at balance level for white flour than for the other proteins studied. It would appear, then, that the lysine and tryptophane contents of white flour are perhaps limiting factors in human protein metabolism also.

The values recorded here must be regarded as purely approximate because of the lack of rigorous definition of the proteins studied and also because of the uncertainties associated with the analytical figures used for the calculations, as well as the effects of digestibility coefficients and biologic values more or less removed from unity. The effect of considering these figures in the light of protein requirements for what Mitchell calls true maintenance and "adult growth" has yet to be determined. Nevertheless, they may serve as useful approximations for interim purposes. The last column of Table 2 lists the minimal quantities of each amino acid so far adduced as required for maintenance of human nitrogen balance.

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Sex Produced in the Protozoa of *Cryptocercus* by Molting

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Twenty-five species of flagellate protozoa live in the hind gut of the wood-feeding roach, *Cryptocercus punctulatus* (*1*). They represent a varied assortment of organisms, ranging from small, fairly simple cells to large, complex ones, and comprise 2 orders, 8 families, and 12 genera. They all exhibit some form of sexual behavior when their host molts, but between molts there is no sexual behavior, they have the haploid number of chromosomes, and division is mitotic. However, under the influence of molting, their chromosome number is doubled, and they remain diploids until two meiotic divisions convert them again to haploids. But this reversible process of mitosis to meiosis does not occur in all of them at the same time: some begin it in the early stages of molting, finishing two or three days before the exoskeleton is shed; others do not begin it until 10–15 hours after the exoskeleton is shed and do not complete it until two to three days later. Also, they do not all employ the same method in changing from haploidy to diploidy.

The sexual process is fertilization in *Trichonympha*, *Leptospironympha*, and *Eucomonympha*, but in each genus there are interesting fundamental differences in the details of the process. In *Trichonympha* gametogenesis occurs within cysts, each gametocyst producing two gametes. In *Eucomonympha*, no cysts have been seen, and the method by which the gametes are formed is unknown. In *Saccinobaculus*, *Barbulanympha*, etc., other processes occur.

In *Trichonympha*, both gametes are the same size, but there are very definite, easily recognized differences between them before fertilization begins; and sometimes these differences may be seen before excystation, although they are usually not evident until later. The female or egg has an area of clearly defined, large, dense granules embedded in a jelly-like matrix. This area lies in the posterior end of the body and occupies about one-tenth of the entire cell. There is a clear, open space in the center of the area into which the male or sperm, which has no area of specialized granules, inserts its somewhat pointed, anterior, rostral end when fertilization begins. In a comparatively short time very firm contact is made between the gametes, and from this point on the egg plays the active role by ingesting the entire cytoplasmic and nuclear contents of the sperm. Soon after ingestion is complete, the extranuclear organelles of the sperm begin to disintegrate. First the nuclear sleeve, the outer and inner caps, and the postrostral flagella go, then the rostral flagella and the parabasals, leaving only the rostral tube, the rostral lamella, and the two centrioles (a long one and a short one at interphase). In the meantime the sperm nucleus becomes free to move, because of the disintegration of the extranuclear organelles holding it in position. It moves toward the egg nucleus, which is not free to migrate because those organelles which keep it in place, like the others of the egg, do not disintegrate. The membranes of the two nuclei touch each other and soon join so firmly that it is impossible, in living material, to separate one from the other without destroying both nuclei. Their chromosomes, which can be seen clearly in the living state, come closer and closer together, and an attraction between homologues is plainly evident. Complete nuclear fusion results, and the two groups of chromosomes become one group, all lying in a common, enlarged nuclear membrane. By this time, or shortly thereafter, the remaining extranuclear organelles of the sperm (rostral tube, rostral lamella, and centrioles) begin to disintegrate and soon disappear. Now a duplication of the male and female chromosomes occurs, and they enter the first meiotic prophase. As they shorten, synapsis and tetrad formation occur. At this point, the centrioles of the egg produce an achromatic figure which functions to separate the shortened, rod-shaped, metaphase chromosomes into two groups, the chromosomes going to the poles as dyads. This is the first meiotic division, and the second, which follows quickly—before the chromosomes have time to divide—is as typical as the first. This returns the chromosomes to the haploid condition.

In *Leptospironympha*, no cytoplasmic differentiation in the gametes has been seen, but their nuclei are clearly differentiated into male and female. As in *Trichonympha*, one gamete begins to enter the other, is ingested, and loses its extranuclear organelles. Nuclear fusion and zygotic meiosis are as in *Trichonympha*. However, *Leptospironympha* differs markedly from *Trichonympha*, in that its gametes are quite unlike the ordinary (somatic?) cells from which they arise. They have only the short rostral portion of flagellar bands. The two long spiral portions, which in nongametic cells extend from the anterior to the posterior end, are absent. The lack of these heavy, rather rigid bands obviously greatly facilitates the entrance of the sperm and the cytoplasmic union of the two cells.

In *Eucomonympha* one gamete does not enter the other; the two join in a manner similar to conjugation in ciliates, although the process is not conjugation in any sense of the word. When the gametes first come together, only a small portion of their

[1] Some of this work was done at the Mountain Lake Biological Station of the University of Virginia. It was aided by a grant from the Penrose Fund of the American Philosophical Society.

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.