

## Seckel Syndrome

Sir,

Although the literature on the 'Seckel syndrome' is very sparse, I was not surprised to see that Sauk *et al* (1973) missed my report (Szalay, 1964) of a patient with the Seckel syndrome. In 1963 and 1964 (Szalay, 1963; 1964), I used the feature of craniofacial disproportion to separate the Seckel and Russell dwarfs from other forms of intrauterine growth retardation (IUGR); I still feel (Szalay, 1972a and b; 1973) that this feature is a useful clinical point in differentiating these dwarfs (Table).

My report (Szalay, 1964) was the second to reveal normal chromosome studies in the Seckel dwarf. However, cytogenetic studies (especially the new banding techniques) are essential in view of the increasing numbers of chromosomal anomalies being found (Table). More recently, acute myeloid leukemia has been reported in a patient with IUGR, microcephalic dwarfism, and a ring 1 chromosome (Bobrow *et al*, 1973).

I last saw my patient in 1963. A recent inquiry revealed that she was employed in a menial position in a protected environment for some time and has remained mentally retarded, microcephalic, and dwarfed. At the present time, she cannot be located but follow-up

attempts are continuing (personal communication, Professor H. Medovy, Winnipeg, Canada).

Yours, etc,

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TABLE\*

Form of intrauterine dwarfism	Craniofacial disproportion	Reported chromosomal anomalies†	Clinical features
Seckel	Yes	Ring 1 chromosome	IUGR; microcephaly
Silver	No	Diploid-triploid mosaicism	IUGR; congenital asymmetry
Russell	Yes	47,XY,+18/46,XY mosaic 45,X/46,XY mosaic	IUGR; pseudohydrocephalus

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† See references in Szalay (1973).

## Cellular Metachromasia with Toluidine Blue O in Cultured White Cells of Cystic Fibrosis Heterozygotes

Sir,

Studies on cultured leucocytes of cystic fibrosis (CF) patients and heterozygotes (Danes and Bearn, 1969; Danes *et al*, 1969) have confirmed the occurrence in

these cells of Toluidine Blue O metachromasia similar to that previously observed in cultured fibroblasts (Danes and Bearn, 1968). In some CF patients, however, the cultured white cells remained ametachromatic and a similar absence of metachromasia was noted in leucocytes from the parents of these individuals. Because of our interest in CF heterozygote detection we examined white cell cultures from 45 parents of CF patients (ie,

compulsory heterozygotes) to confirm if possible the existence of an ametrachromatic group (class III) and to quantitate this class in relation to the numbers of heterozygotes showing vesicular cytoplasmic metachromasia (class I) and generalized metachromasia (class II).

Leucocyte cultures from the 45 CF carriers were set up in parallel with similar cultures from 45 normal healthy controls according to a culture method described by Woodliff (1958). Four cultures from each subject were established in short-necked Leighton tubes. The medium consisted of Eagle's Minimal Essential Medium 75%, human AB serum 20%, bovine embryo extract 5%, penicillin and streptomycin 200 u/ml and 100 u/ml, respectively and preservative-free heparin to a final concentration of 40 u/ml. In each case the coverslip was removed from tube 1 after 7 days, tube 2 after 14 days, tube 3 after 21 days, and tube 4 after 28 days. With their attached cell growth they were briefly washed in warm balanced salt solution, fixed in methanol for 5 minutes and then air dried. After 5-min staining in 0.1% Toluidine Blue O in 30% methanol the preparations were finally cleared in acetone for 1 min, acetone-xylene for 2 min, and xylene for 2 min. Cytological evaluation of all specimens was based on examination of 500 cells at magnification  $\times 1000$ . A culture was recorded as either positive (when the majority of cells showed metachromatic staining) or negative (when the cells did not exhibit metachromasia).

Successful cultures were obtained in all instances. These could be clearly separated into positive cultures which showed metachromasia at 7 days and maintained this appearance in the 14-, 21-, and 28-day cultures and negative cultures which were ametrachromatic at 7 and 14 days but showed some mild metachromasia at 21 and 28 days. The distribution of the different classes of metachromasia in the CF heterozygotes is shown in the table. In six heterozygotes both skin fibroblast and white cell cultures were performed and the classes of metachromasia were identical in both in each instance. Leucocyte cultures were performed in four sets of parents of CF children and all eight heterozygotes exhibited class II metachromasia. Of the 45 control cultures four were positive and 41 were negative according to the criteria outlined above.

These results confirm the value of the Toluidine Blue O metachromasia phenomenon in white cell cultures as a method for heterozygote detection in cystic fibrosis and suggest that it may be justified to replace the tedious, expensive and time-consuming fibroblast technique with the much simpler and more rapid leucocyte culture procedure.

TABLE  
DISTRIBUTION OF  
METACHROMASIA CLASSES  
IN LEUCOCYTE CULTURES  
FROM 45 CYSTIC FIBROSIS  
HETEROZYGOTES

Metachromasia Class	Number
I Vesicular	18
II Diffuse	22
III Ametrachromatic	5
Total	45

The possibility that the strong intrafamilial correlation for the different metachromasia classes may reflect genetic heterogeneity in cystic fibrosis has been pointed out by Danes *et al* (1969). They felt that the evidence strongly suggested that the CF phenotype was due to homozygosity of genes at different loci. The simplicity and apparent reliability of the leucocyte culture procedure as outlined here should facilitate intrafamilial investigation and co-ordination of metachromasia class with other parameters such as sweat electrolytes, ciliary dyskinetic factor and clinical manifestations. Such a study, if it revealed distinct and consistent phenotypic correlations, might furnish valuable additional evidence for genetic heterogeneity in cystic fibrosis.

Yours, etc,

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