between these values, the small differences being imputable to the standard error of the method. The only appreciable difference is in lysine content, and this can be ascribed to its relative instability in acid solutions[6].

Recently HUISMAN *et al.*[7] have analyzed the amino acid composition of alkali resistant Hb separated by chromatography on Amberlite IRC–50 from blood haemolysate of a patient suffering from Cooley's anemia (*Talassemia Major*). Our data are also fully comparable with those obtained by these authors, and reported in the Table for comparison. HUISMAN *et al.* come to the conclusion that Hb F and the alkali resistant fraction of Cooley's anemia are in all probability the same protein.

Our results on crystallization and amino acid composition further support the hypothesis that alkali-resistant Hb present in *Talassemia Minor* and in Cooley's anemia is identical to Hb F.

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Institute of Biological Chemistry, University of Rome (Italy), June 3, 1957.

Riassunto

Dal sangue di un soggetto affetto da *TalassemiaMinor* si sono ottenuti cristalli tipici di Hb F.

L'analisi della composizione in aminoacidi di tali cristalli porta a concludere che la frazione di Hb alcali-resistente presente in questa malattia e l'Hb F sono identiche.

[7] T. H. J. HUISMAN, H. K. PRINS, and P. C. VAN DER SCHAAF, Exper. *12*, 107 (1956).

A Female-sterile Mutant *(Deep Orange)* of *Drosophila melanogaster* Increasing Isoxanthopterine Content

Isoxanthopterine has been found and identified in the tissues of both normal and mutant *Drosophila melanogaster*[1]. In the mutants *white* and *brown*, it disappears from the tissues during the first few days of imaginal life[2], and it is never present at any stage of development in the mutant *rosy*[3]. In the more than 25 eye colour mutants which have been examined[4], none has increased the amount of isoxanthopterine present in the tissues.

The sex-linked recessive female-sterility factor *deep orange* (*dor*) has an effect on eye colour[5]. Eyes of both males and females are light orange in newly emerged flies and darken to a deeper orange with age. A comparison of the isoxanthopterine content of wild type (Sevelen line) and *dor* flies at four different developmental stages shows a clear difference between mutant and wild type flies in isoxanthopterine content (Tables I and II). In *dor* females, the amount of isoxanthopterine in-

[1] E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci., Wash. *37*, 650 (1951). – S. NAWA and T. TAIRA, Proc. imp. Acad. Japan *30*. 632 (1954). – E. HADORN, Exper. *10*, 483 (1954). – H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. *77*, 4865 (1955). – M. VISCONTINI, E. LOESER, P. KARRER, and E. HADORN, Helv. chim. Acta *38*, 2034 (1955).

[2] E. HADORN, Exper. *10*, 483 (1954).

[3] E. HADORN and I. SCHWINCK, Nature *177*, 940 (1956); Z. Vererbungslehre *87*, 528 (1956).

[4] E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci., Wash. *37*, 650 (1951). – E. HADORN, Exper. *10*, 483 (1954). – E. HADORN and I. SCHWINCK, Nature *177*, 940 (1956); Z. Vererbungslehre *87*, 528 (1956).

[5] D. J. MERRELL, Amer. Naturalist *81*, 399 (1947).

Table I

Differences in isoxanthopterine content of + and *dor* males at different developmental stages, expressed in percentage of + value.

Developmental stage	+♂♂*		*dor*♂♂			*t*	*p*
	n	SE	*n*	$\bar{x}$	SE		
Prepupa	26	1·41	28	63·82	2·26	12·54	< 0·001
50 h pupa	21	1·62	21	72·57	2·11	10·63	< 0·001
Emergence (bodies only)	15	1·82	16	99·31	2·21	0·19	not significant
4 day imago (bodies only)	13	1·86	14	74·50	3·59	4·01	< 0·001

* Because of variations from chromatogram to chromatogram it was not always possible to make a direct comparison of fluorescent values; therefore the mean value of the + readings on any one sheet was given a value of 100·0% and all readings converted to equivalent percentage values. The figures in the third column give the standard error of + male readings.

Table II

Differences in isoxanthopterine content of +, *dor* and *ClB/dor* females at different developmental stages, expressed in percentage of + value.

Developmental Stage	+♀♀*		*dor*♀♀			*t*	*p*	*ClB/dor*♀♀			*t*	*p*
	n	SE	*n*	$\bar{x}$	SE			*n*	$\bar{x}$	SE		
Prepupa	8	2·99	17	117·65**	3·41	4·05	0·001					
50 h pupa	12	2·63	11	165·28	9·51	6·73	0·001	11	143·91	3·30	10·48	< 0·001
Emergence (bodies only)	16	3·54	14	207·0	14·21	7·31	0·001	15	175·10	5·21	12·25	< 0·001
4 day imago (bodies only)	25	2·40	23	233·43	6·78	18·38	0·001	19	131·74	4·40	6·30	< 0·001

* As in Table I, all figures are converted to percentage of + mean value on each chromatogram, the + value being equal to 100·0%.
** At this stage, it is not possible to distinguish between *dor* and *ClB/dor* females; this measurement represents their combined values.

creases during development until more than twice the normal content is present. Females heterozygous for *dor* also show increased amounts of isoxanthopterine in all developmental stages studied. In mutant males, on the other hand, the isoxanthopterine level remains below that of normal males during pupal development, and only at hatching is the amount equal to that in + males.

Pigmentation of *dor* testes occurs precociously, and the testes of newly emerged mutant males are often yellow, while those of newly emerged + males are colourless or only faintly tinged with yellow. By 4 days, however, the normal testes are slightly darker than those of mutant males. Differences in content of fluorescent substances in the bodies of + and *dor* males are probably

Table III

Differences in the pterine content of the heads of 4 day + and +/*dor* adult females expressed in percentage of + value.

Pterines (Heads only)	+♀♀		+/*dor*♀♀			t	p
	n	SE	n	$\bar{x}$	SE		
Red	17	2·69	20	121·80	3·56	4·92	$< 0{\cdot}001$
'Xanthopterine'	19	2·96	25	151·88	5·65	8·10	$< 0{\cdot}001$
'Sepia' pterine	12	4·88	14	114·64	2·80	2·60	$< 0{\cdot}05$
$HB_1 + HB_2$	12	4·67	14	148·43	7·70	5·30	$< 0{\cdot}001$

4 days after hatching, the amount in *dor* males has declined to three-fourths that of the + males.

Other fluorescent substances are also affected in the mutant flies. In the bodies of four-day-old male *dor* flies, the green fluorescent substance (tentatively identified in our laboratory as xanthopterine by Dr. ZIEGLER-GÜNDER, unpublished) is reduced to $59{\cdot}8 \pm 4{\cdot}2\,\%$ of the amount of + males of the same age. 'Xanthopterine' measurements were not made of female bodies. At least 3 components of the fluorescent spots designated as 4 and 5 by HADORN and MITCHELL[6] have been identified: the 'sepia' pterine (yellow fluorescence)[7] and two blue fluorescent substances, HB_1 (2-amino-6-hydroxy-pterine)[8] and HB_2[9]. All of these substances are present in amounts about 3 times that of the normal males in newly emerged *dor* males; by 4 days, however, there are no significant differences between normal and mutant males in these substances in the body. The bodies of newly emerged and four-day-old *dor* females contain 3 times as much of the fluorescent substances of the 4/5 spots as do those of + females of the same age.

Heads of newly emerged and four-day-old flies were measured separately. Red pigments and 'xanthopterine' are reduced in both male and female *dor* to about 10% of the normal amounts. The 'sepia' pterine is reduced to about half the amount found in + flies, but there is no significant difference between + and *dor* flies in the blue components 4/5. Red pigments, 'xanthopterine' and the components of 4/5 are all increased in the heads of +/*dor* hybrids (Table III). Measurements were made of +/*dor* because of the reduction in the size of the eye in *ClB*/*dor* flies due to the *Bar* gene; measurements of the pterines in the bodies of +/*dor* flies gave results comparable to those obtained from *ClB*/*dor* flies of the same age. Until the biochemical interrelations of the various pterines are better understood, it is not possible to give an adequate explanation of the results obtained in the heterozygous female.

[6] E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci. Wash. *37*, 650 (1951).

[7] H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. *76*, 5656, 5658 (1954).

[8] M. VISCONTINI, E. LOESER, P. KARRER, and E. HADORN, Helv. chim. Acta *38*, 1222 (1955). – H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. *77*, 4865 (1955).

[9] H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. *77*, 4865 (1955).

related in part to differences in the rate and amount of testes pigmentation.

Malpighian tubules of *dor* flies of both sexes are deep yellow in colour[10] and are thicker and stiffer than normal Malpighian tubules. This is due to the accumulation of large amounts of excretory material in the lumen (*cf. rosy*[3]). The tubules of four-day-old *dor* flies contain 3 to 6 times more of the fluorescent substances than those of + flies of the same age. The blue components of 4/5, which are present only as traces in the tubules of + flies, are present in considerable concentration in *dor* tubules.

The different biochemical patterns exhibited by *dor* males and females are of interest in relation to the fact that lethal embryos from homozygous *dor* females exhibit two characteristic phenocritical periods which are dependent upon the sex of the embryo[10]. Preliminary results indicate that amino acid metabolism is also affected to a different degree in the two sexes.

All measurements were made on one-dimensional chromatograms developed in propanol-amonia. Whole prepupae and 50 h pupae, and heads and bodies of newly emerged and four-day-old flies, were squashed directly on the filter paper. Measurements of the fluorescent intensity of various spots were made by the direct method first used by HADORN and KÜHN[11].

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Zusammenfassung

Der Bestand an fluoreszierenden Stoffen in verschiedenen Entwicklungsstadien der Weibchen-steril-Mutante

[10] S. J. COUNCE, Z. Vererbungslehre *87*, 443 (1956).

[11] E. HADORN and A. KÜHN, Z. Naturf. *8b*, 582 (1953).

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deep orange wurde papierchromatographisch festgestellt. In allen geprüften Stadien von *dor/dor-* und *ClB/dor-*Weibchen ist mehr Isoxanthopterin nachweisbar als bei +-Weibchen. Bei Männchen der Mutante findet sich in Puppenstadien weniger Isoxanthopterin als bei +-Männchen; nur bei frisch geschlüpften Fliegen entsprechen sich normale und *dor*-Männchen in ihrem Isoxanthopterin-Gehalt. Bei *dor/dor-* und *dor/Y*-Tieren sind ausserdem noch andere Pterine betroffen. So zeigen die Köpfe der *dor*-Mutante weniger rote Pigmente, sowie mehr «Xanthopterin» und «Sepia-Pterin» als Fliegen des Wildtyps, dagegen kommen HB_1 und HB_2 in normaler Menge vor. Im Kopf der heterozygoten Weibchen werden für alle diese Pterine Werte gemessen, die sogar den Wildtypus beträchtlich übertreffen. Bei *dor/dor* und *dor/Y* findet sich in den Malpighischen Gefässen eine stark erhöhte Konzentration an fluoreszierenden Stoffen.

Taxonomical Implications of the Properties of a Riboflavin-producing Mutant Yeast

Introduction.—SUBRAMANIAM and RANGANATHAN[1] isolated a top yeast, BY 2, after treatment of a brewery bottom yeast with acenaphthene for 90 days. Studies on the biochemical properties of the mutant revealed[2] that the strain is capable of synthesizing and excreting into the medium a considerable amount of riboflavin under simple cultural conditions. The remarkable stability of this property of the mutant has been varified through continued observation and study[3] during the past 10 years.

Certain yeasts[4] and yeast-like organisms[5] are known to be endowed with this property of producing large amounts of riboflavin. The question was posed whether the artificial alteration of the chromosomal constitution[1] of the parent strain had resulted in the creation of one of these organisms having acknowledged property of riboflavin production. An alternative hypothesis would be that the mutant yeast, BY 2, is a new species of yeast created by an induction of mutation. In order to elucidate this point, the properties of the mutant yeast were analysed with regard to the major characteristics having taxonomical implications.

Observations.—I. Cultural characteristics. Typical giant colony of the mutant in barley malt agar is rough and has a wavy outline with a distinct ring around the periphery. The young colonies are white and dry; they become moist, slimy and brown with age.

In *still* liquid culture, the strain forms distinct pellicles which are white, dull and wavy in character. They appear as islets and subsequently unite to form a distinct surface film; the rest of the medium remains clear. On prolonged incubation, the film gains in thickness and finally settles down forming a flocculant sediment and leaves a distinct ring around the surface. A homogeneous growth is obtained in *agitated* liquid media. Deep yellow colour due to the excretion of riboflavin is evident in appropriate liquid media on prolonged incubation.

II. Morphological characters.—The cells of the mutant show a wide degree of polymorphism. The cell shape varies from bulb-shape through long oval to fairly elongated ones. Polymorphism is less evident in young culture in agitated liquid media where the range of size of the cells is usually (5–9) $\mu \times (2{\cdot}5\text{–}3{\cdot}5)\mu$. The reproduction of the cells is by multilateral budding and the cells have a very distinct tendency to become pseudomycelial. Sporulation is absent.

III. Physiological characteristics

(1) *Sugar fermentation.*—Fermentation tests in Einhorn tubes were carried out with media composed of 1% peptone and 4% of the individual sugars, *viz.*, glucose, galactose, maltose, sucrose and lactose. No gas formation was observed in any of the tubes even on prolonged incubation for 15 days. The mutant yeast is a purely non-fermentative organism.

(2) *Sugar utilization.*—Experiments on the assimilation of sugar in Laurent's medium enriched with the vitamin supplement solution show that the mutant yeast can utilize glucose, galactose, maltose and sucrose. No growth is observed in medium containing lactose as the sole source of carbon.

(3) *Assimilation of nitrogen.*—Lodder's medium with the vitamin supplements was used for these tests. Nitrogen was supplied at a level of 21 mg/ml in the form of peptone, urea, asparagine hydrate, ammonium sulphate and potassium nitrate in the individual media tested. The results of these experiments show that very good growth of the mutant is obtained in medium containing peptone as the sole source of nitrogen. Ammonium sulphate, asparagine hydrate and urea can also serve independently as suitable sources of nitrogen for the growth of the mutant. The mutant is incapable of utilizing potassium nitrate as a source of nitrogen.

(4) *Utilization of ethanol.*—A simple inorganic medium containing $MgSO_4$, $7H_2O$; KH_2PO_4; $(NH_4)_2SO_4$ and enriched with biotin was used for these tests. A positive growth is obtained when ethanol is incorporated at a level of 2% by volume in this medium. The mutant is capable of utilizing ethanol as the sole source of carbon in the medium.

(5) *Effect on litmus milk.*—Tubes containing sterile litmus milk with and without calcium lactate were inoculated with the mutant yeast and incubated at 30°C. Results of observation after 3 weeks of incubation show that: *a*) the colour of litmus milk changes to blue, and *b*) in tubes containing added calcium lactate, there is coagulation along with the changing of the colour to blue.

Discussion. An analysis of the properties of the mutant yeast, BY 2, detailed above shows that the strain resembles in a general way *Candida scotii* DIDDENS et LODDER[6] but differs from it in the following characters:

[1] M. K. SUBRAMANIAM and B. RANGANATHAN, Nature *157*, 49 (1946).

[2] K. K. MITRA, J. sci. industr. Res. *8B*, 236 (1949); *11B*, 109 (1952).

[3] K. V. GIRI and P. R. KRISHNASWAMI, J. Bact. *67*, 309 (1954). – K. K. MITRA, J. sci. industr. Res. *14C*, 21 (1955); *15C*, 257 (1956). – K. V. GIRI and P. R. KRISHNASWAMI, J. sci. industr. Res. *13A*, 106 (1954).

[4] P. R. BURKHOLDER, Arch. Biochem. *3*, 121 (1943).

[5] F. W. TANNER jr. and J. M. VAN LANEN, J. Bact. *54*, 38 (1947). – A. GUILLIERMOND *et al.*, C. r. Acad. Sci. *201*, 1077 (1935).

[6] J. LODDER and N. J. W. KREGER-VAN RIJ, *The yeasts – a taxonomic study* (Amsterdam 1952).