shorter duration with this method. Patients who are not sedated are less apt to show postconvulsive restlessness. The postconvulsive hypertension is reduced, but not entirely eliminated. Observations with different methods of therapy indicate that there are at least four factors in postconvulsive hypertension: (1) anxiety and restlessness, which can be alleviated by means of intravenous sodium pentothal or sodium amytal; (2) muscular exertion, which can be alleviated by means of curare; (3) asphyxia, which can be prevented by the method described; and (4) a cardiac convulsant action. The heart seems to take part in the generalized convulsion, cardiac irregularities having been reported during seizures. These are less marked when asphyxia is prevented. Hypertension does not always accompany partial convulsions, but is an invariable component of generalized seizures, although it is less marked and of shorter duration with the technique described. In the postconvulsive period, especially with metrazol, premature cardiac beats coincide with mild, generalized twitches. These coincide too well temporally to be explained except on the basis of a common stimulus. The fact that this hypertension can be temporarily arrested by carotid sinus pressure suggests that there is a nervous pathway through the autonomic nervous system. Clinically, however, with the prevention of asphyxia, anxiety, restlessness, and severe muscular exertion by means of the technique outlined (barbiturate, curare, intraconvulsive airway) most cardiac patients seem able to stand the convulsion with minimal evidence of cardiac strain.

### References


1. Brill, H., and Kalinowsky, L. *Psychiat. Quart.*, 1942, **16**, 351.
2. Brody, M. *J. nerv. ment. Dis.*, 1945, **102**, 357.
3. Orenstein, L. L. *N. Y. St. J. Med.*, 1939, **39**, 1921; Drinker, C. K. *Carbon monoxide asphyxia.* New York: Oxford Medical Publishing Co., 1938. Pp. 81, 158, 189; Norris, V., and Weiss, S. *J. Pharm. exp. Therap.*, 1927, **31**, 43.
4. Sillman, L. R., and Terrence, C. *Psychiat. Quart.*, 1943, **17**, 241.


## Photoinactivation of Milk Fat Lipase and the Origin of Bitter Flavor in Milk


Vladimir N. Krukovsky

*Department of Dairy Industry, Cornell University, Ithaca, New York*


H. D. Kay recently reported in a letter to the editor of *Nature* (*2*) that an enzyme which hydrolyzes tributyrin in milk (tributyrinase) is sensitive to light and that the atmospheric oxygen plays an important part in the photodestruction of the enzyme. This letter prompts the present writer to release information pertaining to the mechanism of the photochemical inactivation of milk fat lipase and to the origin of bitter flavor in milk, obtained in connection with studies of the interrelationship of ascorbic acid oxidation in milk and the reaction which produces the tallowy flavor (*4*). It has been found that the activity of milk fat lipase, as produced by cooling, warming, and the subsequent holding of milk at 0–5° C. from 24 to 48 hours (*5*), varied with the ascorbic acid content of the milk reduced to various levels by exposure to light (Fig. 1B). The phenomenon suggested the possibility that $H_2O_2$, formed in the course of the photooxidation of ascorbic acid (*1*), might be responsible for the inactivation of milk fat lipase.

This postulation made it necessary to determine the effect of $H_2O_2$, added to milk in different quantities but not in excess of that required to oxidize the ascorbic acid completely, upon the activity of milk fat lipase. The results were that both processes, namely, the oxidation of ascorbic acid and the destruction of lipase, were promoted simultaneously in the milk (Fig. 1A).

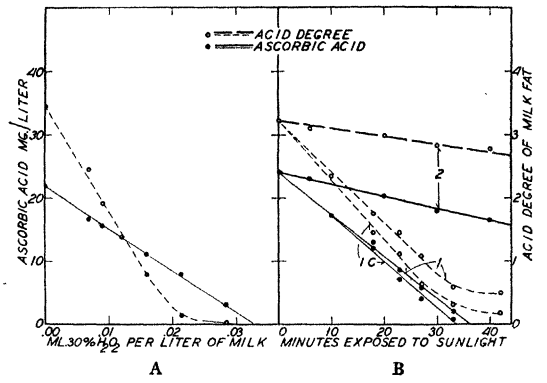


Fig. 1. Simultaneous promotion of ascorbic acid oxidation and of inactivation of milk fat enzyme lipase in the milk either in the presence of added $H_2O_2$ or photochemically by the exposure of milk in Erlenmeyer flasks to direct sunlight (B, 1) or northern daylight (B, 2). Sample 1 C was exposed in the presence of added catalase. Lipolysis was activated by cooling-warming-recooling of milk, and activity measured by increase in the acid degrees of fat at the end of a 48-hour holding period at 0–5°C. Ascorbic acid content of the milk was followed by direct titration with 2,6-dichlorophenol-indophenol in acid solution.

Consequently, to prove that the photooxidation of milk fat lipase resulted from $H_2O_2$ formed in the course of ascorbic acid oxidation, a portion of lipolytically active milk was fortified with catalase prior to exposure to sunlight. The amount of catalase (*6*) added to milk was sufficient to prevent ascorbic acid oxidation in the presence of 0.028–0.03 ml. of 30 per cent $H_2O_2$ solution/l. of milk, added subsequently. The activity of catalase, determined again at the end of 40 minutes of exposure to sunlight by the addition of ascorbic acid to milk depleted of its content and of $H_2O_2$, was found to be the same as at the starting point. The photoinactivation of milk fat lipase was not prevented, however, by the addition of catalase to milk (Fig. 1B). In fact, the enzyme was inactivated at a slightly faster rate in the presence of catalase as compared with that in the control milk. It was apparent, therefore, that the photoinactivation of lipase is an independent reaction, and that $H_2O_2$ formed in the course of ascorbic acid oxidation might play an auxiliary part. This evidence was supported further by data concerning the effect of the depletion of the ascorbic acid content of milk, prior to exposure to light, by cucumber juice oxidase. Since the ascorbic acid–cucumber juice oxidase system uses one atom of oxygen per molecule of vitamin C (*1*), it was safe to assume that no $H_2O_2$ was present in the milk at the time of exposure. Again, the sensitivity to light of the milk-fat-splitting enzyme was found to be approximately the same as in the control portion of milk containing ascorbic acid. It was also found that sensitivity of milk lipase to light varied appreciably between the samples of milk from different cows and that, of the 20 samples studied, only two samples showed from 80 to 90 per cent loss in the lipolytic activity due to 30 minutes' exposure to light. The losses of lipase in the remaining samples of milk varied from 50 to 80 per cent. The inactivation of milk

lipase by added $H_2O_2$ was considerably reduced in the presence of catalase, whereas deoxygenated milk sealed under vacuum showed an increase in lipolytic activity over that in control milk. The continuous exposure to sunlight up to 90 minutes reduced its activity approximately 10 per cent. Fat lipolysis in milk is quite often accompanied by the development of bitter flavor, which can be traced primarily to the gravity cream layer of the milk. This could be demonstrated by draining off carefully the gravity skim milk below the cream layer of milk at the end of a 24-hour holding period at 0–5° C., in which lipolysis was stimulated by cooling and warming prior to storage. It indicated that the changes in the chemical properties both of the fat and of the fat-globules-stabilizing membrane could be responsible for the development of the bitter flavor. Not only has the milk-fat-agglutinin concentrate (gravity cream plasma, obtained by the reseparation of gravity cream forewarmed to 37–40° C. in the cream separator, *3*) a tendency to develop bitter flavor on standing at refrigeration temperatures, but the milk fat from very rancid milk is quite often bitter. The compound responsible for the bitter flavor in the fat could be readily extracted by the re-emulsification of the fat in the skim milk.

### References


1. Hand, D. B., and Greisen, E. C. *J. Amer. chem. Soc.*, 1942, **64**, 358.
2. Kay, H. D. *Nature, Lond.*, 1946, **157**, 511.
3. Krukovsky, V. N. Unpublished data.
4. Krukovsky, V. N., and Guthrie, E. S. *J. Dairy Sci.*, 1945, **28**, 365; 1946, **29**, 293.
5. Krukovsky, V. N., and Herrington, B. L. *J. Dairy Sci.*, 1939, **22**, 137.
6. Sumner, J. B., and Dounce, A. L. *J. biol. Chem.*, 1939, **127**, 439.


## The Origin and Evolution of Meiosis[1]


L. R. Cleveland

*Department of Biology, Harvard University*


The question of the relation of mitosis to meiosis has received as much attention, perhaps, as any in biology. The discussion has been focused, for the most part, on the mechanism responsible for the change from one type of nuclear division to the other, and the manner in which meiosis, which made sex and biparental inheritance possible, originated. Certainly, one difficulty in tracing the origin and development of meiosis from mitosis has been the failure to understand clearly the small but fundamental differences in the two processes. And, of course, the assumption by some biologists that the two processes were almost unrelated has not helped toward an understanding of them. Far too little attention has been given to the role of the centriole and the relation, in time, of its duplication to that of the chromosomes, for herein lies the key to an understanding of the manner in which a cell changes from one process to the other.

For many years I have been studying the chromosomes of 40 genera and more than 500 species of hypermastigote and polymastigote flagellates from termites and the wood-feeding roach, *Cryptocercus punctulatus* (*1*). The protozoa of *Cryptocercus* are profoundly influenced by the molting of their host (*2*). Between molts they have the haploid number of chromosomes, and division is mitotic; during molting their chromosome number is doubled, and zygotic meiosis, which follows, returns them to haploids, where they remain until their host molts again. In three genera, *Trichonympha*, *Leptospironympha*, and *Eucomonympha*, the change from haploidy to diploidy is brought about by fertilization; in others, particularly *Saccinobaculus*, it results from the autogamy. In these genera sexual processes similar to those of higher animals and plants have become fairly well established, but not permanently, for sometimes they revert to a more primitive process, such as the one that has become very well established in *Barbulanympha* and several other genera, which process will be described presently.

Of the total number of genera studied there are six which, in my opinion, are particularly important, since they serve to show the manner in which meiosis has arisen from mitosis. Among these, two processes, permanent mitotic diploidy and meiosis without gametogenesis, deserve consideration. They lie between fertilization (including autogamy) on the one hand, and mitosis on the other.

In the first process, which is seen in the genera *Holomastigotoides* and *Spirotrichosoma*, the chromosomes, in certain instances, have become permanently diploid, since these genera have not been able to develop a method for changing back to haploids. In the genus *Holomastigotoides*, which is present in many genera of termites distributed through most of the tropical and semitropical regions of the earth, the haploid number of chromosomes is 2, and most of the species have this number. However, in each of the species of termites that I have examined from the genera *Prorhinotermes*, *Psammotermes*, *Coptotermes*, and *Heterotermes*, diploids have also been present, together with a 3-chromosome form (1n, 2n); and, in *Psammotermes*, there is in addition a 5-chromosome form (2n, 3n). All of these are derived from the basic 2-chromosome form. This polyploidy is not one of recent origin, since, for example, precisely the same forms occur in widely separated species of *Prorhinotermes* (southern Florida, Java, Madagascar), which termites have been separated at least since the beginning of the Tertiary, and probably much longer. *Holomastigotoides*, then, in a few instances, has been able to survive in a permanent diploid condition.

Another example of a similar situation is found in the genus *Spirotrichosoma*, present in three species of *Stolotermes* (a very primitive termite) from Australia, one from South Africa, and one from New Zealand. The haploid number of chromosomes is 12—the number present in all the species of *Spirotrichosoma* from the Australian and South African species of *Stolotermes*. This same number also occurs in the *Spirotrichosoma* from the New Zealand species of *Stolotermes*, but polyploids with 24, 48, and 60 chromosomes are also present. Thus, with a larger number of chromosomes, the difficulty an organism encounters in permanently adapting itself to polyploidy seems to be greatly increased.

Nuclear division of these polyploids can be seen very plainly, especially those with 4 rod-shaped chromosomes. Every division is exactly alike: synapsis in the prophase, followed by formation of tetrads, and movement of the chromosomes to the poles as dyads, *i.e.* every division is exactly like the first division in meiosis. One may ask: Why isn't this followed by a second meiotic division, thus returning the chromosomes to the haploid condition? The answer is simple: the centrioles are


[1] Aided by a grant from the Milton Fund.