Some Observations upon the Mitotic and Meiotic Divisions in the Wistar Rat. II. Changes in temperature over localised areas

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Introduction

Moore and Chase (1923), Moore (1924) and Crew (1927) studied the effects of heat upon the testes and all agreed that external application of higher than normal body temperatures was followed by degeneration of the testes.

Seiler (1920), quoted by Schrader (1928), reported the experimental and cytological investigations on heat effect upon maturing eggs of *Talaeporia tubulosa*. He found that the X-chromosome showed a tendency to go out into the polar body, while under high temperatures and in stale eggs, the X manifested a tendency to remain in the egg.

Gronsky (1930) worked on the effect of low temperatures on the testes of white rats, six to eight months old. After treatment for three weeks general atrophy of the tubules was observed, only sertoli cells and spermatogonia remaining.

Some effects of temperature on the cytological interpretation have been outlined by the author (1935) but that study was carried out when the complete animals were subjected to the changes. In this work, results were studied from changed temperatures over localised areas in male Wistar rats, the testes being sectioned and examined, any interpretation of figures being based on the partial chiasmatype hypothesis (Janssens 1909 1924 : Darlington 1932).

Observation

(1) Lowered temperature

The temperature was reduced over the scrotum from body temperature to 5°C (room temperature 16°C) by a gradual process using ether and ethyl chloride, the animals being kept under a light general anaesthesia. The process was conducted twice an hour and the test was continued over 8 hours after which time the testes were immediately removed and fixed.

Mitosis was very evident in prophase, metaphase and telophase stages. Practically all these stages were observed in the outer cells of each tubule but as previously described, the metaphase stage is the one

most commonly observed and it appears that the chromosomes are held longer in this position. No cells were observed with the chromosomes in the anaphase stage—either the elements were observed on the metaphase plate or in the compacted nuclear mass typical of telophase. Prophase stages were abundant but the individual nature of the chromosomes could not be made out.

In meiosis the sections revealed a condition somewhat similar to that described previously where the whole animal was subjected to a reduced temperature (author 1935) but the degrees observed here were not as advanced. Looping of the chromosomes with chiasma formation was observed, the stage which appears in the rat characteristic of diplotene. Diakinesis stages were very evident and though some bivalents appeared to assume a form characteristic of metaphase bivalents it was only in cells exhibiting late stages of diakinesis. The metaphase stages were of two types—some were found in which the bivalents were, with one or two exceptions arranged quite regularly, whereas other cells contained irregularly arranged bivalents similar to the case described previously in which the entire animal was subjected to a reduced temperature.

The bivalents observed were of the usual types, some exhibiting terminal, others subterminal, interstitial and interstitial and terminal chiasmata. Fig. 1. The types found in controls with two chiasmata, both terminal, are more prevalent here than in some other treatments and they are often found in the early stages of precocious separation. Terminalisation in some bivalents was apparent, those forms showing both interstitial and terminal chiasmata—the “hat shaped” forms of Pincus (1927)—have the distal ends usually in close association. In diakinesis too, terminal chiasmata are frequent and it would appear that movement of chiasmata from diplotene positions has been more regular here than in those animals subjected to a reduced temperature over the entire body.

Anaphase stages were seen in few instances, but telophase was common in some sections. The divisions of the second spermatocyte were evident but comparatively rare while spermatozoa were not observed in any great quantity. There was slight evidence of giant cell formation in some tubules but the testes generally showed very little signs of any degeneration.

(2) Raised temperature

The rats in this experiment were treated by applying heat to the scrotum by pads of lint wrung in hot water, the temperature being raised to 48°C each half hour over a period of eight hours. At the conclusion of the experiment the testes were removed and placed in fixative. The sections produced all stages of division, similar in many ways to the

stages observed in those animals placed bodily in a temperature of 48°C. In mitosis the metaphase plates were very distinct with chromosomes spread right across the cell; they were not arranged on a circumference with the points of location of attachment constrictions towards the centre as described in the study by the author on the chromosomes of the Wistar rat (1932). In this study the elements were arranged more irregularly and appeared more compacted than in the control forms.

In meiosis the usual stages were seen. The diakinesis bivalents were compact and thick and did not show the same degree of "metaphase formation" as observed in those animals subjected to bodily increase, but some cells showed bivalents usually associated with metaphase stages. Fig. 2. There was little evidence of degeneration of tissue in the tubules and the interstitial tissue epithelium etc., were also normal. Some metaphase plates in tubules from the centre of the testis showed almost normal arrangement, whereas others towards the periphery exhibited a distorted arrangement somewhat similar to that previously described. But all plates exhibited some degree of change from the normal forms seen in control sections. In the extreme forms examined, bivalents were found scattered from the usual equatorial position, some being found well removed from the remainder. There was, however, a more regular arrangement here and bivalents appeared more generally to adopt positions somewhat similar to those observed in the control forms. The early separation of the component parts of bivalents has not been observed here to the same extent as in other treatments. The type usually found to be dividing precociously viz. those small bivalents with two chiasmata, both terminal, are found here but not in such a state of division as found in control forms—compare Figs. 3 and 6. Irregularities in the behaviour of the sex chromosomes were not observed. The anaphase separation was not seen in the sections studied but the more or less condensed chromatin mass, characteristic of telophase was evident while stages of second spermatocyte division were observed in few cases. Spermatozoa though present were not found in very great numbers.

(3) Alternating temperatures

Alternating temperature on the scrotum was carried out by alternating the treatments described in (1) and (2)., the whole experiment lasting eight hours, the testes then being removed and fixed. The mitotic figures appeared much the same as in the other treatments. The chromosomes at metaphase were arranged more like those observed in control forms previously described. The telophase stages were abundant.

The testes showed no obvious signs of degeneration. Meiotic stages were abundant. Fig. 4. In metaphase some very regular plates were observed—the scattered arrangement which was characteristic in the sections from the high and low temperature treatments were not found

Figs. 1-6 Metaphase plate from a rat subjected to a low temperature over a localised area. 2. Diakinesis from a cell of locally heated rat. 3. Two metaphase plates from locally heated animals. 4. Bivalents from a cell exhibiting diakinesis from an alternately heated and cooled animal. 5. Two metaphase plates from animals alternately heated and cooled. 6. Metaphase from control animal.

in these sections. The early and often irregular separation of the sex-chromosomes was observed.

The arrangement of the bivalents on the metaphase plate was often somewhat different from that seen in control forms, Fig. 5 but in other respects this treatment agreed more closely with the figures seen in the sections prepared from untreated animals. The final stages of the first spermatocyte division appeared more numerous than in the other treatments while stages of second spermatocyte division and presence of spermatozoa were also observed.

(4) Control forms

The animals used as controls were litter mates of those used in the experimental sections described. They were subjected to similar general treatment regarding feeding, handling etc. The sections from these forms produced an abundance of dividing cells both mitotic and meiotic stages being plentiful. In mitosis the chromosomes appeared similar in every way to the stages in the male rat previously described. Whereas

in the various treatments recorded in this study no true anaphase stages were observed, in the control sections such stages have been observed, in some cases exhibiting lagging chromosomes observed and described previously.

The meiotic divisions presented appearances similar to those previously observed and described (author 1932).

Table 1. The frequencies of chiasma formation and the coefficients of terminalisation in the various treatments.

Treatment	Diplotene		Metaphase	
	Chiasma freq.	Term. coeff.	Chiasma freq.	Term. coeff.
Lowered Temp.	1.894	0.204	1.48	0.60
Raised Temp.	1.808	0.338	1.44	0.38
Alternate Temp.	1.882	0.254	1.48	0.28
Control	2.022	0.295	1.54	0.79

Discussion

The tests described have been made to observe the general effects on the testes and to determine whether any changes have been occasioned in the chiasma frequencies. To this end the chiasma frequency has been determined in early diplotene in each test along with the coefficient of terminalisation. The frequencies have been calculated by taking cells exhibiting not less than sixteen bivalents and counting the number of chiasmata in each, the cells being taken at random over all the sections prepared. (Table 1).

The frequencies of chiasma formation registered by the temperature treatments do not show a significant difference the one from the other.

Darlington (1932) considers that the chiasmata gradually move down the configuration away from the location of the attachment constriction with consequent terminalisation of chiasmata. If this is so, evidently there is some check in the formation of the terminal associations in those forms exposed to the low temperatures. A suggestion has been offered in a previous paper dealing with frequencies recorded where the whole body of the animal was subjected to the change in temperature (author 1935). It is possible that with the changed conditions, the cell environments become altered and changes take place within the cells to

meet the new conditions. In doing so chiasma formation may be effected as well as time and/or rate and/or amount of terminalisation. The frequency of meiotic stages observed in the sections studied are listed in Table 2 which has been prepared as described in previous papers by

Table 2. The frequency of stages of meiosis recorded from counts in the control sections and in sections from the treatments—in percentages.

Treatments	Pro-phase	Meta-phase	Barren
Lowered Temp.	74.7	4.4	20.9
Raised Temp.	73.3	3.5	23.2
Alternating Temp.	79.1	4.5	16.4
Control	84.5	6.5	9.0

taking complete sections at random and counting the frequency of stages in the tubules.

The results indicate that the treatments have an effect on the progress of the division. Those tubules in which dividing stages are observed exhibit a larger proportion of prophase stages in the temperature treatments than do the actual controls, but when the barren tubules are included the controls exhibit a much more active state. It appears that the changed conditions effect the later more than the earlier stages of the divisions and from observations made it appears that metaphase divisions have in some cases endeavoured to progress to completion without the normal succession of all those bivalents then in stages of diakinesis. In the dividing stages exhibited there are often obvious differences in the configurations of bivalents suggesting that some are able to withstand the new conditions without effect, whereas others appear to be checked in their normal cycle of development. This fact would further suggest individual differences in the nature of the configurations and consequently in the original chromosomes. It is only in the control sections that true anaphase stages have been observed. Such a state could be imagined if divisions had been checked, the chromosomes adjusting themselves to suit the new environment by assuming a state in which they may rest until conditions return to normal, a condition suggested in mitosis as well as in meiosis. In those metaphase plates of meiosis in which the bivalents are not arranged regularly, differences in degree of terminalisation would be sufficient to account for them. If some bivalents under the new conditions were to terminalise more quickly than others, or if some were slower than normal, bivalents would appear, some ready for anaphase separation, others still in the metaphase state. The change in position of bivalents from diakinesis, where the arrangement is irregular, to metaphase, with the regular arrangement of bivalents on the equatorial plate, must be accompanied by the action of forces acting within the cell. If the new conditions under which the animals were placed, were sufficient to cause the forces acting on the bivalents to bring them to the plate position to be checked, the irregular arrangement at metaphase would be accounted for.

In previous studies, a parallel has been drawn between the genetical and cytological results of crossing over. If chiasmata at early diplotene, the first stage at which they are here observed, can be considered to bear some relation to the points of crossing over in genetical studies, then from the results recorded in this work, it would be possible to postulate significant differences in crossing over values when animals are subjected to changes in temperature over localised parts as described in this study.

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