55. *On Certain Effects of Some Fixatives on the Constitution of Chromosomes.*

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In his study on the Mammalian chromosomes, hitherto considered difficult to fix, Minouchi (1928) studied various effects of many fixatives, employing them either according to their original formulas or changing various conditions. He notes the injurious effect of acetic acid, and recommends especially Champy's fluid and Flemming's solution without acetic acid. He further recommends for fixing the chromosomes of grasshoppers and crickets a very dilute solution of Flemming's mixture without or with a very small quantity of acetic acid.

I have experimented according to his suggestion and have come to know two extraordinary effects produced by the dilution of the solution and the omitting of acetic acid. This applies to certain species of Gryllodea. Among the Gryllodean insects, in some species it is very easy to fix the chromosomes, but others are not so easy to treat. For these insects Flemming's mixture diluted with an equal or a double volume of water with a certain ammount of acetic acid proved very good.

According to my former experience Champy's fluid gave no good results. My later experiments with the same and Flemming's mixture without acetic acid proved the same. In materials fixed with them, cytoplasmic inclusions become very prominent and stain very dark, but the chromosomes become very difficult or even impossible to stain, even after treatment with Chura's fluid.

Then I tried diluted Flemming's solution with a very small quantity of acetic acid. In materials fixed in this way, the chromosomes are stained rather pale but are very clear. On the other hand its effects on the constitution of chromosomes are very remarkable. Fig. 1 is a spermatogonial metaphase of *Loxoblemmus frontalis*, treated in the new method.¹⁾ This consists of eight V's and two rods with

1) In this instance, I employed Flemmings strong solution diluted to half of its original concentration, to 3 c. cm of which is added a drop (one twenty-fifth of 1 c. cm) of acetic acid.

an X-chromosome when fixed with ordinary fixatives. As is seen in the figure, all the V's are split at the apex into the component two arms, and moreover what I thought to be a simple rod is, in reality, proved to consist of two components; one of them is very small and is attached to one end of the other. Further there were observed pale staining sutures in the middle of some of the rods, though too slight to be shown in the figure. These effects are entirely new observations hitherto undiscerned.

Fig. 1.

The same effect on the chromosomes of *Brachytrupes portentosus* has been mentioned in my preceding note.¹⁾

One more striking effect of these fixatives is on the characteristics of X-chromosomes. In ordinary materials the X-chromosomes become granular and stain paler as they advance in their spermatogonial stages. But this effect become decidedly conspicuous when it is fixed with these fixatives. In extreme cases the X-chromosomes almost entirely lose their affinity for stain. See Figs. 10, 11 of my preceding paper.

In short, Flemming's mixture when diluted and with the quantity of acetic acid diminished has two remarkable effects on the constitution of chromosomes; which serve to analyse the chromosomes into their component elements and to distinguish the X-chromosomes very clearly. These effects are produced by changing the concentration and by diminishing the quantity of acetic acid.

1) In this case, Flemming's strong solution is diluted to one fifth of its original concentration, to 5 c.cm of which is added a drop of acetic acid.