قسم : التشريح والهستولوجي - كلية الطب البيطرى - جامعة الأسكندرية . رئيس القسم : أ . د . / رود ولف بسيرج .

دراسة يستوفوتومترية لحامض ديزوكس ريبونيكليك على الأذن الأيسر في الفأر الأبيض بعد انسداد تجريبي في عضلات القلسب

# أنسور قاسسم

اجـرى البحـث علـى عشـرة فـئران بيضـا \* ذكور خصـه منهـم قلبهـم سـليم ، والخصـة الأخـرى قلبهـم به انسـداد فـى الشـريان التـأجـى الموصل للأذين الايسـر ، ووجــد أن الفئران الطبيعيـة ؟ ٩ ٪ مــن الانويـه تحتـوى علـى كميـه مضـاعفة حـامضى ديزوكس ريـونيكليك وبعــد ضــى من ٢ ـ ٣ شــهر من الانسـداد وجد أن سبة الانويـه الــــتى تحتـوى علـى كميـة هــذا الحــامغ المضـاعفـة تقـل الـى ؟ ٧ ٪ .

Pept. of Anatomy, Histology & Embryology, Faculty of Vet. Med. Alexandria University, Head of Dept. Prof. Dr. H.R. Berg.

FEULGEN-CYTOPHOTOMETRIC DEOXYRIBONUCLEIS ACID DETERMINATION ON THE LEFT ATRIUM OF THE ALBINO RAT AFTER EXPERIMENTAL MYOCARDIAL INFARCTION (WITH 5 FIGURES)

BY
A.M. KASSEM
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## SUMMARY

Cytophotometric measurements of DNA - content were performed in the left atrium of normal and at 2-3 months after experimental, left myocardial infarction in adult albino rats.

In normal rats 94% of the nuclei contain the diploid amount of deoxyribonucleic acid (  $2\ N$  ) and 6% of tetraploid nuclei (  $4\ N$  ).

After 2-3 months of the experimental infarction, the proportion of the polyploid nuclei were increased up to 74%, while the diploid amount was decreased to reach 26%. The cell nuclei of muscle fibers with tetraploid ( 4 N ) were increased up to 44.5%, octoploid ( 8 N ) DNA - content reach to 24.1% and with 16 ploid (16 N) about 5.4%.

## INTRODUCTION

Cytophotometric determinations in the myocardial cells of mammlian species were performed by VENDRELY and VENDRELY (1956); SANDRITTER and SCOMAZZONI (1964); KOMPMANN <u>ET AL</u>. (1966); MIRAKYAN and RUMYANTSEV (1968); GROVE. NAIR and ZAK (1969); SASAKI <u>ET AL</u>. (1970) and PFITZER (1971, 1972).

SANDRITTER and SOOMAZZONI (1964) and KOMPMANN ET AL. (1966) were able, to demonstrate polyploid nuclei in normal and hypertrophic human hearts.

As, it is known the cell nuclei of different tissues and organs, except in polyphloidy, contain double the amount of DNA, present in the sperm of the same species. The present work was aimed, to observe the behaviour of the polyploidization in the left atrium of the albino rat, after 2-3 months of massive left myocardial infarction.

## MATERIAL AND METHODS

A total of 10 hearts were examined in this study, 5 hearts of normal (not oparated) male albino rats (120-170 gm body weight) and 5 hearts with a passive left ventricle infarction, obtained by means of the ligation of the left coronary artery (SELYE ET AL., 1960).

After 2-3 months, five intact (control) and five operated rats were used to determine the DNA - content in the left atrium cell nuclei.

The material was fixed in cold Carnoy's fluid, embedded in paraffin and 8 - 12 um sections were prepared. Feulgen nucleal reaction for DNA, using a 12 minute hydrolysis time in / N-Hcl at 60 c and Schiff's reagent, prepared with basic fuchsin was relized.

Each atrial myocyte nucleus was photographed on V T - 31 film, by using a MYF - 6 microscope (monochromatic light of 546 mu, isolated by using an interference filter, 10 mu, and a stabilized tungsten filament - light source).

The nuclear areas were determined by planimetry of the enlargements. The photometer apparatus attached to a computer was applied in the DNA measurement.

DNA - values were obtained in an arbitrary unit (AU), by multiplying the surface area of the nucleus by the average extinction (DNA in arbitrary unit = average extinction x area in u). Spermatozoa nuclei were used as reference standards for establishing the diploid nuclear DNA values (DNA - content of spermatozoa nuclei x 2 = diploid nuclear DNA).

#### A.M. KASSEM

#### RESULTS AND DISCUSSION

Shortly after birth (WALKER and ADRIAN, 1966; RUMYANTSEV and SINGIREVSKAYA, 1968) or several weeks after brirth (SASAKI ET AL. 1968) the mitotic activity of the cardiac muscle appears to become rare or even absent. In addition, many investigations, using thymidine H<sup>3</sup> - radioautography have indicated, that the DNA - synthesis occurs in the cardiac muscle fibers of the rats postnatally (MESSIER and LEBLOND, 1969; RUMYANTSEV, 1963).

Capers (1964) described, that the relative amount of DNA in cardiac muscle nuclei, when studied by Feulgen microspectrophotometry, in the normal and hypertrophied heart, was found to be not significant, concerning the quantitative difference of DNA in these two conditions of the heart. Although, an activation of the DNA synthesis and mitoses was observed, it persisted as long as up to 40th day after an experimental left myocardial infarction (RUMYANTSEV and MIRAKYAN, 1968; KASSEM, 1977).

Contrary, to what revealed in the human heart by SANDRITTER and SCOMAZZONI, (1964); KOMPMANN ET AL. (1966), the cytophotemetrical examinations in the present investigation had shown, that in normal control rats, the left atrial muscle cell population consists mainly of diploid nuclei (about 94%) (Fig. 1), while the polyploid nuclei occurred only in a few percentage (about 6% of tetraploid cell nuclei) (Fig. 2).

SANDRITTER and SCOMAZZONI, (1964) determined the measurements of DNA - content in the human heart. They found, that tetraploid (4 N ) cell nuclei of normal or slightly hypertrophic heart muscles were found in 97%. In addition, KOMPMANN ET AL. (1966), had described a very substantial amount of polyploidy in the normal human heart. They showed more than 80% of its muscle cell nuclei were polyploid. On the other hand, RUMYANTSEV (1966); MIRAKYAN and RUMYANTSEV (1968) obtained the same results as observed in the present work. They had shown, that in control animals, the atrial muscle cell contains mainly diploid nuclei (2 C). Another important observation was made under pathological conditions. In the cases of a hypertrophy, a higher degree of polyploidization was attained (PFITZER and SCHULTE, 1972). They were found in the hypertrophic heart, due to hypertension, less than 1% 2 C nuclei and a predominating group of 86% 8 C and 16 nuclei. Also, RUMYANTSEV (1966); MIRAKYAN and RUMYANTSEV (1968) had shown, that in the 40th day after infarction the DNA - content involves gradual polyploidization (up to 90% of polyploid nuclei).

The weight of the albino rat heart increased after experimental left myocardial infarction, and the DNA activation in the left atrium involves polyploidization. Two to three months after this operation the present study had shown, that the muscle cell population in the left atrium consists mainly of polyploid nuclei (about 74% of polyploid cell nuclei) (Fig. 3, 4). The number of the diploid nuclei (2 N) decreased in comparison to normal (till to 26%), while the tetraploid nuclei (4 N) increased up to 44.5% (Fig. 2). And also, the cell nuclei of muscle fibers with octoploid (8 N) DNA - content reach to 24.1%. Moreover, cell nuclei of 16 ploid (16 N) appeared (about 5.4%).

In the human hypertrophic heart, SANDRITTER and SCOMAZZONI (1964); KOMPMANN ET AL. (1966), had shown, that the DNA - content increased as the heart increased in weight, with some nuclei containing up to 16 times the diploid amount. But, in adult mammlian species, the polyploid nuclei were low in ventricular muscle cells (GROVE, NAIR and ZAK, 1969; PFITZER, 1971; GRIMM, 1971). Their observations were similar to what was described in the present study. They were shown from about 85 to 98% of myocardial cell nuclei was diploid.

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#### LEGENDS

- Fig. 1: Section through the left atrium of control rat showing two myocyte nuclei. Feulgen stain.
- Fig. 2: The distribution of the nuclear DNA content in 500 myocyte nuclei of the left atrium of five control non operated rats. (each by 100 nuclei).
- Fig. 3: Section through the left atrium of operated at 2 3 months after experimental myocardial infarction.

  Note the diameter of the myocyte nuclei. Feulgen stain.
- Fig. 4: Section through the left atrium of oparated rat after 2-3 months of experimental myocardial infection.

  Note the paird nuclei which contain similar DNA content. Feulgen stain.
- Fig. 5: The distribution of the nuclear DNA content in 500 myocyte nuclei of the left atrium of five rats (each by 100 nuclei) at 2-3 months after experimental left myocardial infarction.

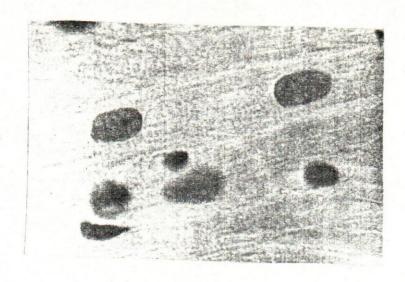


Fig. 1

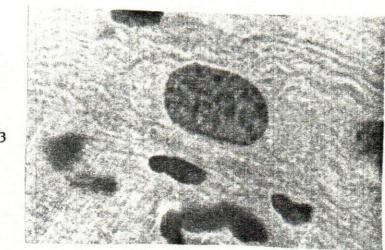


Fig. 3

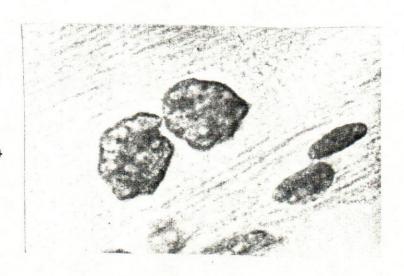


Fig. 4

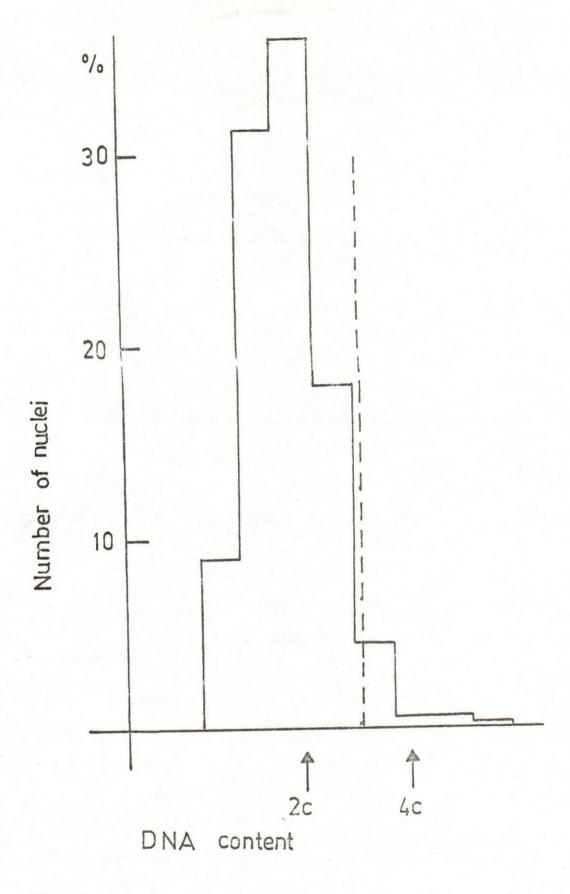


Fig. 2

