sRNA, slightly reduces fixed radioactivity at each stage, but does so neither selectively nor sufficiently to indicate that the rates of protein synthesis decrease at metaphase (Table 1).

The first cleavage cycle is unusual, for, during the early part of it, sperm penetration and nuclear fusion must take place. The second and subsequent cleavages are, however, normal mitotic divisions (except that the cells are not "growing" in the sense of increasing in mass). The total uptake of amino acid and the protein synthesis during the second cycle were also measured. In Fig. 2 are plotted data points for two separate experiments, all normalized to a rate of 1.0 at the time that 50 percent of the cells showed the first cleavage furrow. Also represented is the relative rate of DNA synthesis during the first cycle, as it is reflected in the incorporation of C^{14}-thymidine. The rate of DNA synthesis is normalized to 1.0 at the observed maximum.

The DNA synthetic period ("S") of the first interphase is discrete, to the extent, at least, of a very sharp drop in the incorporation rate at metaphase. If we assume that DNA synthesis in any one cell stops at metaphase, these data indicate that the departure from perfect synchrony and the length of the incubation period interact in such a way as to cause a decrease in the observed incorporation rate of about 60 percent of that during the synthetic period.

A reduction of the same magnitude in the rate of protein synthesis and in the number of polyribosomes is seen in HeLa cells (*2–4*). We might therefore expect a similar decrease in the observed rate of protein synthesis of sea urchin blastomeres if it behaved as does that of DNA, but there is no detectable decline in protein synthesis and no detectable decrease in the rate of penetration by the amino acid.

During the first cell cycle, changes in the rate of protein synthesis follow changes in the permeability rather closely, which suggests that penetration may limit the number of counts incorporated into protein (that is, that proteins are being synthesized from a pool of leucine of increasing specific activity). During the second cycle, the permeability becomes almost constant, and there is evidence that thereafter it remains so at least to the gastrula stage (*12*). The rate of protein synthesis, however, increases very greatly during the second cleavage.

The suppression of protein synthesis at metaphase in mammalian cell cultures has been attributed to stopping of transcription at the time of chromosomal condensation, which allows the population of functioning cytoplasmic messengers to decay. Polypeptide assembly is assumed to be limited by the availability of template RNA, and therefore the rate of protein synthesis decreases until transcription starts again, perhaps in the G_1 period of the next interphase. This explanation is favored by the observation that cells arrested in metaphase manifest an increasingly severe inhibition of H^3-histidine incorporation (*1*).

Scharff and Robbins (*3*) and Salb and Marcus (*2*) reject this explanation on the ground that the half-life of messenger RNA, estimated from the decay of polyribosomes after treatment with actinomycin (*5*), is much longer than the duration of normal mitosis. In addition, there is evidence that ribosomes from metaphase populations are less competent to function in vitro than are those from interphase cells (*2*). The metaphase ribosomes can be "restored" by a trypsin treatment; this is interpreted (*2*) as indicating that the nucleus, as it breaks down in prometaphase, releases an inhibitor of ribosomes which is sensitive to trypsin. However, the claim that mitotic HeLa cells support the replication of normal yields of polio virus (*4*) does not corroborate this explanation.

The inhibition of protein synthesis during metaphase of HeLa cells is in any case a fact; our results show that it is not so in another cell type. Continuity of protein synthesis at metaphase of sea urchin blastomeres neither supports nor denies the explanations offered for inhibition in the cell cultures. The exceptionally long half-life of messenger RNA in the embryo (*8*) may account for the observed continuity; this supports the idea that it is decay of the messenger that causes the metaphase inhibition in exponentially growing cells.

On the other hand, the ratio of the nuclear to the cytoplasmic volume is very much smaller in a blastomere than it is in a HeLa cell. Agents released from the nuclei during early cleavage may have a reduced effect on the relatively enormous population of cytoplasmic protein-synthesizing units. However it is clear that the rates of protein synthesis in sea urchin blastomeres have no obvious relation to the cytological changes of mitosis, and therefore the cessation of synthesis is not an obligatory result of the entry of cells into karyokinesis.

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Zinc and Cobalt: Effect on the Iron Metabolism of Ustilago sphaerogena

Abstract. *The ferrichrome content of cells of the smut fungus* Ustilago sphaerogena *during growth in a medium deficient in zinc increases with increase in the cobalt of the medium from 0 to 3 × 10^{-5}M. The addition of zinc to such cultures prevents the accumulation of ferrichrome. The results suggest that zinc is involved in the utilization of iron via a process which can be blocked by cobalt.*

Healy *et al.* (*1*) noted a striking similarity between cobalt toxicity and iron deficiency in the pattern of enzymatic activities in *Neurospora crassa*, and Padmanaban and Sarma (*2*) reported the production of a new "iron-binding compound" by the same species during growth under similar conditions. We have found that when the smut fun-

gus *Ustilago sphaerogena* is grown in a medium containing high levels of cobalt ions, large quantities of ferrichrome accumulate in the cells. The action of cobalt is completely reversed by zinc ions (see below). From the known effect of zinc on the cytochrome (*3*) and ferrichrome (*4*) content of *U. sphaerogena*, it appears that zinc ions are required for the utilization of iron by this organism.

Iron (as chloride) was added at a level of 16 mg/liter to a basal medium that was identical to that used previously (*5*), except that zinc was omitted and phosphorus was furnished as KH_2PO_4. The medium was dispensed in 50-ml quantities for sterilization [15 lb (6.8 kg), 15 minutes] and inoculated with 1 ml of a 2-day culture grown in the basal medium, and the cultures were incubated for 48 hours at 30°C with vigorous aeration by agitation. Total ferrichrome was extracted from the washed cells with 1*M* acetic acid and assayed as previously described (*6*). Cell mass was estimated by turbidity measurements and reference to a standard curve of dry weight versus absorbancy at 650 mμ.

The ferrichrome content of cells grown in the medium containing various concentrations of cobalt ions, and the effects of various concentrations of zinc on the accumulation of ferrichrome in the presence of cobalt ($3 \times 10^{-5}M$) are shown in Fig. 1. The dry weights of the cell mass in the cultures varied from 2 to 4 mg/ml. Neither manganese nor nickel ions at $3 \times 10^{-5}M$ concentration promoted the production of ferrichrome, nor did these ions reverse the effect of cobalt ion ($3 \times 10^{-5}M$). Since zinc does not inhibit the production of iron-binding compounds by iron-deficient cultures of *U. sphaerogena* (*5*), it seems likely that zinc is required for the utilization of iron. Further indirect evidence which supports the hypothesis that zinc deficiency, at least in some aspects, is essentially iron deficiency was the observation that diminished porphyrin synthesis occurs when cultures are grown in a medium low in zinc but containing normal amounts of iron. The speculation of Grimm and Allen (*3*) that depressed porphyrin synthesis occurs in zinc-deficient medium was proven correct by the extraction and measurement of porphyrins in the cells, and by the detection of a low specific activity of δ-aminolevulinic acid dehydrase in such zinc-deficient cells. Diminished porphyrin synthesis and low specific activity of δ-aminolevulinic acid dehydrase had been observed previously by Burnham (*7*) with iron-deficient cells of *U. sphaerogena*.

Ferrichrome is believed to act in microbial metabolism as a "coenzyme" for the transfer of iron. The present work suggests that the movement of iron from ferrichrome to the iron-containing enzymes and other iron-requiring sites within the cell involves the participation of zinc in a mechanism which can be blocked by cobalt.

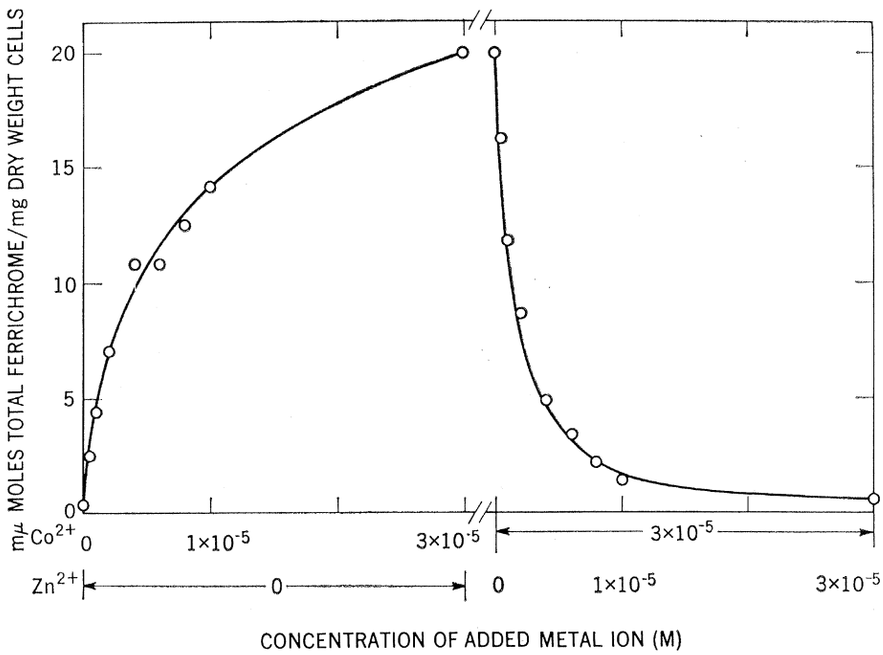

Fig. 1. Accumulation of ferrichrome by cells of *Ustilago sphaerogena* in a medium containing cobalt, and its counteraction by zinc.

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Laboratory Culturing of a Thermophilic Alga at High Temperature

Abstract. *Conditions for laboratory growth of the thermophilic alga* Cyanidium caldarium *at elevated temperatures have been developed. Growth characteristics of the organisms are described.*

Thermophilic algae that exist in hot springs and geysers are among the most resistant of all organisms to the deleterious effects of high temperature. It has been claimed that these microorganisms can grow at temperatures as high as 85°C in their native environment (*1–3*). However, growth above 70°C has not been reported for any thermophilic alga under laboratory conditions on a defined medium (*4*). Thermophiles appear to be a potential source of heat-stable enzymes. Our specific need for thermostable aminoacyl-sRNA synthetases with acidic *p*H optima (*5*) led us to investigate a species of algae that had been reported to grow at environmental temperatures as high as 82.5°C and to survive intermittent scaldings with boiling acidic water at 15- to 30-minute intervals (*3*). We now describe culture-growth characteristics of the thermophilic alga *Cyanidium caldarium* under laboratory conditions approximating its natural habitat of high temperatures and low *p*H.

Tilden first described this alga in 1896 and reported them growing at several locations in Yellowstone National Park (*6*). They have subsequently been found elsewhere, including the hot