

Table V. Dosage/germination, and dosage/seedling height correlations and regression

Cultivar	Dosage/germination		Dosage/seedling height	
	Correlation coefficient	Regression equation	Correlation coefficient	Regression equation
C-612	-0.406	$\hat{y} = 84.275 - 0.12 X$	-0.742*	$\hat{y} = 8.092 - 0.067 X$
Sanyasi	-0.944 <sup>b</sup>	$\hat{y} = 80.339 - 1.326 X$	-0.884 <sup>b</sup>	$\hat{y} = 8.010 - 0.190 X$
Chhola	-0.922 <sup>b</sup>	$\hat{y} = 74.789 - 0.686 X$	-0.994 <sup>b</sup>	$\hat{y} = 7.788 - 0.204 X$

\*Significant at  $p \geq 0.05$ . <sup>b</sup>Significant at  $p \geq 0.01$ .

equal to control). The lower lysine content and fluctuation in other amino acids may also improve, as it is known<sup>12</sup> that mutants having high amino acid contents can be obtained after mutagenic treatments.

In the radiosensitivity studies, the seedlings emerged earlier in 2.5 and 5.0 kR in all cultivars, but the final germination count did not depict a significant stimulation for these treatments (Table II), where a significant ( $p \geq 0.05$ ) germination depression occurred from 15.0 kR. The effect of 15.0, 20.0 and 25.0 kR was identical. Varietal germination differences indicated a significantly greater radioresistance for C-612 than Sanyasi or Chhola. The germination of the latter two was identical. Seedling height measurements (Table III) manifested the radiation effect by a significantly delayed seedling growth from 15.0 kR, with the higher dosages offering a seedling height nonsignificance. The seedling height varietal trend was also manifested by estimated seedling performance<sup>8</sup> (Table IV). Such data yielded  $D_{50}$  values of 24.0, 10.0 and 12.1 kR respectively for C-612, Sanyasi and Chhola. Related decreased germination and reduced growth characteristic results with increased radiation exposures have been earlier reviewed, and reported for *Phaseolus vulgaris* L. cv. Blue Lake by MUJEEB and GREIG<sup>8</sup>.

Except for germination of cv. C-612, significant negative correlations with dosage of this characteristic were obtained for Sanyasi and Chhola. Seedling height and dosage were negatively correlated for all cultivars (Table V). These were significant. The significance level of Sanyasi and Chhola was greater than C-612. Non-significant correlation for such traits have been considered by SIDDIQUI and MUJEEB<sup>13</sup> to be a part result of growth stimulation. In the present study, the nonsignificant germination/dosage and 0.05% level seedling height/

dosage correlation of cv. C-612 has been considered a function of greater radioresistance.

These initial findings for developing a mutation breeding programme with cv. C-612 provided a  $D_{50}$  value of 24.0 kR, a protein content of 20.6%, and a varied amino acid composition compared to cultivars Sanyasi and Chhola. This composition could be improved with greater emphasis attached to isolating variants depicting breaks in the negative high yield/protein/lysine correlations.

**Résumé.** Etat nutritif et radiosensibilité de 3 cultures de *Cicer arietinum* L. (C-612, Sanyasi et Chhola). Le pourcentage de protéine était de l'ordre de 19.9, 20.6 et 22.6 pour Sanyasi, C-612 et Chhola respectivement. Chez C-612 le niveau de lysine est bas et celui d'arginine élevé. Le rapport de pourcentage entre le total des acides aminés et les protéines fut le même dans toutes les cultures. Le C-612 était significativement ( $p \geq 0.05$ ) plus résistant aux rayons gamma, comme l'ont montré les relations: dosage/germination et dosage/hauteur de plantule.

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<sup>12</sup> W. F. TONG, Y. E. CHU and H. W. LI, *Improving Plant Protein by Nuclear Techniques* (I.A.E.A., Vienna 1970), p. 71.

<sup>13</sup> S. H. SIDDIQUI and K. A. MUJEEB, *Stimul. Newslet.* 4, 12 (1972).

<sup>14</sup> Acknowledgment. The help provided by Prof. C. W. DEYOE, Kansas State University, Kansas 66506, USA, for the nutritional estimations is gratefully acknowledged.

## Identifying Y Chromosome in Interphase Nuclei of the Human Brain

A technique has been suggested for identifying certain specific species of heterochromatin by means of fluorescent staining with quinacrine mustard and quinacrine dihydrochloride. This technique permits us to identify the Y chromosomes in metaphase plates and in interphase nuclei (CASPERSSON et al.<sup>1</sup>, PEARSON et al.<sup>2</sup>). In consequence it is possible to identify the fluorescent site of the Y chromosome among the nuclear structures, especially with respect to the nucleolus and the nuclear membrane.

There are advantages in using in neural tissues in such studies, for it is known that the neurons do not divide and this produces a relatively homogeneous mass of interphase nuclei with a very conspicuous nucleolus.

In order to obtain a monolayer, prints were produced by applying to slides surfaces of blocks obtained from the

brain cortex, the hypothalamus, the medulla and the pontal tissue of 9 women and 8 men who have died 6–24 h before the material was taken. The material was fixed for 15 min in Carnoy's fluid. The slides were then washed with 96% alcohol and dried. Staining was carried out for 5 min in 0.5% solution of quinacrine dihydrochloride (National Institute of Health, USA), which was kindly provided by Professor M. GREEN, California University, USA, or quinacrine mustard (National Cancer Institute, Bethesda, USA) prepared in a phosphate

<sup>1</sup> T. CASPERSSON, L. ZECH, C. IOHANSSON, I. LINDSTEN and M. HULTEN, *Expl. Cell Res.* 67, 472 (1970).

<sup>2</sup> P. L. PEARSON and M. BOBROW, *Nature, Lond.* 226, 79 (1970).

buffer at pH 5.7. The preparations were rinsed in tap water and mounted in distilled water. The observations were carried out under the ML-2 fluorescent microscope (USSR) using phase contrast, light and fluorescent microscopy.

The observations have shown that fluorescent site of the Y chromosome is associated with the nucleolus. In the neurons, especially in the large ones, during fluorescent microscopy the nucleolus appears differently to the rest of the nucleoplasm and is frequently surrounded by a fluorescent rim of chromatin (Figure 1a) against the back-

ground of which contrasts the brightly fluorescent body of the chromosome. After staining the preparation with methyl green pyronine according to the standard technique, it was examined through passing light and the preliminary suggestions have been confirmed that the body in the nucleus corresponds to the nucleolus (Figure 1b).

In order to identify the nucleoli rapidly in studies of a large number of nuclei under the fluorescent microscope, phase contrast microscopy was used (Figures 2b, 3b and 4b). Staining using methyl green pyronine and phase contrast were compared. Both techniques proved to be equally good for nucleoli identification. In addition, phase contrast microscopy made it possible to discern cases when lipofuscin or lipids due to the type of their fluorescence resemble the Y chromosome.

The fluorescent body of the Y chromosome usually has the figure of a crescent lying close to the concave part of the nucleolus. It seems that the Y chromosome is one of the accumulations in the nuclei of the neurons, the so-called paranucleolar chromatin are identical.

Sexual dimorphism in the preparations was clear-cut. In the brain of female origin, only weak fluorescence of the chromatin structures was observed, including the nuclear chromatin. Counts of nuclei in the neurons of the brain of male origin containing both the fluorescent site of the Y chromosome and the nucleolus show that these 2 structures are often associated.

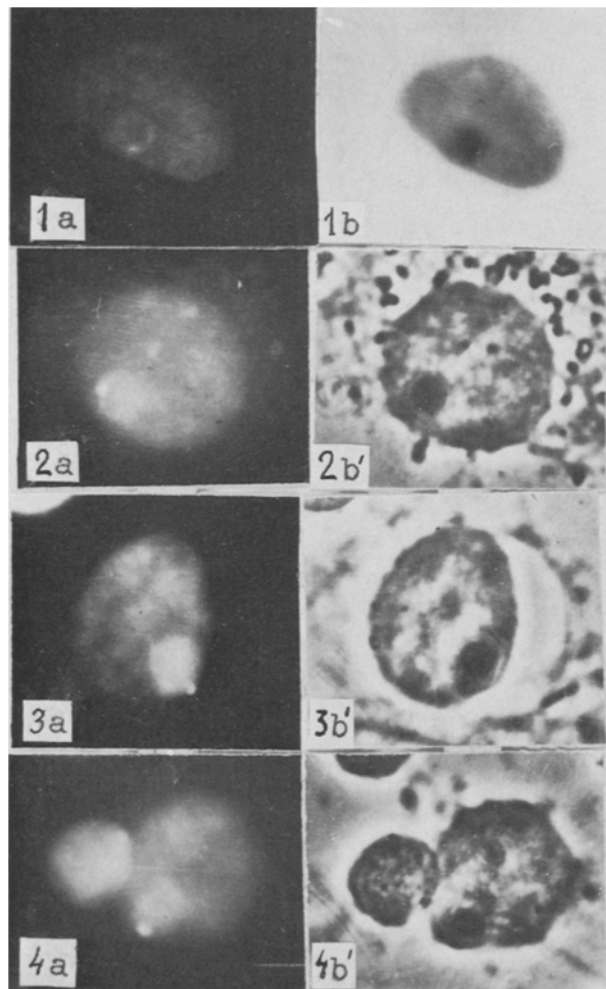
An association between the nucleoli and the Y chromosome in the interphase cells of cultures of human fibroblasts was reported by BOBROW et al.<sup>3</sup>, and the same association in the lymphocyte tissue by GAGNE and LEBERGE<sup>4</sup>.

Thus, the association of the fluorescent site of the Y chromosome and the nucleolus seems to be a general rule for the tissues of man, just as the localization of sex chromatin on the nuclear membrane.

**ВЫВОДЫ.** Люминесцентной микроскопией изучалась локализация У-хромосомы в интерфазных ядрах клеток различных отделов головного мозга человека после окраски акрихином или акрихин-ипритом. Обнаружена преимущественная ассоциация У-хроматина с ядрышком, подтверждаемая цитохимически и фазово-контрастной микроскопией.

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Nuclei of neurons of the human brain. 1. The hypothalamus. 2. The cortex. 3. The medulla. 4. The pont. a) staining by quinacrine dihydrochloride and quinacrine mustard; b) staining by methyl green-pyronine; b') phase contrast.  $\times 1400$ .

<sup>3</sup> M. BOBROW, P. L. PEARSON and H. E. A. C. COLLOCOT, *Nature*, Lond. 232, 556 (1971).

<sup>4</sup> R. GAGNE and C. LEBERGE, *Can. J. Genet. Cytol.* 13, 128 (1971).

## Étude génétique de mutants Mal induits par la nitrosoguanidine chez *Escherichia coli*

Deux loci, *malA* et *malB*, sont impliqués dans le métabolisme du maltose chez *E. coli*<sup>1</sup> alors que les gènes *uvrA*<sup>2,3</sup> et *exrA*<sup>4,5</sup>, localisés dans la région *malB*, confèrent à la souche B, déjà plus sensible aux ultraviolets que la plupart des autres souches de cet organisme, une sensibilité encore plus grande à cet agent.

Après avoir évalué la fréquence de mutation de différents marqueurs génétiques de même que le taux de réversion de mutants Mal induits dans B251 par NG, nous avons déterminé l'appartenance des mutants Mal UV<sup>s</sup> à la région *malA* ou *malB* et avons procédé à une analyse génétique des mutants de type *malB* UV<sup>s</sup>.