Some Observations upon the Mitotic and Meiotic Divisions in the Wistar Rat

I. The effect of changes in temperature

by

Wm. Bryden

Technical College, Christchurch, New Zealand

Received August 17, 1934

Introduction

Some results from genetical studies on the changes in crossing over values in *Drosophila*, produced by temperature, have been given by Plough (1917), Plough and Strauss (1923), Stern (1926), Young and Plough (1926), Crew (1927) and Dobzhansky (1928). These workers found varying degrees of change taking place in the cross-over value for definite characters within certain ranges of temperature.

With mammals Castle and Wachter (1924) studied the effect of temperature on the crossing over values in rats and mice but in their experiments only seasonal influences were studied and negative results were reported, conclusions regarded by Eloff (1932) as unsatisfactory.

Moore (1922), Fukui (1923), [from Crew] Moore and Chase (1923) and Crew (1927) reported similar results—i.e. that application of a higher than normal body heat to the testes produced degeneration of those parts.

Kemp and Juul (1931 a b) subjected embryonic fowl tissue to heat (40°–50°C) and found that the high temperature caused death while a temperature of 44°–46° produced normal mitoses which remained a long time at metaphase apparently unable to enter anaphase.

Vintemberger (1930) kept hens eggs at 39°C for several hours and then subjected them to a falling temperature to 4°C. It was found that very marked temperature reduction produced nuclei which remained at the stage they were in before reducing the temperature.

Bleier (1930) recorded a change in the orientation of chromosomes producing resting nuclei instead of the dividing forms when the plants of *Triticum vulgare* were exposed to temperatures of from 0°–35°C in reduction division.

Heilborn (1930) placed flower buds of apple trees in various temperatures and found that with rising temperature there was a

larger number of univalents.

The present work was carried out in conjunction with cytological studies on chiasma frequencies and allied investigations concerning rats, and any interpretation of figures presented, is based on the theories of Janssens (1909 1924) and Darlington (1932).

Materials and Methods

The material used was provided in Wistar rats from the stocks maintained at the Institute of Animal Genetics, University of Edinburgh. All the animals were of similar genetic constitution, were closely related, and were of similar age—9 weeks. Three rats were used in each test. Immediately after treatment the testes were removed and fixed in 2BE and 2BD (La Cour 1931). Sections were cut at 12μ and stained with the standard gentian violet stain.

The drawings were made with a Zeiss camera lucida, at a magnification of 3500 diameters, the figures being later reduced. In some metaphase plates the bivalents have been separated for clearness.

Observations

Most of the previous work has been in connection with temperatures higher than normal. In this study both higher and lower than normal temperatures have been employed as well as a temperature alternating first to high and then to low. Each treatment is compared with a control.

(1) The control animals produced stages of mitosis and meiosis similar to those described previously by the author (1932). The mitotic figures in prophase, metaphase and anaphase were observed, the metaphase stage appearing much more prolonged than the other stages.

Figs. 1 and 2 show typical control sections.

(2) The animals subjected to a reduced temperature were placed in an environment in which the temperature gradually dropped from the room temperature (average 17°C) to 3°C , the whole process lasting four hours, the lowest temperature being maintained for 30 minutes. Mitosis was observed and in almost all cases chromosomes were seen at metaphase or telophase, results which appear to parallel those of Vintemberger (1930).

The chromosomes in the early prophase stages of meiosis appeared shorter with very evident looping. Diakinesis bivalents were compact and some exhibited forms more like those usually seen at metaphase. This feature was observed in most sections examined. Fig. 3. shows bivalents from a typical cell exhibiting diakinesis stages. The bivalent on the extreme left shows a subterminal chiasma

whereas two on the right show forms similar to those typical in metaphase configurations.

In metaphase many features were noticed strikingly different from similar stages of the control animals. Small bivalents with only two chiasmata, both terminal, have often been observed to exhibit precocious separation in control sections. In this treated material similar bivalents were found sometimes removed from the remaining bivalents but without having first precociously separated. Fig. 4 shows a decided contrast to the regular arrangement of the

Fig. 1. Control diakinesis bivalents. Fig. 2. A metaphase plate from a control animal. Fig. 3. Diakinesis configurations from a cell of rat subjected to lowered temperature. Fig. 4. Metaphase plates from low temperature treatment.

bivalents shown in the control plate in Fig. 2. The arrangement is odd and many forms have divided precociously while the normal configuration of some "hat shaped" forms appears to have been checked.

Other workers have suggested that the apparent increase in bulk of the metaphase bivalents is possibly due to absorption from the cytoplasm. If this is so it would seem that in this study, with the

lowered temperature, such absorption has been checked and bivalents of apparently smaller volume result. The reduced temperature appears to affect both the rate of division and the completion of the divisions already in progress.

Anaphase stages, so commonly seen in control forms, were observed in but few cases.

(3) The animals subjected to an increased temperature were placed in an incubator, the temperature being maintained at 43°C for 72 hours. Mitotic division was observed and appeared similar to that described in (2). Metaphase stages were observed and it is suggested, from lack of anaphase and telophase, that mitotic divisions may have been somewhat checked by the new environment.

The sections examined for meiotic division did not show chromosomes in prophase stages as definitely as in the previous treatment in which the threads were very clear. In diakinesis some bivalents appeared to have terminalised quickly and presented configurations somewhat similar to those recorded in

Fig. 5. A cell at diakinesis from animal subjected to high temperature treatment.

(2). In Fig. 5 the chromosome pairs aggregated and some forms seem more typical of metaphase, at which stage the bivalents do not seem to occupy the usual equatorial plate position but were observed in most cases scattered throughout the cell. vide Fig. 6.

Fig. 6. Metaphase plates from high temperature.

The condition here and in the previous treatment may be compared with the results recorded by Kostoff and Hadjidimitroff (1931) and the interpretation may be to some extent similar. Along with the cells exhibiting division different from the usual, others were found in which the general form and arrangement of the nuclear parts were nearly normal. These were not confined to any particular part of the tubules but were found scattered at random; these cells show the bivalents closer together in the metaphase plate but not

quite similar to those shown in the control forms. (4) Whereas other workers have recorded the effects produced by either the raising or lowering of the temperature, no results have been found dealing with the effects produced by submitting to an alternating temperature. Rats were placed in an environment of 43°C for an hour and then removed to a decreasing temperature descending to 3°C , the process lasting an hour and then reverting to the higher temperature again, the process being carried on for 10 hours.

Mitotic divisions were similar to those observed in (2) and (3) but there were more cases in which anaphase stages were observed.

The spermatogonial divisions again appeared fewer in number. In meiotic stages the bivalents at diakinesis did not exhibit the forms found in metaphase and reported in (2) and (3). The bivalents in Fig. 7 are similar to those observed in the diakinesis stages of the

Fig. 7. Alternate heat and cold treatment—diakinesis stage.

Fig. 8. Metaphase stages of alternate heating.

controls. cf. Fig. 1. No forms were seen in which the contraction of chromosomes progressed still further with the production of bivalents typical of metaphase. At metaphase the arrangement was again irregular but not to the degree shown in either of the previous treatments. Fig. 8 shows two plates with metaphase bivalents.

Some bivalents were found separated as in anaphase while yet others were still in the state quite typical of metaphase. Some appeared unable to complete the early division carried out by others.

Discussion

The scrotum appears to be a temperature regulating mechanism, the presence of which is necessary for the elaboration of functional spermatozoa. If the temperature is raised above the optimum or decreased greatly below the optimum, the normal function is hindered.

It would appear that the testes are able to elaborate spermatozoa only between a certain optimum range; at either extreme the normal elaboration is disturbed. It is reasonable to assume that the primary spermatocytes will continue to be formed unless all cell division is completely checked and in all three environments here discussed the divisions did not seem to be completely stopped. The early prophase stages were, however, present in a much greater proportion than in the control. Some idea of the relative proportion of the stages present in the treatments may be gained from the counts made; the ten sections have been taken from each treatment, one section of

Table 1. The percentages of the two stages in the division of the first spermatocyte.

Treatment	Prophase	Metaphase
Control	90.8	9.2
Low temperature	92.4	7.6
High temperature	97.8	2.2
Alternating temperature	94.4	5.6

which exhibits metaphase stages, the other nine being taken at random over all the slides prepared. The number of stages at prophase and metaphase have been recorded and percentages calculated. The results are shown in Table 1.

The control shows a greater percentage of metaphase configurations and a correspondingly smaller number of prophase counts. The high temperature appears to exert the greatest effect on the divisions and as shown by the table, the metaphase divisions are comparatively rare.

A further count has been made of the frequency of stages in the tubules. 750 tubules have been counted and note made of those showing stages of prophase or metaphase the final stages of the first spermatocyte division and stages of second spermatocyte division being recorded with the metaphase counts. Note has also been made of these tubules showing no evidence of divisions. The results are given in Table 2.

In common with the previous table, the control shows the greatest number of metaphase stages, the high temperature the lowest. The high temperature also shows a greater frequency of barren tubules and a smaller number of prophase stages bearing a relation to the state found in the seventeen months old rat sections. (Bryden 1933). Whereas the two tables show figures which seem definite, it must be remembered that

Table 2. The percentages of stages of division from 750 tubules in each treatment.

Treatment	Prophase	Metaphase	Barren
Control	80.5	4.9	14.6
Low temperature	73.4	3.8	22.8
High temperature	64.5	1.4	34.1
Alternating temperature	73.0	3.0	24.0

in all probability in certain treatments some divisions have commenced normally and stopped, whereas others may have started during treatment. Some data have been collected from the slides examined of the frequency of chiasma formation in diplotene and metaphase and the results are given in Table 3.

From the differences in values when comparing the treatments with the control it appears that chiasma frequencies, as well as general observations on cell divisions, are affected. The presence of types more typical of metaphase, in the diakinesis stages, and the greater percentage of forms separating precociously points to the suggestion that the metaphase stage is a suitable resting stage and with varying conditions, the cell endeavours to protect its own interests by assuming the stage of rest as quickly as possible. Such a change, acting more or less quickly within the cell would probably create changes from normal in the crossing over of the chromosomes, with consequent changes in the mechanism of chromosome movement, hence the production of irregular figures and irregular chiasma frequencies.

Table 3. The chiasma frequencies in the various treatments.

	Diplo- tene	Term. coeff.	Meta- phase	Term. coeff.
Control	2.0	.25	1.59	.77
Low temper- ature	1.79	.11	1.41	.57
High temper- ature	1.76	.16	1.54	.77
Alternating temperature	1.81	.16	1.53	.6

Conclusions

The results that have been recorded by Kemp and Juul, Bleier, and Heilborn appear to be paralleled in this study. Changed environments have the effect of checking the divisions and from observations made it appears that the chromosomes are urged to take up a position on the metaphase plate or the completed anaphase division to escape any deleterious effects from the enforced new conditions.

Observations on the chiasma frequencies show some divergence from the control figures. The counts and interpretation of figures are merely given (for what they may be worth) to compare the cyto-genetical side with the purely cytological aspect.

From the chiasma frequencies and frequencies of bivalent types it appears that the mechanism of chromosome movement, chiasma formation, terminalisation etc., are all affected by the experimentally produced environments.

No degeneration of testes was noticed in the tests carried out but it must be remembered that the treatments were never continued for more than 10 hours.

Acknowledgments

I am indebted to Professor F. A. E. Crew of the Institute of Animal Genetics, University of Edinburgh, for providing me with the material used in this study and for the hospitality extended to me in his laboratory.

To Dr. F. W. Sansome of the John Innes Horticultural Institution, Merton, London, I extend my hearty thanks for helpful criticism and friendly advice.

I should also like to thank my friend and colleague Dr. P. Koller for his friendly criticism.

References

- Bleier, H. (1930) "Experimentell-zytologische Untersuchungen. I. Einfluß abnormaler Temperatur auf die Reduktionsteilung". *Ztschr. f. Zellforschung* 11.
- Bryden, Wm. (1932) "Cytogenetic studies on the rat. Somatic chromosome complex, meiosis and chiasma frequency". *Jour. Genetics* 26 394.
- (1933) "The relation of age to the frequency of chiasma formation in the Wistar Rat. *Ibid.* 27 415.
- Castle, W. E. and Wachter, W. L. (1924) "Variations in linkage in mice and rats". *Genetics* 9.
- Crew, F. A. E. (1927) "The Scrotum: A temperature-regulating mechanism". *Internat. Kongres. f. Sexualwis.* I.
- Darlington, C. D. (1932) "*Recent advances in cytology*". London: Churchill.
- Dobzhansky, T. (1928) "Effect of temperature on the viability of suppler females in *Drosophila melanogaster*". *Proc. Nat. Acad. Sci.* 14 671.
- Eloff, G. (1932) "A theoretical and experimental study of the changes in the crossing over value, their causes and meaning". *Genetica* 14.
- Janssens, F. A. (1909) "Spermatogénèse dans les Batraciens. V. La théorie de la chiasmotypie, nouvelle interprétation des cineses de maturation". *Cellule* 25 387.
- (1924) "La chiasmotypie dans les insectes". *Ibid.* 34 135.
- Kemp, T. and Juul, J. (1931a) "Der Einfluß der Wärme auf die Zellteilung, untersucht in Gewebekultur". *Arch. exper. Zellforschg.* 11 602.
- (1931b) "Influence de la chaleur sur la division cellulaire, dans un tissu cultivé in vitro". *C.R. Soc. Biol. Paris* 108 139.
- La Cour, L. (1931) "Improvements in everyday technique in plant cytology". *Jour. Royal Mic. Soc.* 51 119.
- Moore, C. R. (1922) "The effects of gonadectomy in the guinea pig on growth, bone lengths and weight of organs of internal secretion". *Biol. Bull.* 43 285.
- Moore, C. R. and Chase, A. D. (1923) "Heat application and testicular degeneration". *Anat. Rec.* 26.
- Plough, H. H. (1917) "The effect of temperature on crossing over in *Drosophila*". *Am. Nat.* 60 192.
- Plough, H. H. and Strauss, M. B. *Journal Gen. Physiol.* 6 167.
- Stern, C. (1927) "Ein genetischer und zytologischer Beweis für Vererbung in Y-Chromosom von *Drosophila melanogaster*". *Zeitschr. ind. Abst. u. Verb.* 44.
- Vintemberger, P. (1930) "Etude expérimentale sur la mitose envisagée comme facteur de radiosensibilité". *Arch. d'Anat.* 12 229.
- Young, W. C. and Plough, H. H. (1926) "On the sterilisation of *Drosophila* by high temperature". *Biol. Bull.* 51 3.