surfaces is joined, and they present a picture like that of very late cleavage; later, after joining more completely, they present a picture closely resembling early cleavage. Finally, the furrow between their joined surfaces disappears, and what was once two separate cells now looks exactly like a larger but single cell except for the presence of two nuclei and two rostra. In the early stages of cytoplasmic fusion, the rostra, each with a nucleus held in a fixed position at its base, point more or less in the same direction; however, as fusion progresses, the rostra move in opposite directions, finally taking up positions at opposite ends of the zygote. If the situation is examined more closely, it will be seen that the male gamete which, like the female, has flagella over its entire body except for a very small area at the posterior end, began to lose its flagella about the time the furrow between the fusing gametes disappeared. This process continues until the axostyles and all the flagella with the exception of those on the small, anterior, rostral portion of the body disappear. At this time, or slightly earlier in some instances, the nucleus of the male gamete becomes free of its fixed position at the base of the rostrum and begins to migrate toward the nucleus of the female gamete, which, since *Eucomonympha* is a very large cell, may lie 200–300 μ away. However great the distance may be, the male nucleus succeeds in making contact with the female nucleus, which never moves from its fixed position at the base of the rostrum of the female gamete. By the time the nuclei begin to fuse, or thereabouts, the rostrum, lamella, and centrioles of the male gamete are extruded from the cell, pinched off with the loss of practically no cytoplasm. As in *Trichonympha*, the female gamete never loses any of its organelles, while the male loses all except the nucleus.

In the large polymastigote *Saccinobaculus*, the process is autogamy and begins by simultaneous division of many of the nuclei. The daughter nuclei may move a considerable distance apart in the cell and return to the interphase condition, yet the cytoplasm makes no effort to divide. When the nucleus divides, the flagella and the very large, broad, heavily staining axostyle are discarded and renewed, one new set being produced for each nucleus. This occurs three to five days before the roach sheds its exoskeleton, and the cells remain in this condition for one to two days. Then the posterior ends of the axostyles begin to move together. This process continues until the axostyles lie side by side, from end to end, so closely that one has to look carefully to see that two are present. Since the nuclei are securely anchored to the axostyles near their anterior ends, this brings them close together, and fusion follows, but not immediately. It usually occurs shortly before the roach sheds its exoskeleton, or about two days after daughter nuclei and axostyles come together. It should be noted that *Saccinobaculus*, unlike the three genera already considered, does not lose its extranuclear organelles when the nuclei fuse, but at a considerably later time. The duplication of chromosomes occurs 10–20 hours after nuclear fusion. This is followed by synapsis, formation of tetrads, first and second meiotic divisions. Since the extranuclear organelles do not begin the process of disintegration until the prophase of the first meiotic division, and a long time is required for the large, heavily staining axostyle to be dissolved, the presence of two of these structures, instead of one as in all other divisions, serves as a perfect label for the first meiotic division.

Since the processes occurring in *Barbulanympha* and related genera supply valuable information on the origin and evolution of meiosis, these will be dealt with in another paper.

The writer does not know whether the effect of the molting fluid of the roach on the protozoa is direct or indirect, although the experiments carried out so far suggest that it is direct. Withholding of food, addition of CO_2, and removal of some O_2, conditions present at molting, fail to produce any of these sexual phenomena. However, irrespective of whether the effect is direct or indirect, the results set forth here indicate that the evolution of sexual and asexual phases in the life cycles of protozoa began as an environmental response.

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Hexosediphosphoric Acid in Living Yeast

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Some years ago, Deuticke and Hollmann (*1*) made the sensational report that they had been unable to find hexosediphosphoric acid in the intact muscle. The role of hexosediphosphate as an intermediate in the normal glycolysis was thus again made questionable, and by workers of great authority. Almost simultaneously Macfarlane (*3*), however, showed hexosediphosphate in the living brewer's yeast, where its amount increases during fermentation—a result which Rapoport (*5*) had been unable to obtain two years earlier. Considering the fundamental role of hexosediphosphoric acid in the Embden-Meyerhof-Parnas scheme, it was deemed necessary to reinvestigate the problem, especially as it has been continuously under discussion (*4*). We have therefore made some determinations on baker's and brewer's yeast according to Young's principle as adapted by Deuticke and Hollmann (*1*). According to our control tests, glucose-1-phosphate does not interfere.

TABLE 1

	Hexosediphosphoric acid Total P content γ/1 gram dry weight	
	Fresh yeast	Fermented 15 min. at + 20° C.
Baker's yeast	12	124–125
Brewer's yeast	5–8	49– 86

To 700 (1,000) ml. fermenting solution, including about 0.5 kg. baker's (brewer's) yeast and 35 (50) grams glucose, 150 (210) ml. ice-cold 50 per cent trichloroacetic acid were added in order to stop fermentation. After standing 20 minutes in ice water, the solution was centrifuged. The liquid was freed from nucleotides according to Kerr (*2*) by precipitation with mercuric acetate. The hexosediphosphoric acid was then separated, according to Macfarlane (*3*), as the acid barium salt at pH 3.6 by an addition of 3 vols. alcohol. This was repeated after the removal of inorganic phosphate by magnesium mixture, whereupon it was reprecipitated at pH 8.2, adding .1 vol. alcohol. Determinations on resting yeast were made in the same manner, only without sugar.

The results are compiled in Table 1.

When hexosediphosphoric acid was precipitated only twice, for the first time as the acid barium salt at pH 3.8 and a second time as the neutral salt at pH 8.2, and when removal of inorganic phosphate was omitted, the results were as indicated in Table 2. These values may also more truthfully describe the quantitative proportions of hexosediphosphoric acid in both yeasts.

TABLE 2

	Hexosediphosphoric acid Total P content γ/1 gram dry weight	
	Fresh yeast	Fermented 15 min. at + 20° C.
Baker's yeast	62	214
Brewer's yeast	57	568

Yeasts employed contained few dead cells, the baker's yeast less than 0.5, per cent and the brewer's yeast 1.8–2.6 per cent.

Hexosediphosphoric acid must accordingly be considered as a normal intermediate in the fermentation of sugar both by baker's and brewer's yeast, even in an undestroyed fermentation system.

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Inactivation of Penicillins G and K by Liver and Kidney

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It has been shown (*1*, *2*) that penicillin K disappears more rapidly from the blood of rabbits and men than do penicillins G, F, and X in comparable dosage. It has also been stated (*1*) that the recovery of penicillin K in the urine of rabbits and men was 30–35 per cent as compared with an average recovery for G, F, and X of 74 per cent in rabbits and 91 per cent in men. In agreement with these data, the curative dose for experimental rabbit syphilis has been found to be very much greater for penicillin K than for the other penicillins (*1*). Eagle and Musselman have ascribed the rapid disappearance of penicillin K from rabbit blood and its comparatively low recovery in the urine to its inactivation by a "relatively thermolabile, nondialyzable constituent of plasma." The following experiments were undertaken in an effort to discover whether or not the liver plays a role in the inactivation of penicillin K.

Crystalline penicillins K and G were used in these experiments.[1] These compounds were characterized by carbon, hydrogen, and nitrogen analyses and by determination of the subtilis-staphylococcus ratios. The assays for penicillin were made by a modification of the Rammelkamp method (*5*). In some of the *in vivo* studies and in the *in vitro* experiments intermediate dilutions were made to detect small differences in penicillin concentration.

Rabbits anesthetized with sodium pentobarbital were used in the first phase of this study. Initially the rate of disappearance from the blood of penicillin K as compared with penicillin G was determined in renal-ligated preparations. In harmony with previously published reports (*1*, *2*), penicillin K was found to disappear from the circulating blood far more rapidly than penicillin G (Table 1).

TABLE 1

Penicillin Levels in Blood of Rabbits Two Hours After the Intravenous Administration of 1,000 O.U./Kg. of Penicillin G or K

Penicillin G O.U./ml. of blood		Penicillin K O.U./ml. of blood	
Eviscerate, renal-ligated	Renal-ligated	Eviscerate, renal-ligated	Renal-ligated
7.5*	5.0	2.50	0.312
7.5*	2.5	2.50	0.156
5.0	5.0	2.50	0.156
5.0	2.5	1.25	0.156
2.5		1.87*	0.156
2.5			
2.5			
Mean 4.64	3.75	2.12	0.187
S.E. ±0.84	S.E. ±0.72	S.E. ±0.25	S.E. ±0.031

* Obtained by modifying the Rammelkamp method to include intermediate values in the usual geometric progression of serial dilution.

The disappearance rates of penicillins G and K were then compared in renal-ligated, eviscerated preparations. The dose of penicillin was 1,000 O.U./kg. of intact body weight as in the first experiment. The results, as shown in Table 1, suggest that the evisceration procedure slowed the inactivation of penicillin K considerably, although the rate of disappearance of penicillin K in the renal-ligated, eviscerated preparations may have been more rapid than the rate of disappearance of penicillin G under the same circumstances. In this connection it should be noted that in the eviscerate preparation used in this study the liver was allowed to remain in the animal with patent hepatic veins, and therefore the animals may have retained some residual liver function (*4*). This interpretation of the data is strengthened by studies of surviving liver slices under anaerobic conditions, to be reported below.

Experiments were undertaken to test the ability of surviving rabbit liver slices to inactivate penicillins K and G *in vitro*. In each experiment approximately 100 mg. wet weight of liver slice were suspended in 3 ml. of Krebs and Henseleit (*3*) phosphate buffer (pH 7.4) containing 0.5 O.U./ml. of either penicillin K or G and 200 mg. per cent of fructose. The Q_{O_2} of the tissue was determined by the direct method. The flasks were filled with oxygen, incubated at 37.8° C., and shaken for 90 minutes at a rate of 120 per minute in a Barcroft-Warburg apparatus. A comparison of the inactivation of penicillin K with that of penicillin G under the same circumstances (Table 2) reveals that the total change in 90 minutes in penicillin K/100 mg. of liver tissue was 0.713 O.U., whereas the corresponding mean figure for penicillin G was 0.18 O.U.

Subsequent experiments showed that 1.05 O.U. of penicillin

[1] The pure penicillin fractions were kindly supplied to us by Dr. W. C. Risser, Bristol Laboratories, Inc.