multiplied by a factor of 20 (number of 50-nm sections per micrometer) to give the number of vesicles per square micrometer. The average diameter (30 μm) in vivo of a contractile vacuole just prior to systole does not take into account the shrinkage in electron microscopy preparation, and thus 30 μm sets an upper limit on the dimensions of a contractile vacuole observed with the electron microscope; 26 μm, the average diameter of postsystole hemispheres in electron micrographs, includes the shrinkage, and therefore sets a lower limit on the dimensions of the hemisphere in vivo.
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2 August 1972; revised 10 October 1972

Natural Selection of Parental Ability to Vary the Sex Ratio of Offspring

Abstract. *Theory and data suggest that a male in good condition at the end of the period of parental investment is expected to outreproduce a sister in similar condition, while she is expected to outreproduce him if both are in poor condition. Accordingly, natural selection should favor parental ability to adjust the sex ratio of offspring produced according to parental ability to invest. Data from mammals support the model: As maternal condition declines, the adult female tends to produce a lower ratio of males to females.*

Fisher (*1*) showed, and others (*2*) reformulated, that natural selection favors those parents who invest equally in both their sons and their daughters. When the parents invest the same in an average son as in an average daughter, natural selection favors a 50/50 sex ratio (ratio of males to females) at conception (*3, 4*). (For simplicity, we assume here that parents are investing equally in average offspring of either sex.) Individuals producing offspring in sex ratios that deviate from 50/50 are not selected again as long as these deviations exactly cancel out and result in a sex ratio at conception of 50/50 for the local breeding population. Such a situation is highly unstable, since random deviations from the 50/50 ratio in local populations rapidly favor those individuals producing their young in ratios of 50/50. We show here that under certain well-defined conditions, natural selection favors systematic deviations from a 50/50 sex ratio at conception, and that these deviations tend to cancel out in the local breeding population.

Imagine a population of animals (for instance, caribou) in which the condition of adult females varies from good to poor (as measured, for example, by weight). Assume that a female in good condition is better able to bear and nurse her calf than is a female in poor condition, so that at the end of the period of parental investment (PI), the healthiest, strongest, and heaviest calves will tend to be the offspring of the adult females who were in the best condition during the period of PI. Assume that there is some tendency for differences in the condition of calves at the end of the period of PI to be maintained into adulthood. Finally, assume that such adult differences in condition affect male reproductive success (RS) more strongly that they affect female RS. That is, assume that male caribou in good condition tend to exclude other males from breeding, thereby inseminating many more females themselves, while females in good condition, through their greater ability to invest in their young, show only a moderate increase in RS. Under these assumptions, an adult female in good condition who produces a son will leave more surviving grandchildren that a similar female who produces a daughter, while an adult female in poor condition who produces a daughter will leave more surviving grandchildren than a similar female who produces a son.

In short, natural selection favors the following reproductive strategy. As females deviate from the mean adult female condition they should show an increasing tendency to bias the production of their young toward one sex or the other. Whenever variance around some mean condition is a predictable attribute of adults in a species, natural selection will arrange the deviations away from a 50/50 sex ratio at conception so that the deviations will tend to cancel out. Other things being equal, species showing especially high variance in male RS (compared to variance in female RS) should show, as a function of differences in maternal condition, especially high variance in sex ratios produced.

The model we are advancing depends on three assumptions, for which there are both supporting data and theoretical arguments.

1) The condition of the young at the end of PI will tend to be correlated with the condition of the mother during PI. This has been shown for many species (*5–7*) and is probably true of almost all animals with small brood sizes. It is sometimes true of species with large, highly variable brood sizes but need not be (*7*).

2) Differences in the condition of young at the end of the period of PI will tend to endure into adulthood. Although animals show some capacity for compensatory growth, we would be surprised if this claim were not often true. It has been demonstrated experi-