

tailed description of the histological findings, together with the physiological effects, of the ovarian grafts and ovarian tumors induced in the present experiments will be reported elsewhere.

It is of especial interest that the pure granulosa-cell tumors and luteomas developed only in the castrated male and female mice, respectively, bearing the intrasplenic ovarian transplants. This may be due to structural and physiological differences in the pituitaries of male and female mice similar to those observed in rats (8).

Experimental induction of ovarian tumors in mice following X-ray irradiation has been reported previously (5, 6, 11), although no explanation as to the cause of the neoplastic formations was given. Because the histological structure of granulosa-cell tumors and the luteomas appearing in the ovaries transplanted into the spleen were similar, if not identical, to the ovarian tumors induced with roentgen rays, it seems that the same mechanism, namely, gonadotropic overaction, might be responsible for the tumors under both circumstances.

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Human Amino Acid Requirements

ROBERT A. HARTE and JOHN J. TRAVERS

Research Laboratories, The Arlington Chemical Company, Yonkers, New York

A recent report (2) from Mitchell's laboratory presented data from which were calculated the requirements of normal young adult female humans for nitrogen balance for three different proteins: milk, soy flour, and white flour. From these figures and the analytical data summarized by Block and Bolling (1) it is possible to follow the pattern of estimation described by Stare, *et al.* (3) for egg protein, and calculate some of the individual amino acid requirements for humans on these bases.

For convenience of comparison, Mitchell's protein requirement figures for nitrogen balance, based on a 59.55-kg. female subject consuming a total of 1,300 calories, were extrapolated linearly to 70-kg. subjects. The corresponding data of Stare's estimate, which so far lacks experimental verification, are in-

cluded in Table 1, while the calculated results for the individual amino acids are given in Table 2.

Theoretically, such calculations should have validity only if the biologic value of the protein is essentially unity. The fact that agreement is quite good, despite the wide range of biologic values reported by Mitchell (0.41-0.74), suggests that

TABLE 1
HUMAN PROTEIN REQUIREMENTS FOR NITROGEN BALANCE

Protein	Grams required for equilibrium in females (59.55 kg.) on 1,300-cal. diets (N X 6.25)	Coefficient of true digestibility	Grams protein corrected for digestibility* for 70-kg. individual (N X 6.25)
Milk†.....	22.4	0.94	24.7
Soy flour†.....	23.4	0.92	25.3
White flour†.....	38.7	0.97	44.1
Egg†.....	—	1.00	28.0

* For the purpose of these estimates it is assumed that the effect of digestibility is the same on all amino acids. Since the digestibility figures are close to unity, the error thereby introduced is not likely to be greater than the various experimental errors in the analytical data, etc.

† Mitchell's data.

‡ Stare's estimate.

biologic value may be determined largely by the "essential" amino acid content, and the fraction of absorbed nitrogen excreted in the urine (which reduces the biologic value from unity) may merely reflect the excess of "nonessential" amino acids which cannot be utilized for lack of the necessary quantity of "essentials."

TABLE 2
HUMAN AMINO ACID REQUIREMENTS FOR NITROGEN BALANCE (Grams/day)

	Protein source				Minimal quantity
	Milk	Soy flour	White flour	Egg	
Arginine.....	1.2	1.5	1.7	1.8	1.2
Cystine.....	0.8	0.2	0.8	0.5	0.2
Histidine.....	0.7	0.6	1.0	0.5	0.5
Isoleucine.....	1.3	1.2	1.6	1.3	1.2
Leucine.....	3.0	1.7	5.3	3.6	1.7
Lysine.....	1.8	1.4	0.8	1.4	0.8
Methionine.....	0.5	0.5	1.3	1.4	0.5
Phenylalanine.....	1.5	1.4	2.4	1.4	1.4
Threonine.....	1.1	1.0	1.2	1.2	1.0
Tryptophane.....	0.5	0.4	0.4	0.4	0.4
Tyrosine.....	1.3	1.0	1.7	1.0	1.0
Valine.....	1.4	1.1	1.5	1.3	1.1

Of particular interest is the relatively good agreement of these several estimates. Most striking, perhaps, are the relatively low quantities of cystine and methionine in the case of soy flour and of lysine in the case of white flour. Bearing in mind the well-known observation that lysine seems to be the limiting amino acid in the utilization of white flour by the growing rat, it is not surprising that more of that protein is required for balance than either milk or soy flour. Table 2 also reflects this state of affairs by offering, in every other case except tryptophane, substantially more of the individual amino acids

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

L. R. CLEVELAND

Department of Biology, Harvard University¹

Twenty-five species of flagellate protozoa live in the hind gut of the wood-feeding roach, *Cryptocercus punctulatus* (I). 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*, *Leptospiromyxa*, 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 *Leptospiromyxa*, 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, *Leptospiromyxa* 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

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