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Y-Body in Cell Nuclei of Parenchymatous Organs

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Summary. Smears from 5 parenchymatous organs of 26 corpses were studied with quinacrine hydrochloride fluorescent staining to detect Y-body in cell nuclei. The occurrence of Y-body in all male organs tested exceeded 15%. Detection of Y-body is a reliable method for identification of male sex.

Zusammenfassung. Ausstriche aus 5 Organen von 26 Leichen wurden mit der Quinacrine Hydrochloride Fluorescenz-Färbung zum Nachweis von YB in den Zellkernen untersucht. Die Auswertung der Resultate ergab, daß die Häufigkeit der Y-bodies in allen untersuchten männlichen Organen über 15% lag. Demgemäß kann behauptet werden, daß die Darstellung der Y-bodies eine zuverlässige Methode zur Identifizierung des männlichen Geschlechtes ist.

Key word: Y-body, sex identification.

Caspersson *et al.* [2, 3] have reported the presence of fluorescent bands of different intensity in chromosomes stained with various fluorchromes especially with quinacrine mustard dichydrochloride, known to be bound specifically by DNA. A very bright fluorescence was observed on the distal part of the long arm of the Y chromosome in human cells (Zech, 1969). Pearson *et al.* [8, 9] applied the antimalaria drug atebrin (quinacrine dichydrochloride) as fluorescent stain. Using this method the Y chromosome appears as a brightly fluorescent body (YB) in interphase nuclei of buccal smears. Similar observations were made on the YB by Schwinger *et al.* [11] in cells of freshly pulled hairroot sheats. Barlow and Vosa [1] and Sumner [13] *et al.* detected the Y-body in semen smears. Demonstration of the YB by fluorescent staining was reported by Phillips and Gaten [10] and by Müller *et al.* [6] in leukocytes from fresh and dried blood stains. These authors extended their examinations also on buccal mucosa and hairroot cells (Müller *et al.* [7]).

As far no systematic studies have been reported on the YB in the cell nuclei of parenchymatous organs. The detection of YB in parenchymatous organs may have a practical interest for sex identification in mutilated bodies or in fragments of a body with poor sex characteristics.

Material and Method

The occurrence of YB was examined in autopsy specimens from a total of 16 normal male and 10 normal female corpses. The specimens were obtained from 5 organs: brain, heart, liver, spleen and bone marrow. 3 male corpses each were examined 1, 2, 3, 5 and 8 days after death and 1 after 23 days. Female bodies died 1, 2, 3, 5 and 8 days prior to examination served as controls. Smears were prepared from the cut surface of the organs. A total of 100 cells were counted in each smear, representing a total sum of 13000 cells evaluated in this study.

The air-dried smears were fixed in methanol and stained (8—10 min) with an 0.5% aqueous solution of quinacrine hydrochloride. The slides were then washed under a flow of citrate buffer of pH 6.5 and wet-mounted. The stained smears were examined for fluorescence under a HBO 200 fluorescence microscope (Ergaval, Zeiss, Jena) using exciter filters BG3, BG12, barrier filter OG4. Photographs were taken with ORWO Documenten film.

Results

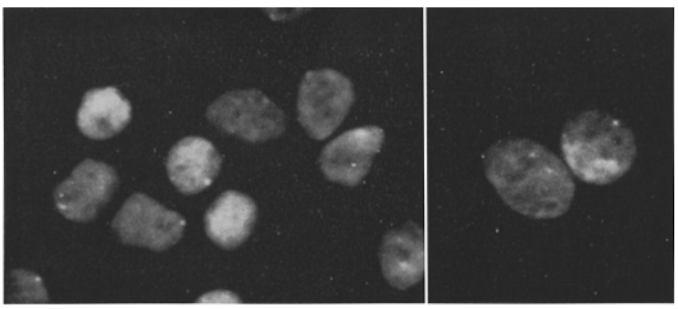

Fig. 1 Fig. 2

Fig. 1. Y-bodies in several nuclei. Smear from spleen. 3 days after death
Fig. 2. Both nuclei with YB. Smear from myocardium. 2 days after death

Smears should be used for the study of YB, since up to now no reproducible results were obtained in frozen or embedded tissue sections (Sellyei and Vass [12]). In male smears the YB appeared in the nuclei as a clearly distinguishable bright spot of 0.3—0.5 micron diameter (Figs. 1 and 2). The incidence of YB was more than 15% in all specimens from all organs studied (Table 1). In some further cases not included in the present report the evaluation was limited and even made impossible by the heavy bacterial contamination. Bacteria stained by quinacrine interfered with clear recognition of YB.

Nuclei from the female controls contained but very rarely fluorescent spots showing resemblance with true YB's. The average incidence of such fluorescent spots was about 1.04% in female specimens. Their incidence in the various organs was as follows: brain 0.5% (0—2), myocardium 1.3% (1—4), liver 1.3% (1—5), spleen 1.1% (0—5), bone marrow 1.0% (0—3). (In parentheses the range.) So, the low incidence of female nuclei with YB-like fluorescence does not interfere with correct sexing of a human specimen.

Discussion

The detection of YB's in the nuclei in smears from all organs tested seems to be a reliable method for sexing male human specimens.

Autolysis of nuclei is expected to limit the detectability of YB [10]. Nevertheless even 23 days after death we found a 23.6% incidence of YB in all specimens of male origin. On the other hand, except the brain all other organs exhibited higher incidence of YB in 2 days old than in fresh corpses (Table 1). This might, however, be explained by the fact that at different times different bodies were examined and the incidence of YB is known to vary in different individuals (Caspersson *et al.* [4]; Manolov *et al.* [5]).

Table 1. *Results*

Days after death	Brain	Myocardium	Liver	Spleen	Bone marrow	Average of YB in 5 organs
1	44.0% (39—49)	33.6% (26—42)	30.6% (18—43)	44.0% (40—48)	28.0% (26—30)	36.04%
2	42.0% (38—46)	38.0% (27—49)	47.6% (43—56)	47.6% (41—53)	30.0% (26—34)	41.04%
3	37.3% (30—45)	21.0% (16—26)	32.0% (17—46)	37.3% (34—41)	20.3% (18—22)	29.58%
5	22.0% (17—30)	20.0% (18—22)	21.6% (19—25)	21.3% (15—27)	21.0% (17—25)	21.18%
8	21.3% (20—23)	18.3% (15—20)	20.6% (20—22)	17.6% (15—22)	19.0% (18—20)	19.36%
23[1]	30.0% (—)	26.0% (—)	25.0% (—)	25.0% (—)	15.0% (—)	23.60%
Average of YB in the organ	32.76%	26.15%	30.73%	32.13%	22.21%	

1 One case.

The detection of YB in smears from various organs tested appeared to be a reliable test for identification of male sex. The effect of the examination is limited by autolysis, heavy bacterial contamination and a weak fluorescence of YB known as a rare anomaly in normal males (Caspersson *et al.* [4]). The sexing of a male human specimen can be based on the presence of YB's. The absence of YB's may not be accepted as a sign of female sex. For that purpose the presence of Barr-bodies should be demonstrated. The latter too, might be misleading in certain chromosome anomalies e.g. Klinefelter syndrome (karyotype 47 XXY), where only the detection of fluorescent YB will give a clue to determine correctly the male phenotype.

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