THE SYNTHESIS OF DNA AND NUCLEAR PROTEIN BY GONOCYTES IN THE TESTES OF NORMAL AND X-IRRADIATED RATS

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Summary

It had previously been found that treatment of the testes of rats at 4 days of age with X-rays (300 r) suppressed mitotic activity in gonocytes and between 5 and 8 days of age these cells, together with their nuclei and nucleoli, became markedly increased in size.

The present investigations showed that the nuclear hypertrophy in irradiated gonocytes was associated with the synthesis of both DNA and nuclear protein. Although DNA content and nuclear surface area were strongly correlated, it was found that DNA gradually became diluted as nuclei enlarged. The concentration of nuclear protein, however, remained constant, and production of this substance was regarded as being responsible for the nuclear hypertrophy.

Hypertrophy of gonocyte nuclei in the testes of normal rats from 5 to 8 days of age was also found to be due to nuclear synthetic activity. At 5 days of age, enlargement, being associated with the premitotic synthetic phase of gonocytes, could be regarded as a normal process. Excessive hypertrophy of some gonocyte nuclei which took place in the 5–8-day period, however, was regarded as being abnormal. This hypertrophy was due to the accumulation of DNA and protein synthesized by nuclei which had failed to complete the division process.

I. Introduction

In a previous paper (Sapsford 1965) it was shown that after treatment with X-rays at 1 or 4 days of age, the majority of gonocytes in the testis of the rat failed to divide, and by 8 days of age these cells, together with their nuclei and nucleoli, had become markedly increased in size. A number of gonocytes still present in the testes of 8-day normal rats had also exhibited this generalized enlargement.

It was thought possible that the nuclear hypertrophy in both irradiated and normal testes had been due to the accumulation of nuclear substances synthesized by cells unable to complete the final stages of mitosis. This possibility was further investigated by determining the DNA content of and protein concentration in gonocyte nuclei. The present paper gives the results of these determinations. A quantitative assessment of the increase in nucleolar volume in irradiated gonocytes is also included.

II. Materials and Methods

(*a*) *Source of Material*

The source and the method of fixation of the testes used in the present study have already been described (Sapsford 1965). The testes had been embedded in paraffin and sections, 20 μ and 5 μ in thickness, were cut from each paraffin block used.

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(*b*) *DNA Estimations*

(i) *Microspectrophotometry.*—The 20 μ sections, stained by the Feulgen procedure of De Tomasi as described by Pearse (1960), were used for the determination of the DNA content of germ and Leydig cell nuclei. The microspectrophotometer and general methods used for these determinations have been described by Cleland (1965). The two-wavelength method was adopted, the wavelengths used being 603·5 and 565 mμ. DNA estimations were carried out only on nuclei wholly contained within the sections. Nuclei within the tubules of the testes were somewhat crowded and the method of correction for "cytoplasmic chromophore" as described by Cleland (1965) could not be adopted. To minimize the effect of these substances, therefore, the photocell diaphragm opening was set to the equivalent of three-quarters of the minor diameter of individual nuclei. Since Cleland (1965) found that nuclei simulated flat plates of even absorption, the total DNA content of a nucleus was calculated by multiplying the L_1D value (extinction corrected for distributional area) by the surface area of the nucleus. The same procedures for the determination of DNA were adopted in the case of the Leydig cell nuclei.

(ii) *Nuclei Examined in the Testes of 5-day-old Normal Rats and of 5-, 6-, and 8-day-old Irradiated Rats.*—The DNA contents of the following were determined: 25 gonocyte nuclei and 20 Leydig nuclei from the testes of each of two normal rats and two irradiated litter mates at 5 days of age; 25 gonocyte nuclei and 20 Leydig nuclei from the testes of each of two irradiated rats at 6 and at 8 days of age. The gonocyte and Leydig nuclei examined from each rat were contained in the same sections. In the case of all irradiated rats, the dose of X-rays (300 r) had been given at 4 days of age.

Two series of DNA estimations were carried out. In the first of these, the sections used, which were from the testes of one 5-day-old normal and one 5-, 6-, and 8-day-old irradiated rat, had been stained simultaneously. Sections from the testes of the remaining rat in each age–treatment group, examined in the second series, had not been stained simultaneously.

(iii) *Nuclei Examined in the Testes of 8-day-old Normal Rats.*—In the testes of 8-day-old normal rats, attention was confined to germ cells with enlarged nuclei. The examination of 24 sections from the testes of two rats yielded a total of only 25 such cells with nuclei suitable for photometry. The DNA contents of 40 Leydig nuclei in the same sections were also determined.

(*c*) *Nuclear Protein Estimations*

(i) *Microspectrophotometry.*—The 5 μ sections, stained for 4 hr in a 1% solution of orange II in 0·01N HCl, were used for the determination of the concentration of protein in germ and Leydig cell nuclei. To minimize variation in staining, treatment of sections with the orange II solution was simultaneous. The wavelengths used for the germ cell nuclei were 533 and 546 mμ. To avoid inclusions of cytoplasmic protein, the photocell diaphragm was set to the equivalent of three-quarters of the minor diameter of individual nuclei. A constant diaphragm opening, which was subsequently found to be equivalent to one-third of the average nuclear diameter, was used in

the case of Leydig cell nuclei. This restricted opening was necessary because of the small size of these nuclei in comparison with the section thickness. Because of the small diaphragm opening and the homogeneity of staining, absorptions at only one wavelength (546 mμ) were determined. Only those germ and Leydig cell nuclei which had cut surfaces at both the upper and lower planes of the sections were used in the protein estimations.

(ii) *Nuclei Examined.*—The L_1D values of the following were determined: 20 gonocyte nuclei in sections from the testes of each of the two irradiated rats at 5 and at 8 days of age, and 10 gonocyte nuclei in sections of the testes from each of the two normal rats at 5 days of age. All nuclei from one individual were contained in one section of the testis from that individual. The extinctions of 20 Leydig nuclei in each section used were also determined. Single sections from the testes of each of the two normal rats at 8 days of age were searched for large germ cell nuclei. 10 such nuclei were found in one section, 7 in the other. The L_1D values of these nuclei and the extinctions of 20 Leydig nuclei in each section were calculated.

(*d*) *Estimations of Nucleolar Volume in Irradiated Gonocytes*

A number of the 20 μ sections used during the DNA estimations were subsequently counterstained with yellowish eosin in order to estimate the volume of the nucleoli in irradiated gonocytes. The nucleoli in 20 nuclei from each of the two irradiated rats at 5 and at 8 days were examined. The number of nucleoli per nucleus were counted and the volume of each nucleolus calculated from the formula $\frac{4}{3}\pi r^3$, r being the mean radius determined from camera lucida drawings.

III. Observations

(*a*) *DNA Content of Nuclei in the Testes of 5-day-old Normal Rats and of 5-, 6-, and 8-day-old Irradiated Rats*

(i) *Corrections to Apparent DNA Contents of Nuclei*

During the period between 5 and 8 days after birth, Leydig cells rarely divide and it could be assumed that their nuclei would have a stable DNA content. The microspectrophotometrical data obtained from these nuclei thus provided a means of assessing variability of staining by the Feulgen procedure.

In the first series of DNA estimations in which all sections were stained simultaneously, the mean DNA content of Leydig nuclei did not vary significantly from individual to individual (extremes of range $4{\cdot}17 \pm 0{\cdot}12$ to $4{\cdot}26 \pm 0{\cdot}11$ DNA units). In the second series, in which sections had not been stained simultaneously, this was not the case, and the means ranged from $3{\cdot}49 \pm 0{\cdot}09$ (5-day-old normal rat) to $4{\cdot}43 \pm 0{\cdot}11$ (8-day-old irradiated rat) DNA units.

In order that all data from both series could be pooled, the total DNA contents of Leydig and gonocyte nuclei from each second-series individual were corrected by a factor which made the mean DNA content of Leydig nuclei of the individual equal to the mean DNA content of Leydig nuclei from all first-series individuals.

(ii) *Ranges for Diploid, Tetraploid, and Octoploid Nuclei*

The distribution of the DNA contents of Leydig nuclei from first-series individuals and that obtained by the addition of corrected data from second-series individuals are given in Figure 1. The combined distribution is normal ($\chi^2 = 7{\cdot}77$ on 9 d.f., $P>0{\cdot}5$) as is also the first-series distribution ($\chi^2 = 10{\cdot}99$ on 9 d.f., $P>0{\cdot}2$). The absence of any skewing confirmed the observation that there is little mitotic activity in Leydig nuclei during the stages of testicular development concerned.

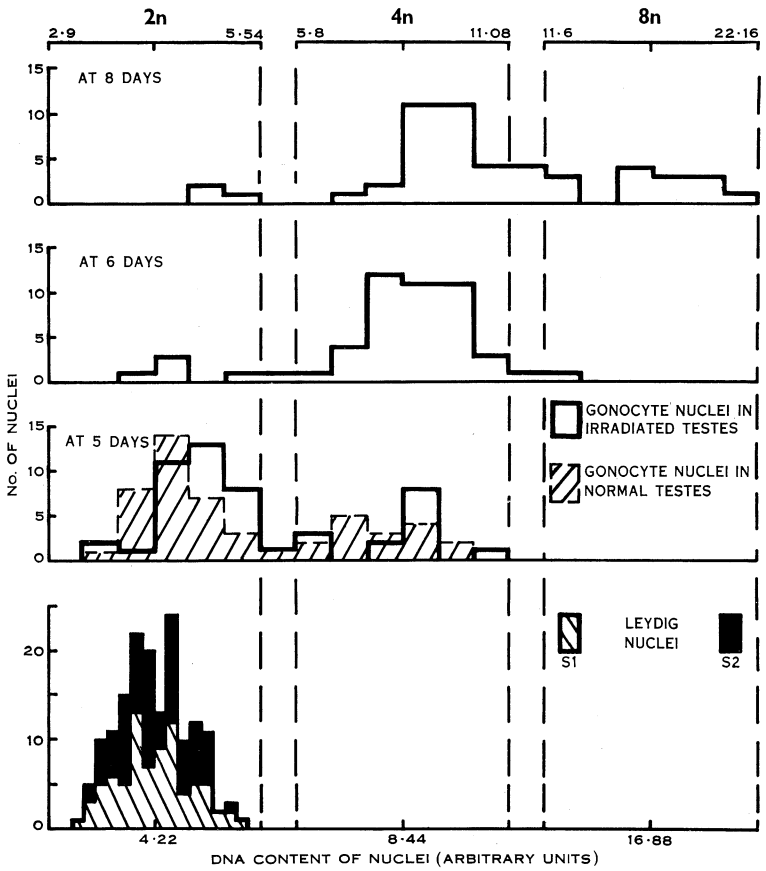

Fig. 1.—Distributions of the DNA contents of Leydig nuclei and of gonocyte nuclei in normal rats at 5 days of age and in irradiated rats at 5, 6, and 8 days of age. S1 and S2, Leydig nuclei examined in first and second series of DNA estimations respectively.

The mean and standard deviation of the DNA contents of the 160 Leydig nuclei of the combined distribution were 4·22 and 0·44 DNA units respectively. These statistics were then used to set the likely limits of the diploid, tetraploid, and octoploid ranges of gonocyte nuclei. The arbitrary limits decided upon for each of these ranges were $1\times$, $2\times$, and $4\times$ $[4{\cdot}22\pm(3\times0{\cdot}44)]$ DNA units respectively. These procedures assumed that both Leydig and gonocyte nuclei had the same DNA

content and that the coefficient of variation of the DNA content of different ploidy classes of nuclei was constant.

(iii) *DNA Content of Gonocyte Nuclei*

The distributions of the DNA content of gonocyte nuclei (corrected in the case of nuclei from second-series individuals) are given in Figure 1. In plotting these histograms, the class interval used for the $2n$, $4n$, and $8n$ ranges was one standard deviation of each respective range.

(1) *DNA Content of Gonocyte Nuclei in Testes of Normal 5-day-old Rats.*—At 5 days of age, the presence of frankly tetraploid nuclei and the shift to the right in the distribution of diploid nuclei indicated that DNA synthesis had been going on, presumably in preparation for the mitotic activity which produced the first spermatogonia during the next few days (Sapsford 1965).

(2) *Effect of Irradiation on the DNA Content of Gonocyte Nuclei.*—The data in Figure 1 indicates that irradiation did not cause any delay in DNA synthesis in gonocytes. At 5 days of age the proportion of tetraploid nuclei in irradiated testes was the same as in normal testes and the shift to the right in the distribution of diploid nuclei was possibly more pronounced. The DNA content of irradiated gonocytes continued to increase over the next few days. At 8 days only a few nuclei were still diploid and approximately 30% were octoploid.

(3) *Relationship between DNA Content and Nuclear Size.*—The correlation coefficient between surface area and DNA content, calculated using uncorrected data from the 75 gonocyte nuclei examined in first-series irradiated rats, was $+0{\cdot}81$. It was permissible to use this data for the calculation of a correlation coefficient because analysis of variance of the means of the surface areas of Leydig nuclei indicated that shrinkage of nuclei during processing had been the same in all sections examined from the three rats concerned.

The correlation coefficient of $+0{\cdot}81$ was highly significant ($P<0{\cdot}001$), demonstrating that the hypertrophy of nuclei had been closely associated with increases in DNA content. Further examination of data, however, indicated that while nuclear enlargement had been taking place, actual concentration of DNA had fallen. In first-series irradiated individuals, the ratios between mean nuclear volume at 5, 6, and 8 days of age were $1 : 1{\cdot}36 : 2{\cdot}68$. The ratios between mean DNA content at these ages were $1 : 1{\cdot}44 : 1{\cdot}74$.

(*b*) *DNA Content of Enlarged Germ Cell Nuclei in the Testes of 8-day-old Normal Rats*

Of the 25 enlarged germ cell nuclei examined for DNA content in the testes of 8-day-old normal rats, six had surface areas ($64{\cdot}3$–$79{\cdot}5\ \mu^2$) less than that of the smallest gonocyte nucleus encountered in the testes of irradiated rats of the same age. It was unlikely therefore that these six nuclei were of gonocytes which had failed to divide. Reference to the distributions of the surface areas of germ cell nuclei in normal testes of 8-day-old rats (Sapsford 1965) indicated that these nuclei were spermatogonial in type. On the basis of the DNA content of Leydig nuclei examined in the same sections, these spermatogonial nuclei were found to be tetraploid,

i.e. their DNA content had doubled in preparation for division. It has already been pointed out that dividing spermatogonia could be found in the testes of normal 8-day-old rats (Sapsford 1965).

The remaining 19 nuclei were placed in the following ploidy classes: diploid—one nucleus with a surface area of $88 \cdot 7\,\mu^2$; near-tetraploid—one nucleus with a surface area of $99 \cdot 8\,\mu^2$; tetraploid—12 nuclei with a mean surface area of $112 \cdot 5\,\mu^2$; near-octoploid—two nuclei with surface areas of $137 \cdot 9$ and $155 \cdot 5\,\mu^2$; octoploid—three nuclei with a mean surface area of $155 \cdot 7\,\mu^2$.

The presence of diploid nuclei indicated that DNA synthesis in some gonocytes is normally considerably delayed. It is unlikely, therefore, that the diploid gonocyte nuclei encountered in the testes of irradiated rats of this age had failed to enter into DNA synthesis because of irradiation. The occurrence of the very large nuclei with DNA content beyond the tetraploid range showed that hypertrophy of the nuclei of some gonocytes in normal testes was associated with a change in ploidy. These gonocytes must have failed to divide in the normal way. The identity of the 12 tetraploid nuclei was a matter of conjecture. Some, like the tetraploid nuclei in irradiated testes, would undoubtedly have been those of gonocytes which had failed to divide. A relatively small proportion could have recently become tetraploid. It is not impossible that some of the smaller nuclei (five had surface areas of $90 \cdot 5\,\mu^2$ and less) were those of tetraploid spermatogonia.

The small number and uncertain identity of the nuclei examined prevented any assessment of possible changes in concentration of DNA which might have accompanied gonocyte nuclear hypertrophy in normal testes.

(*c*) *Protein Concentration in Gonocyte Nuclei*

Data obtained during the determinations of the protein concentration in gonocyte and Leydig nuclei is given in Table 1. The means given were obtained by pooling values from the two individuals constituting each age–treatment group.

Examination of data from Leydig nuclei provided the only possible means whereby variation due to treatment of sections could be assessed. No significant differences were found between the four Leydig surface area means given in Table 1. Likewise the four Leydig extinction means constituted a homogeneous group. As there was little reason to suppose that the concentration of protein in Leydig nuclei had varied in the 5–8-day period, the simplest explanation of these results was that mean section thickness, together with shrinkage and protein staining of Leydig nuclei, had not varied between the different age–treatment classes. It was possible but rather improbable that compensatory fluctuations in the above variables, including protein concentration, would have produced the results obtained.

Thus, as far as it was possible to determine, shrinkage and protein staining of gonocyte nuclei would have been the same for all age–treatment classes. Analysis of variance showed that the four means, given in Table 1, of the L_1D values of gonocyte nuclei did not differ significantly from each other. Since the light paths, i.e. mean section thickness, had not varied, it followed that the concentration of protein in gonocyte nuclei in normal and irradiated testes at all ages had remained constant.

This meant that the hypertrophy of gonocyte nuclei had been accompanied by the synthesis of nuclear protein and that nuclear volume and protein content had increased at the same rate.

During the DNA estimations, it could not be decided with certainty whether some germ cell nuclei in the testes of normal 8-day-old rats were spermatogonial or gonocyte in type. This difficulty was not encountered during the protein estimations because the smallest nucleus examined in normal testes at this age had a surface area of 103·1 μ^2. All nuclei were accordingly classified as being those of gonocytes.

TABLE 1

PROTEIN CONCENTRATION IN LEYDIG AND GONOCYTE NUCLEI

Surface areas and extinctions of Leydig nuclei; surface areas and L_1D values of gonocyte nuclei

Age (days)	Treatment	Leydig Nuclei			Gonocyte Nuclei		
		No. of Nuclei	Mean Surface Area (μ^2) (± S.E.)	Mean Extinction (± S.E.)	No. of Nuclei	Mean Surface Area (μ^2) (± S.E.)	Mean L_1D Value (± S.E.)
5	Normal	40	32·43 ± 0·65	0·6077 ± 0·0151	20	94·88 ± 2·81	0·8104 ± 0·0250
5	Irradiated	40	31·00 ± 0·89	0·6293 ± 0·0156	40	81·92 ± 2·28	0·8212 ± 0·0194
8	Normal	40	30·55 ± 0·62	0·6083 ± 0·0139	17	164·63 ± 12·25	0·8155 ± 0·0358
8	Irradiated	40	30·43 ± 0·58	0·6509 ± 0·0168	40	158·89 ± 5·12	0·8262 ± 0·0252

(*d*) *Nucleolar Volume in Irradiated Gonocytes*

In view of the evidence which indicates that increases in nucleolar volume in normal cells are correlated with increases in cytoplasmic protein (see Mirsky and Osawa 1961), it was considered to be of interest to obtain a quantitative estimate of the increases in nucleolar volume which accompanied the hypertrophy of gonocyte nuclei (Sapsford 1965). Attention was confined to the nucleoli of gonocytes in irradiated testes.

The number of nucleoli per nucleus in these cells at 5 and at 8 days was found to vary from 1 to 4, with means of 2·42 and 2·38 at each respective age. The mean total nucleolar volume per nucleus was 7·93 ± 0·49 μ^3 at 5 days and 31·26 ± 1·62 μ^3 at 8 days.

IV. Discussion

(*a*) *Synthesis of DNA following Irradiation*

Although the observations described in this paper were carried out to investigate the reasons for the enlargement of gonocyte nuclei in normal and irradiated testes, some of the results obtained were of more general interest.

Conflicting opinions concerning the relationship between two effects of irradiation, namely the inhibition of DNA synthesis and arrest of mitoses, have recently

been reviewed by Abrams (1961). Present evidence suggests that in most instances irradiation has a primary effect of inhibiting, at least temporarily, the synthesis of DNA. This inhibition is not due to arrest of mitoses. Because the two phenomena generally take place concurrently, it has been postulated that the antimitotic effect of X-rays is due to the inhibition of DNA synthesis. Goutier (1961) has pointed out that this proposition is not universally accepted because in a number of experiments it has been possible to block mitoses without impeding DNA synthesis.

The experiments quoted by Goutier (1961) were, with the exception of those of Holmes (1956), carried out on tumour cells, on cells *in vitro*, or on microorganisms, and, apart from the observations of Kelly *et al.* (1957), involved the use of labelled DNA precursors. Quastler and Sherman (1958), and Cattaneo, Quastler, and Sherman (1959), using labelled precursors, found that irradiated mouse small intestine and hair follicle cells, although ultimately synthesizing DNA, generally failed to divide. Goutier (1961) has pointed out that implicit in conclusions drawn from experiments involving the use of labelled precursors was the assumption that incorporation was not associated with metabolic turnover of these substances.

The reaction of the gonocytes to irradiation described in the present paper provides one further demonstration that the *in vivo* blocking of mitoses is not necessarily due to the suppression of DNA synthesis. The results are free from any objections arising out of the use of labelled precursors. There is, of course, no guarantee, as Kelly (1957) pointed out, that the DNA synthesized after irradiation is normal.

(*b*) *Hypertrophy of Nuclei in Irradiated Testes*

The results presented in this paper, together with the fact that DNA and nuclear protein constitute all but 1–2% of the dry weight of a variety of nuclei (Mirsky and Osawa 1961), permit the conclusion that synthesis of these substances must have played a major part in the hypertrophy of irradiated gonocyte nuclei. The synthesis of nuclear protein must have been of greater importance in this regard than the synthesis of DNA, for production of the former substance must have been in excess of that of the latter. This may be deduced from the finding that the concentration of nuclear protein remained constant while that of DNA diminished. The fact that the concentration of nuclear protein had been maintained at a constant level eliminates the possibility that the hypertrophy of nuclei had been due to hydration.

The finding that the synthesis of nuclear protein was mainly responsible for the nuclear hypertrophy is in accord with the conclusion of Schrader and Leuchtenberger (1950) and of Alfert, Bern, and Kahn (1955) that this substance is the chief determinant of nuclear volume. Schrader and Leuchtenberger expressed the opinion that DNA could only be of minor importance in this regard because it might be expected that as a general rule, the protein/DNA ratio of nuclei would be high. Mirsky and Osawa (1961) estimate that this ratio varies from 2·7 : 1 to 7·0 : 1 in nuclei of different types of cell.

It was initially thought that the changes taking place in irradiated gonocyte nuclei might have been similar to those occurring when nuclei in normal tissues undergo a change in ploidy. In the latter change, however, increases in mean nuclear

volume are accompanied by proportionate increases in both mean nuclear protein and mean DNA content (Alfert, Bern, and Kahn 1955). In irradiated gonocytes, nuclear protein alone increased at the same rate as nuclear volume.

The products of synthetic activity in cells which failed to divide and which increased in size after treatment with X-rays have been examined by a number of previous workers. However, these workers [e.g. Klein and Forssberg (1954) and Kelly *et al.* (1957)] studied products which, with the exception of DNA, were cellular rather than specifically nuclear in location. It could not therefore be decided whether production of nuclear protein and DNA in disproportionate amounts was a phenomenon peculiar to irradiated gonocytes.

In a previous discussion of the distributions of the surface areas of irradiated gonocyte nuclei (Sapsford 1965), it was suggested that the departures from normality of these distributions at 5 and at 8 days of age could have been due to variation in the degree of hypertrophy of nuclei associated with variation in the degree of nuclear synthetic activity. This suggestion receives support from the present observations, which showed that irradiated gonocyte nuclei were split into at least two distinct ploidy classes at 5 and at 8 days of age (Fig. 1). In addition it was found that nuclear surface area was strongly correlated with DNA content. It might therefore be expected that populations of nuclei at these stages would exhibit signs of inhomogeneity when the distributions of their surface areas were examined.

(*c*) *Hypertrophy of Gonocyte Nucleoli in Irradiated Testes*

It was evident from histological examination that one of the effects of irradiation on gonocytes was to increase cytoplasmic volume. Since the accompanying nuclear hypertrophy was due to the synthesis of DNA and nuclear protein, it is not unreasonable to postulate that the increases in amounts of cytoplasm were associated with the production of cytoplasmic protein. The confirmation, by quantitative means, of the fact that the nucleoli in these cells increased in volume in the 5–8-day period further supports this possibility.

Schrader and Leuchtenberger (1950) found that the fourfold difference in volume of spermatocyte nuclei in different lobes of the testis of the pentamid insect *Arvelius albopunctatus* was paralleled by a comparable difference in nucleolar volume and in cytoplasmic volume. The differences in cytoplasmic, nuclear, and nucleolar volumes were paralleled by comparable differences in cytoplasmic, nuclear, and nucleolar protein. Further evidence which strongly indicates that increases in nucleolar volume are associated with increases in cytoplasmic protein has been reviewed by Mirsky and Osawa (1961).

(*d*) *Hypertrophy of Gonocyte Nuclei in Normal Testes*

A detailed examination of the reasons for the hypertrophy of gonocyte nuclei is very pertinent to the study of the development of the testis of the rat. This is because many previous authors believed that any such enlargement, either before or after the postnatal onset of mitotic activity of gonocytes, was indicative of a degenerative change in these cells. The recent reiteration of this belief by Clermont and Perey

(1957) was seemingly acceptable to Harding (1961) and Hughes (1962). Sapsford (1962, 1964), however, concluded that enlargement of gonocyte nuclei during foetal and early postnatal life is part of the normal differentiation of gonocytes. The present study permits the making of further comments on the hypertrophy of gonocyte nuclei at various stages of the development of the testis.

Since the premitotic synthetic phase in the majority of gonocytes does not begin until rats are 5 days old (Fig. 1), it can be concluded that the normal nuclear enlargement taking place in gonocytes prior to this age (Sapsford 1962, 1964) is not associated with the synthesis of DNA. In view of the previously cited evidence concerning the dependence of nuclear volume on protein content, it is considered likely that the production of the latter substance is the cause of the nuclear hypertrophy during these early stages of development.

That entrance, by gonocytes, into the premitotic synthetic phase is accompanied by increases in nuclear size greater than those taking place during the preceding phase of normal differentiation, was demonstrated by calculating the correlation coefficient between surface area and DNA content of the 25 nuclei examined in the testes of each of the two normal rats at 5 days of age. At this age both diploid and tetraploid nuclei were encountered in each rat. The coefficients for each individual, being $+0{\cdot}71$ and $+0{\cdot}51$, were significant ($P<0{\cdot}01$).

The possibility that a normal nuclear hypertrophy occurs in gonocytes during the premitotic synthetic phase has not been considered by previous workers. The demonstration that it does occur supports the suggestion that departures from normality observed above the 90% level in the distribution of the surface areas of gonocyte nuclei in the testes of normal rats at 5 days of age (Fig. 1 of Sapsford 1965) were due to the fact that a proportion of nuclei in the population had begun to synthesize DNA and consequently had enlarged.

If gonocytes presumably fail to complete the normal division process, still further increases in nuclear size take place. This is indicated by the very large size of the octoploid nuclei encountered in the testes of normal rats of 8 days of age. On the basis of available quantitative evidence, this is the only form of nuclear enlargement which can be considered indicative of gonocyte abnormality.

The behaviour of these abnormal gonocytes in normal testes resembled in many respects that of the majority of gonocytes in irradiated testes. Excessive nuclear enlargement in both normal and irradiated gonocytes had occurred by 8 days of age, was associated with the synthesis of DNA and nuclear protein, and was accompanied by nucleolar enlargement. Hypertrophied gonocytes in normal testes and gonocytes in irradiated testes exhibited similar degenerative changes and had all virtually disappeared by 12 days of age (Sapsford 1962, 1964, 1965). It is unfortunate that it was not possible to decide whether DNA became diluted in the hypertrophied nuclei in normal testes as it had in gonocyte nuclei in irradiated testes. Information on this point would have provided an indication of whether production of disproportionate amounts of nuclear protein and DNA by irradiated gonocytes was due to the effects of X-rays or was a peculiarity of any gonocyte which failed to divide.

(e) *Lysis of Gonocytes*

Harding (1961) and Beaumont and Mandl (1963) divided gonocytes into normal types and those undergoing lysis. Harding concluded that at and after 4 days of age, the population of gonocytes undergoing lysis was about twice that of normal gonocytes. The number of these cells undergoing lysis was found to be higher in irradiated than in normal animals. It would seem that this author has incorrectly identified gonocytes with enlarged nuclei as cells undergoing lysis. Beaumont and Mandl (1963), examining normal rats from the same colony, concluded that the number of gonocytes undergoing lysis in rats aged 4–6·5 days averaged only 12% of the population.

The signs, stated by Beaumont and Mandl (1963) to be indicative of lysis of gonocytes examined by the light microscope, were occasionally observed by the present author, but were interpreted as being artefacts produced by the treatment of tissues. The only changes in foetal and postnatal gonocytes regarded as being degenerative were characterized by irregular clumping of chromatin, together with intense acidophilia of cytoplasm and nuclear sap (Sapsford 1962, 1963). These changes were not described by Beaumont and Mandl (1963).

These authors stated that although qualitatively the nuclei of gonocytes undergoing lysis appeared to enlarge, measurements of normal (467–646 μ^3) and degenerating (465–678 μ^3) nuclei, present between 4 and 6·5 days of age, did not confirm this impression. They added, however, that accurate measurements of degenerating nuclei were difficult to obtain because the onset of lysis was not easy to recognize and because when nuclear membranes had broken down, measurements were apt to be unreliable.

It would appear therefore that nuclei of gonocytes thought to be undergoing lysis did not become very much larger than those of normal nuclei before disintegration hampered an accurate assessment of their size. This is in marked contrast with the enlargement of nuclei due to the synthesis of nuclear constituents. At 8 days of age the outlines of hypertrophied nuclei in normal and irradiated testes were clearly discernible. The mean volume of these nuclei was considerably greater than that of the nuclei measured by Beaumont and Mandl (1963). For example, in the first series of DNA estimations, in which degree of shrinkage of nuclei from all irradiated individuals was comparable, mean nuclear volume was found to have increased from 565 μ^3 at 5 days of age to 1513 μ^3 at 8 days.

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