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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References

1. E. Witschi, *Wilson Bull.* **47**, 177 (1935); *Anat. Record* **64**, suppl., 59 (1936).
2. S. J. Segal, *Recent Progr. in Hormone Research* **12**, 298 (1956).
3. E. Witschi, *Mem. Soc. Endocrinol.* **4**, 49 (1955); S. J. Segal and E. Witschi, *J. Clin. Endocrinol. and Metabolism* **15**, 880 (1955); S. J. Segal, *Science* **126**, 1242 (1957).
4. S. A. Ali, *The Book of Indian Birds* (Natural History Society, Bombay, ed. 4, 1946).
5. S. Baker, *Fauna of British India* **3**, (1926).
6. P. D. Tewary and J. P. Thapliyal, unpublished information.

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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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References and Notes

1. R. C. Staples, H. P. Burchfield, J. G. Baker, *Contribs. Boyce Thompson Inst.* **21**, 97 (1961).
2. R. Kasting, A. J. McGinnis, W. C. Broadfoot, *Nature* **184**, 1943 (1959).
3. R. C. Staples and L. H. Weinstein, *Contribs. Boyce Thompson Inst.* **20**, 71 (1959).
4. R. Syamananda and R. C. Staples, *Phytopathology*, in press.
5. M. B. Yarmolinsky and G. L. De La Haba, *Proc. Natl. Acad. Sci. U.S.* **45**, 1721 (1959).
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