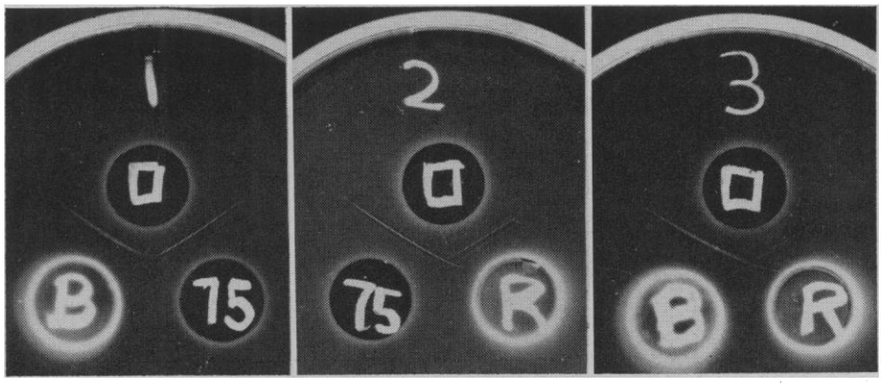

Fig. 2. Ouchterlony agar diffusion plates showing the reaction of anti-OTC with purified OTC, and extracts from arginine repressed and derepressed *E. coli* W cells. Well □: 0.18 mg of anti-OTC serum protein; Well 75: 28 μg of OTC containing 1040 units per milligram of protein [one unit of OTC activity equals 1 μmole of citrulline produced per minute at 37°C under the conditions of assay previously reported (*3*)]; Well B: 0.3 ml of crude extract of derepressed *E. coli* W cells containing 45.6 units of OTC and 3.1 mg of protein; Well R: 0.3 ml of a crude extract of repressed *E. coli* W cells containing 0.8 units of OTC and 3.1 mg of protein. The distance between wells was 14 mm. Plates were incubated for 6 days at 27°C, and contact prints were made. Duplicate plates were run in all experiments.

from the antibody by treatment with acetic acid at *p*H 5 or by ammonium sulfate fractionation.

Extracts from derepressed *E. coli* W cells with a high level of OTC and extracts from arginine-repressed *E. coli* W cells with low levels of OTC were compared on the basis of the precipitation reaction with the specific antiserum. Since the preparations of repressed cells demonstrated a small precipitation reaction which could not be accounted for on the basis of observed OTC activity alone, the Ouchterlony agar diffusion method was employed (*7*). With a 200-fold purified preparation of OTC, formation of a large distinct precipitation line was demonstrated along with two minor lines by this method.

Diffusion agar plates were then prepared with crude extracts of repressed and derepressed *E. coli* W, and purified OTC was opposed to the rabbit anti-OTC serum. Figure 2 illustrates contact prints of three diffusion plates obtained. On diffusion plate 1 (Fig. 2), the purified enzyme (Well 75) formed a definite reaction of identity with the crude extract of derepressed cells (Well B), both in the major heavy precipitation line (OTC) and in the minor lines due to contaminating proteins. On plate 2 the same amount of protein from a crude extract of arginine-repressed cells (Well R) formed no reaction of identity with respect to the major heavy line of purified OTC reacting to anti-OTC serum. The contact print of plate 3 shows the lack of identity of protein from repressed cells (with respect to the major line) when allowed to diffuse simultaneously with the extract from derepressed cells against the anti-OTC serum.

The evidence above strongly favors the hypothesis that arginine, or a repressor formed from arginine, blocks in some yet unknown way the formation of the entire OTC-protein molecule at the site of synthesis (presumably the ribosomes). However, it should be pointed out that the lack of a precipitation reaction or the absence of a displacement reaction as shown by Perrin *et al.* (*4*) does not eliminate the possibility of synthesis of at least part of the OTC molecule or β-galactosidase molecule, as the case may be, at the enzyme-forming site during repression (*8*).

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Plumage in Lal Munia (Amandava amandava)

Abstract. An Indian ploceid finch, *Amandava amandava*, develops brilliant nuptial plumage in males during the breeding season. The females and males outside of the breeding season have modest brown hen plumages. The fact that castrates, whether originally of male or of female sex, assume nuptial plumages during the breeding season of the species indicates that plumage character is under the control of hypophyseal hormones and that hypophyses are passing through an activity cycle which is independent of presence or absence of gonads.

Plumage of African weaver finches is under hypophyseal control (*1*), specifically under luteinizing hormone (*2*), and this fact has been made use of in developing an assay for luteinizing and chorionic gonadotropic hormones (*3*). These finches are natives of Africa and are imported at great cost in the United States, where many laboratories are now maintaining them. As quite a few species of finches occur naturally in India (*4*, *5*), it was, therefore, decided to investigate their plumage control.

We have investigated some species of Indian finches and one of these, lal munia (*Amandava amandava*), which is a very beautiful bird (family, Ploceidae), has yielded encouraging results. Males are brilliantly colored and have vermillion red bills during the nuptial phase. The head and upper plumage are crimson colored. The tail is black and the outer feathers are tipped with white. From chin to breast it is deep crimson, sparsely spotted with white. Females are dull brownish with wing coverts and inner secondaries tipped white and upper tail coverts crimson with small terminal white specks. The tail is brownish black, the chin yellowish white, while the throat and upper breast are grey. Abdomen and under tail coverts are light brownish white (*5*). Males in eclipse resemble females.

A number of birds of both sexes were castrated during the fall, and their feathers were subsequently examined every month. During March and April, males, castrated males, and castrated females regenerated male nuptial plumage. This coincides with the progressive phase of their sexual cycle (*6*). Intact females maintain hen plumage all year round.

The appearance of nuptial plumages in normal males and castrates of both sexes suggests that cock plumage is gonad independent in this species and that the ovarian hormone suppresses

its manifestation in normal females. This is just as it is in the African weaver finches, and so the plumage may reasonably be expected to be under the control of some hypophyseal factor. If this is proved, *Amandava* could also be used for assay purposes as African finches are now.

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Puromycin-Induced Changes in Uredospores of Puccinia sorghi Schw.

Abstract. Puromycin stimulates substrate consumption and initiates an accumulation of amino acids in uredospores of the corn rust fungus. The results indicate that under suitable conditions uredospores should be able to synthesize appreciable quantities of amino acids, but must be stimulated to do so.

Uredospores of the rust fungi synthesize amino acids and other metabolic intermediates very slowly compared to the common saprophytes (*1*). Although many intermediates will eventually become radioactive when uredospores are stirred with radioactive substrates, the amount of radioactivity is small (*1–3*). Since germination of saprophytic fungi is accompanied by the synthesis of proteins and polynucleotides while that of the rusts is not (*2*), inducing uredospores to provide sufficient intermediates for the synthesis of these macromolecules may prove to be a partial solution of growth failure in these obligate parasites. Consequently, a search was initiated for a compound which would stimulate uredospores to consume carbon compounds at a more rapid pace. Carcinogens, like 3,4-benzpyrene, structural analogs, like *p*-fluorophenylalanine, and antibiotics, like chloromycetin, were tested, but puromycin alone induced an accumulation of radioactive amino acids in germinating corn rust uredospores.

Table 1. The effect of puromycin on acetate utilization by corn rust uredospores. The medium included 0.1 g glucose, 5 μc sodium acetate-2-C^{14} (0.21 mg), and 50 mg spores suspended in 200 ml of 0.01-percent vol/vol aqueous Tween 20. The spores were exposed to the substrate on a shaker for 6 hours. The results are counts per minute per milligram of protein N.

Characteristic	Puromycin (ppm)	
	0	80
Fraction		
Amino acids	77,100	295,200
Organic acids	211,000	184,500
Sugars	193,100	310,700
Nucleic acids	55,700	236,700
Protein	65,500	161,200
Residue	2,200	5,400
Total	604,600	1,193,700
Protein N	0.42 mg	0.66 mg
Germination	94%	90%

The spores were germinated as described previously (*4*). A complete description of methods employed for extraction and analysis of components appears elsewhere (*1*). The results of a typical experiment are presented in Table 1. In the presence of puromycin the total acetate consumption was nearly doubled, radioactivity of the amino acid fraction was increased approximately fourfold, while that of the organic acid fraction decreased. The specific activity of the protein and nucleic acid fractions increased nearly threefold and fourfold, respectively. Radioactivity in the amino acid fraction increased logarithmically with increasing puromycin concentration up to 80 ppm. The specific activities of the free and protein-bound amino acids increased in approximately the same order of magnitude as did the total activities of their respective fractions. The source of nitrogen for the increased synthesis of these amino acids is unknown. However, there was but little net synthesis of proteins; the protein nitrogen increased with the increasing concentration of puromycin until about 20 ppm puromycin were added, after which no further increase was observed.

Puromycin is normally considered to be an inhibitor of protein synthesis (*5*), and it was readily found to inhibit the incorporation of radioactive L-leucine, L-glutamate, and D-glucose into the protein fraction of the uredospores (Table 2). Despite such inhibition, total consumption of these materials and their rates of conversion to free amino acids were augmented just as when acetate was employed. It was therefore interesting to study the effect of exposing the spores to puromycin, washing out as much of it as would come out in two washings, and then shaking the uredospores in the presence of L-leucine-U-C^{14}. After pretreatment for 1 hour with puromycin, the spores incorporated radioactivity from leucine and from acetate into the protein fractions more than twice as fast as spores pretreated with water.

Table 2. The effect of 80 ppm of puromycin on substrate utilization by corn rust uredospores under the same conditions as described in Table 1. The values show the specific activity in the protein as percent of the control.

Radioactive compound used in substrate	Puromycin present in medium	Puromycin pretreatment
L-leucine-C^{14} (2 μc)	64	203
L-glutamate-1-C^{14} (2 μc)	54	
Glucose-U-C^{14} (5 μc)	48	
Sodium acetate-2-C^{14} (5 μc)	550	230

It seems clear that regardless of the cause of the changes occurring in the presence of puromycin, the spores do have a large capacity at least for amino acid synthesis, and under the proper conditions can respond to the environment with a vigorous consumption of substrate materials. The results after pretreatment of the uredospores with puromycin suggest that the uredospore normally has the means for an adequate synthesis of materials required for growth, but this capacity is partially suppressed under the usual environmental conditions. Puromycin thus appears to overcome partially the suspected inhibition.

Yarmolinsky and De La Haba have pointed out the analogy between the structure of puromycin and short-chain nucleic acids (*5*). Using these findings as a guide, we shall investigate the effect of soluble nucleic acids on rust uredospores (*6*).

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