The weight of cellular material present in the logarithmic growth phase of a microbial fermentation may be expressed as

$$W = W_0\, 2^{(t/g)},$$

where: W is the weight of cells present at time t, W_0 is the weight of cells present at time $t = 0$, and g is the generation time. Converting to natural logarithms and differentiating,

$$\ln W - \ln W_0 = (t/g) \ln 2,$$

$$\frac{dW/dt}{W} = \frac{0.693}{g}.$$

Thus, the instantaneous rate of cell increase per unit weight of cells present is inversely proportional to the generation time. Taking the observed generation time for *Rh. gracilis,* namely, $g = 2.8$ hr, we find that the rate of dry weight increase in grams per hour per 100 g of dry yeast present is $100 \times 0.693/2.8 = 25$. The cellular material being synthesized at this rate is composed of carbohydrate and protein; that is, almost no fat is being produced.

If we assume that, on a weight basis, the energy content of fat is 2.5 times that of carbohydrate or protein (*3*), we find the maximum rate of fat production to be

$$\frac{25 \text{ g carb.} + \text{prot.}}{100 \text{ g yeast/hr}} \times \frac{1 \text{ cal/g carb.} + \text{prot.}}{2.5 \text{ cal/g fat}}$$

$$= 10 \text{ g fat per } 100 \text{ g nonfat dry yeast per hour.}$$

Therefore, assuming that the energy transport enzyme system of the cell is as efficient during fat production as when carbohydrate and protein are being formed, we may conclude that the maximum rate of fat formation by *Rh. gracilis* would be 10. Calculations on other microorganisms, for example, *Torulopsis utilis,* showing the same generation time, should give the same results.

The converse of this conclusion must also be examined. If the rate of fattening is below the theoretical value calculated in preceding paragraphs, then we may state that the slowest or rate-determining steps occur in the fat-forming process and not in the respiratory or energy-yielding system leading up to it.

We may now compare this theoretical maximum fat rate with results obtained under experimental conditions. Enebo *et al.* (*4*) presented data on the fattening of *Rh. gracilis* at two levels of inoculum under otherwise similar conditions. These data were recalculated in order to determine fat rates. At both levels of inoculum, the calculations showed the fat rate to be 2.0. Kleinzeller (*5*) reported that *T. lipofera* increased in fat content from 23 to 33 percent in 9 hr. If we assume that these data came from the linear portion of the fattening curve, the calculated fat rate would be 2.2. The maximum fat rate found in our work, using *Rh. gracilis,* was 3.1. It is to be noted that these several experimental fat rates are about one-fourth of that theoretically possible as derived in preceding paragraphs. This suggests that further investigations should reveal the conditions required for more rapid synthesis of fat by microorganisms.

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The Fertile Life of Mouse and Rat Eggs

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Recently, Runner and Palm (*1, 2*) have reported ingenious experiments on the transfer of unfertilized mouse eggs at various times after induced ovulation to previously mated recipient mice. The transplanted eggs thus underwent fertilization in the host, and the foetuses developing from these eggs were recognized by eye color. They recorded a mean of 1.1 living young at term from eggs transplanted at the time of ovulation and 0.2 and 0 young from eggs transplanted 4 and 8 hr, respectively, after ovulation. Runner and Palm interpreted their results to mean that a delay of 4 hr or more between the ovulation and the fertilization of mouse eggs would cause a large reduction in the number of eggs capable of normal development. They remarked that their findings were consistent with those previously reported for other species in indicating that fertilization must commence during a "precariously short" interval after ovulation if mammalian eggs are to develop normally to term.

Some of the observations that we have made also bear upon the problem of the length of the fertile life of mouse and rat eggs. Our findings, while confirming the reduction in fecundity after delayed fertilization, indicate that most of the eggs retain their capacity for normal development for a much longer period than that implied by Runner and Palm. It also appears that the losses observed after delayed fertilization can be partly imputed, in the rat but not in the mouse, to the occurrence of polyspermy.

Our evidence is as follows. In one experimental series, 115 adult female rats that had mated during the early evening were killed in groups of 10 to 30 animals at hourly intervals from midnight to 7 A.M. the following morning (*3*). Ovulation occurred between midnight and 4 A.M. The number of eggs ovulated and the proportion of these that had been penetrated by sperms were recorded. When the percentage of rats that yielded eggs and the percentage in which all eggs were penetrated are plotted against time, the mean interval between the two curves is found to be about 5½ hr. This is interpreted to mean that, in any one rat, the mean time between the beginning of ovulation and the penetration of all the eggs by sperms was about 5½ hr.

The procedure was repeated with 100 rats that had

been kept under conditions of controlled illumination, the times of light and dark being such that ovulation took place between 11 A.M. and 3 P.M. (*3*). The average interval, in any one rat, between ovulation and the penetration of all the eggs was estimated in the same manner and again was found to be about 5½ hr.

Similar observations, as yet unpublished, on 150 mice kept under controlled lighting conditions showed that, for any one mouse, the average interval between ovulation and the penetration of the majority of the eggs was 6 to 7 hr.

In a fourth experiment, 14 oestrous rats that had been kept under normal colony conditions were not permitted to mate until 9 to 9:15 A.M.—that is, between 5 and 9 hr after ovulation. We have shown elsewhere (*3, 4*), that, under such circumstances, sperm penetration into the eggs does not begin until 2 to 4 hr after mating and then reaches completion, in any one rat, in 1 to 2 hr. The eggs of these rats must, therefore, have been penetrated by sperms between 8 and 15 hr after ovulation. The rats were allowed to carry their litters to term; a total of 91 apparently normal young (45 ♂♂ and 46 ♀♀) were born and later weaned, corresponding to a mean litter size of 6.5. The mean litter size at weaning of normally mated rats in this colony is 7.7.

Blandau and Jordan (*5*) obtained comparable results from rats artificially inseminated about 6 hr after ovulation; they recorded a mean litter size of 4.6 as compared with 6.7 when insemination was effected before ovulation. When allowance is made for the interval of 3 to 6 hr, which, as already mentioned, occurs in rats between late mating and the penetration of all the eggs, it is seen that the actual time of sperm penetration in their experiments must have been 9 to 12 hr after ovulation.

Mating between 8 and 10 A.M. on the morning of ovulation has been found to result in dispermy in about 9 percent of fertilized rat eggs, and this condition almost certainly leads to triploidy in the embryo (*6*). Chromosome counts on testis squash preparations from 40 to 45 male rats born in the fourth experiment just described revealed no instance of triploidy. This supports the conclusion of Beatty (*7*) that triploid embryos seldom survive to term and partly accounts for the decreased litter size observed in rats after delayed mating.

In short, we have found that, when mating was unrestricted, sperm penetration of all eggs in any one rat was not completed until nearly 6 hr after ovulation, and even when penetration was experimentally delayed until 8 to 15 hr after ovulation, the prenatal loss attributable to the delay was less than 16 percent. The fertile life of most of the eggs in our rats, therefore, was at least 10 hr. The same may have been true also for the mouse eggs, but this was not tested; however, it was shown that in mice allowed free mating, as under colony conditions, the mean interval between ovulation and the penetration of most eggs in any one mouse was as long as 6 to 7 hr. Now, Runner and Palm (*2*) found that when the results of transferring eggs 4 hr after ovulation were compared with those of transferring eggs at the time of ovulation, there was a loss of more than 80 percent of the young. This is in strong contrast to prenatal losses from all causes of 23 percent (unpublished data) and 30 percent (*8*) observed in apparently normal mouse colonies. The much larger loss observed by Runner and Palm must have been in some way associated with manipulation of the eggs *in vitro*, and we conclude that the resistance of eggs to the injurious effects of conditions *in vitro* must diminish rapidly with age.

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Nonidentity between Pepsitensin and Hypertensin Revealed by Paper Electrophoresis

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Croxatto and Croxatto (*1*) reported the production of a new vasoconstrictor and hypertensive substance when plasma globulins were incubated with pepsin at 38°C and *p*H 2 to 6. This substance, which was named pepsitensin, was subsequently studied by several authors and found to present chemical and pharmacological properties very similar to those of hypertensin (*2*). The only difference between the two substances was found by Braun-Menendez and coworkers (*3*), who showed that crude preparations of pepsitensin are not destroyed by hypertensinase from dog's red blood cells (RCH). However, this was shown to be due probably to the presence in crude pepsitensin of hypertensinase-inhibiting substances; in fact, Vasquez (*4*) succeeded in inactivating as much as 50 percent of a purified pepsitensin by incubating it with a dose of RCH sufficient to destroy completely an equipressor amount of hypertensin. Subsequently, we (*5*) were able to purify further pepsitensin and *completely* abolish its pharmacological activity by a dose of RCH that destroyed hypertensin as well. The similarity between both substances was further strengthened by our work when we found that a mixture of both could not be resolved in its components by paper chromatography with three different pairs of solvents (butanol : acetic acid : water, 4 : 1 : 5; acetone : acetic acid : water, 5 : 1 : 4; dioxane : butanol : water 10 : 10 : 5).

We now report the results of the comparative study