EVIDENCE AGAINST A STRAIGHT END-TO-END ALIGNMENT OF CHROMOSOMES IN DROSOPHILA SPERMATOZOA [1]

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IN order to understand the response of the hereditary material in the mature spermatozoa of *Drosophila melanogaster* to physical and chemical mutagens, it is necessary to know the form and arrangement of the chromosomes in the sperm head. For, depending on the plan of its organization, one would expect different relative frequencies of point mutations and gross chromosome alterations after a given mutagenic treatment.

The view that the chromosomes are arranged end to end in a straight line along the long axis of the sperm head has been based on cytological observations. GOWEN and GAY (1933), working with Drosophila, measured the sizes both of the sperm head and of the individual chromosomes during oögonial metaphase. The close correspondence between the length of the sperm head (approximately $7\frac{1}{4}\mu$) and the total length of a haploid set of metaphase chromosomes containing either the X or Y chromosome (approximately 7μ), together with the close similarity in chromosome and sperm-chromatin widths, led them to conclude that the chromosomes are arranged end to end (presumably in a straight line). WOLF (1939) reported similar observations.

Recently, COOPER (1952) reported that the chromosomes in the nearly mature sperm head of Drosophila can be seen in certain fixed preparations. In one case, where a complete set of chromosomes could be recognized, they resembled ordinary mitotic metaphase chromosomes in sizes, shapes, and proportions, and were arranged in single file along the long axis of the sperm head. In general, however, according to figures and information kindly furnished us by DR. COOPER after we had sent him a copy of the present paper as originally submitted, the chromatin of the nearly mature spermatozoa had the appearance not of a straight line but rather of a coarse helix with more or less side-by-side arrangement of its several coils, and these when squeezed apart were seen to be made up of chromosomes of metaphase appearance—observations which we regard as not inconsistent with our own data and interpretations here presented. RAPOPORT (1941), in a paper (dealing primarily with another subject) which has come to our attention only after the completion of our present work, makes the following statement concerning

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Drosophila spermatozoa: " we examined four kinds of preparations stained after Feulgen of the following objects: normal sperm cells, sperm cells without a sex-chromosome, with an extra sex-chromosome and, finally, with a closed X-chromosome. Neither data in favour of a linear end to end arrangement, nor in favour of the existence of a fixed localisation order of the chromosomes (especially in closed X) in the sperm cells were found." Unfortunately, no data or other details were included in his paper.

The view that sperm-head chromosomes do not have a straight end-to-end arrangement but as a rule overlap each other has seemed to us to be indicated by genetic evidence. After irradiating sperm with fast neutrons, MULLER and VALENCIA (1951) found that the frequency of translocations varied linearly with dose even at doses sufficient to produce several proton tracks per sperm. MULLER (1954a) interpreted this to mean that those broken ends which underwent union with one another were derived from breaks caused by the same proton track, and that breaks therefore had to be very close together before there was a good chance of union between their broken ends. This implied that the non-homologous chromosomes which underwent mutual translocation of large pieces (the usual type of translocation) overlapped each other very considerably in the sperm head at the time of irradiation. Recent experiments on the incidence of translocations and sex-linked recessive lethal mutations after treatment of spermatozoa with two doses of neutrons, reported in papers by MULLER now in press (1954b) and in preparation, confirm the earlier data and interpretation.

In order to obtain additional cytological evidence concerning the arrangement of the chromosomes in the sperm head, determinations were made of the length of the chromatin mass in individual spermatozoa containing different chromosome assemblages. Two stocks of *D. melanogaster* were used, an ordinary wild-type stock, Oregon-R, and a special stock made available by DR. E. NOVITSKI and described by LINDSLEY and NOVITSKI (1950). Whereas one-half of the sperm from Oregon-R males contain an X chromosome and one-half a Y, half the sperm produced in NOVITSKI's stock contain neither the X nor the Y while the other half carry both, attached together, and with a part of the heterochromatic material even further duplicated. The autosomal content, however, is quantitatively identical in all these sperm.

EXPERIMENTAL PROCEDURE

To obtain and prepare sperm heads for measurement, the seminal receptacle was dissected from a fertilized female into a small drop of saline (prepared according to BUCK and MELLAND 1942) which was then quickly flooded with the usual aceto-orcein fixative-stain and covered with a coverslip. A current of fresh stain was kept flowing beneath the coverslip for at least ten minutes, after which the excess stain was removed and the coverslip margins sealed with white Karo syrup. To avoid, as much as possible, distortion due to compression, enough aceto-orcein was left beneath the coverslip and sufficient Karo was applied so that the receptacle was barely held in position be-

tween slide and coverslip. Slides were usually used 2–4 hours after preparation, at which time the sperm heads showed the most contrast with their surroundings; there was little precipitation of stain around the receptacle and none within it, and the Karo had not diffused into this region. Such preparations of sperm are especially adequate for this type of study since the heads are distributed over a considerable length of the receptacle, usually lying parallel to the long axis of the tube, and are often separated from each other, straight, and almost completely in one focal plane. Tails of sperm are unstained so that only the chromatin of their heads is readily visible. Beginning at the blind end of the receptacle and proceeding to its opening into the vagina, measurements were made of all suitable sperm on each slide. Sets of measurements from slides of the two stocks were taken alternately, whenever possible, by means of a camera lucida, using a green Corning glass filter and compensating ocular (15×) and apochromatic objective (90×, N.A.1.40) lenses. The magnification at table-level was about 1850 diameters.

THE RESULTS AND THEIR FORMULATION

Seven slides of Oregon-R sperm and thirteen slides of sperm from NOVITSKI's stock were examined, and 244 and 322 measurements of the length of the chromatin mass were made, respectively. The individual determinations from each of these slides are shown in figure 1, as well as the mean values for each slide. It may be noted that there is considerable variation in these means both in the Oregon-R and the NOVITSKI stock. A statistical analysis has shown that this variation of means is several times as great as would be expected as a result of random sampling if the sperm on the different slides of the same stock had represented the same material. This interslide variability must be due, in large measure, to differences in fixation and in compression of the sperm heads in different receptacles. The mean lengths of chromatin mass for the Oregon-R and NOVITSKI stocks are, at table level magnification, 18.3 and 17.6 mm, respectively. The difference between these means is, however, without significance, because of the wide, determinate variation between the means of the different slides of each stock.

In addition to this variation which exists in the actual length of the fixed chromatin masses on different slides, there are five sources of variation resulting from the measurement process. These depend on the depth of focus of the optical system, the straightness of the sperm head, the degree to which it is horizontal, the marking of the limits of length seen using the camera lucida, and the reading of these limits with a ruler. The first three of these sources of error are considered to have been largely randomized, and probably do not often have an error exceeding 1 mm per chromatin mass at table-level magnification. There may not have been so random an error in the marking and reading of the lengths. For example, it may be noticed that in the NOVITSKI stock (figure 1) there are 10 measurements at 16.0 mm but only one at 16.1. This type of error is on an exceedingly small scale, however, and would not affect the results from different slides, still less those from different stocks,

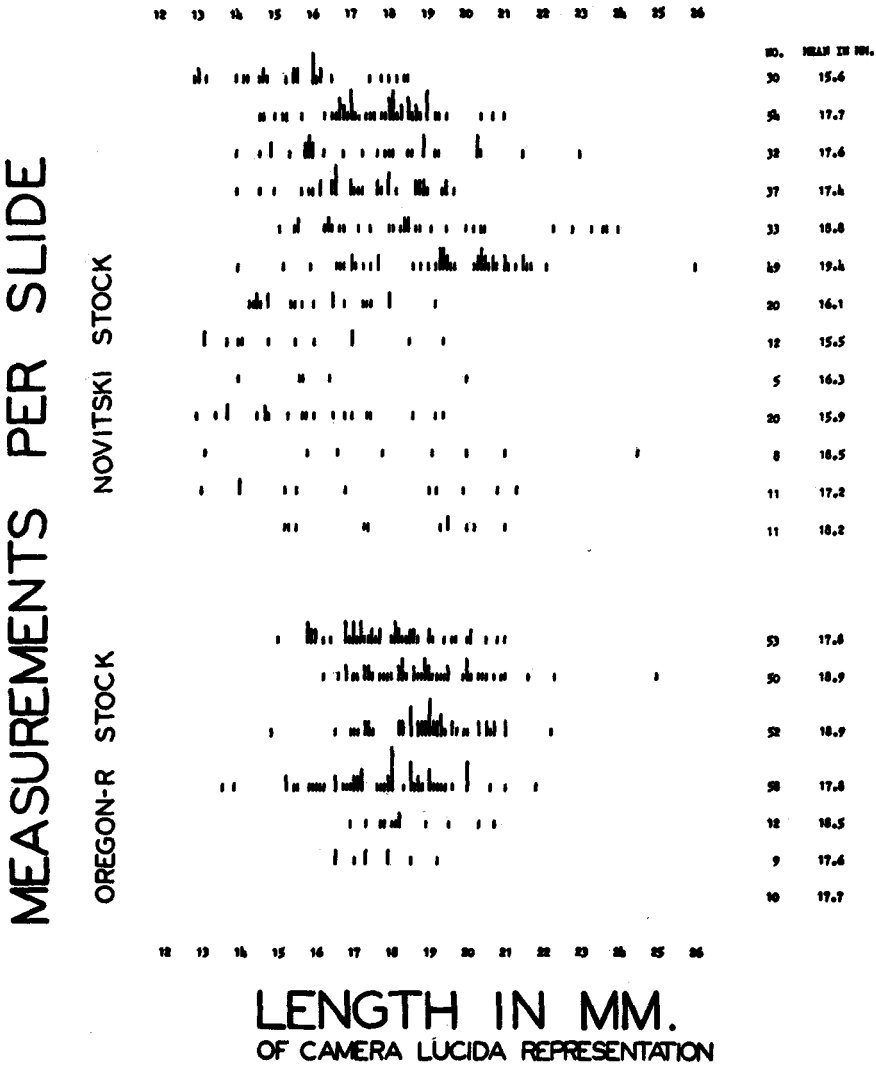

FIGURE 1.—The individual camera lucida lengths of 244 sperm chromatin masses from the Oregon-R stock and 322 from NOVITSKI'S stock are arranged according to slide. For each stock, slides measured later are placed above those measured earlier.

in a differential manner to any significant extent. Moreover, all these five errors of measurement are insignificant in relation to table-readings of the order of half a millimeter for indivdual sperm or of 0.2 millimeters for averages.

Since the high interslide variation, caused by differences in technique of preparation, greatly increases the dispersion and tends to invalidate comparisons of the distributions in the two stocks, a procedure was employed which eliminated this variation. The first step was to calculate the mean value for

TABLE 1

Distribution of measurements of the length of chromatin in sperm heads containing different numbers of chromosomes. (Length expressed in terms of percentage of mean length of sperm on the given slide.)

Stock	No. chromatin masses measured	Percent of sperm having lengths designated												
		70.1–75.0	75.1–80.0	80.1–85.0	85.1–90.0	90.1–95.0	95.1–100.0	100.1–105.0	105.1–110.0	110.1–115.0	115.1–120.0	120.1–125.0	125.1–130.0	130.1–135.0
Novitski	275	0.4	0.7	6.9	9.1	14.6	18.2	18.9	17.1	5.5	5.1	1.8	1.1	0.7
Oregon-R	213	0.0	1.4	0.5	10.3	13.2	24.4	24.9	14.6	7.1	2.8	0.5	0.0	0.5

each slide on which 20 or more measurements had been made. This comprised 4 slides of the Ore-R and 8 of the NOVITSKI stock, including 275 and 213 measurements, respectively. Slides with fewer measurements were omitted in order to avoid introducing an appreciable error caused by the statistical unreliability of these means. It was calculated that for the groups of 20 or more this error would be so small as to have a negligible influence on the results sought. All the individual measurements were then expressed as percentage deviations from the mean value for their slide, and all the measurements of a

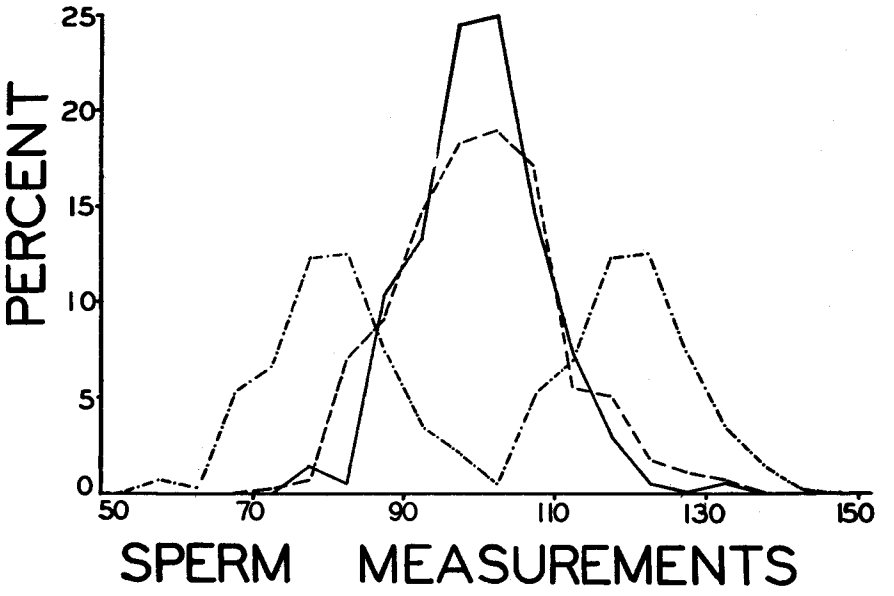

FIGURE 2.—The graphs shown are those for the Oregon-R (continuous line) and the NOVITSKI (dashed line) stock, drawn from the values of table 1, and the one expected for the latter stock (dot-dash line) on the hypothesis of a straight-line end-to-end arrangement in the sperm head of chromosomes proportioned like those at metaphase. Sperm frequencies of each stock are expressed in terms of percent of measurements lying within each 5% interval to right and left of mean of slide from which the given measurement was taken. (Only slides of 20 or more measurements were used.)

given stock, thus expressed, were then combined into one distribution, for which the values of the standard deviation and of the quartiles were calculated.

In table 1 the results for each stock are grouped into class-intervals corresponding to each difference of 5% from the mean sperm length, which is taken as 100%. Figure 2 represents the same results as a graph for each stock. In both table and figure the frequencies are expressed in terms of the percentage of total observations made on the stock in question which fell within each 5% interval. In making the figure these observed percentage frequencies were shown as dots in the middle of their respective 5% intervals of sperm length, the ordinate representing the frequency and the abscissa the length. These dots, for each stock, were then connected by a line. It is evident that

both distributions are effectively unimodal; but the NOVITSKI stock appears to have a greater dispersion.

On further analysis of the distributions, that for Oregon-R proves to have a standard deviation of 8.21 ± 0.40% of its mean. The calculation has been based on the observed measurements when these were grouped in classes having an interval of only 1% instead of the 5% used in the table and figure. The error of the determination has been based on the assumption of a normal distribution. That the Oregon-R distribution is approximately a normal one, as expected of a random sample of items whose deviations have been determined by numerous small factors which vary in large measure independently of one another, is indicated by a number of measured characteristics of the distribution. Among these are the positions of median and quartiles in relation to mean and standard deviation, and the proportion of observations with deviations, in each direction, greater than the standard deviation and greater than 1.5 times and twice the standard deviation, respectively. All these values agree as closely with those calculated for a normal distribution as would be expected for a random sample of the given size taken from an actually normal distribution. This does not yet prove the distribution to be entirely normal, however, for much the same fit might be obtained if the curve represented a combination of two simlar normal curves having means only a short distance apart, as will be discussed later.

The data for the NOVITSKI stock, subjected to the same type of calculation, show a standard deviation of 10.07 ± 0.43% of the mean. Here again the standard deviation and mean have the same positions relative to the median and quartiles as in a normal curve, and the frequencies of observations exceeding 1.5 times and twice the standard deviation are also not very far from those expected for a normal curve having the given standard deviation. On the whole, however, the frequencies in the more extreme regions tend to be higher, in relation to the standard deviation, than for the Oregon-R curve, though this difference in shapes is not enough to be convincing in itself. What is clearly significant in a comparison of the two curves is the difference in their degree of dispersion, as measured by either their standard deviations or quartiles. Thus the difference in their standard deviations, being 1.86 ± 0.59, is 3.2 times its own standard error, and had a chance of occurrence, by random sampling, of less than 1 in 1000 (in this "expected" direction), if the sperm of the two stocks had really possessed the same dispersion.

INTERPRETATION

If the reality of the difference in dispersion between the distributions of sperm lengths for the two stocks be admitted, it follows with high probability that the heterochromatin of the sex chromosomes, or more specifically, the heterochromatic blocks (MULLER and GERSHENSON 1935), have an appreciable size, relative to the euchromatin, as they do in mitotic chromosomes, although they are not necessarily as large as in the latter. For if the blocks and the heterochromatin of the sex chromosomes in general were as minute, rela-

tively to the rest of the chromatin, as they are in salivary and other interphase stages, no appreciable difference in the size of sperm would be expected from their addition or subtraction. Hence the sperm of the NOVITSKI stock would possess no wider dimorphism, and would show no greater dispersion, than those of the Oregon-R stock, in view of the fact that the sperm of these two stocks differ only in respect to the distribution of their sex-chromosome heterochromatin.

In the attack on the problem of whether the arrangement of the chromosomes in the spermatozoa is a straight end-to-end one, let us first assume, as a working hypothesis, that the blocks, and therefore the heterochromatin, are about as large, relatively to the rest of the chromatin, as they are at mitosis. In that case, the amount of chromatin in a sperm with an X would be sensibly equal to that in a sperm with a Y; hence the Oregon-R stock would have no quantitative sperm dimorphism. But in the NOVITSKI stock, the amount of chromatin in the sperm with X and Y attached would bear a ratio to that in the sperm lacking a sex chromosome lying somewhere between 7:4 and 6:4 (i.e., 3:2). Moreover, if the chromosomes were arranged in straight single-file this would also be the ratio of the means of the sperm-head lengths of the two classes of sperm.

The figure 7:4 comes nearer the apparent value as shown in most figures, in which the Y is approximately as long as the X and each of them is about 1½ times as large as one of the arms of the major autosomes. However, the objects are so small that the error, even of the relative sizes, is very large. Because of stretching, the error of relative salivary chromosome lengths is also large. There is reason, based on the lower frequency of induced lethal mutations in the X than in the arms of the major autosomes, to suspect that the euchromatic portion of the X may be not more than 0.8 of the size of the euchromatic portion of an arm of a major autosome. The proximal heterochromatin of the X at mitosis is not more than half as long as the euchromatin of the X, and since it is more or less tapering it might become packed down, in the sperm, to occupy less than half the length of the euchromatin. It is also to be taken into consideration that the heterochromatin blocks of the major autosomes occupy an appreciable space at mitosis (being in total about ¼ as long as the euchromatin of these autosomes) and that those of the small fourth chromosome are probably as long or longer than the euchromatin of that chromosome. Taking all these items into consideration at once (but not the fact that the attached X-plus-Y has somewhat more mitotic heterochromatin than a normal X and a normal Y added together) we arrive at the minimum value of 3:2 for the ratio of lengths of the two classes of sperm of the NOVITSKI stock, on the assumptions of heterochromatin blocks as large as at mitosis and lengthwise, end-to-end arrangement of straight chromosomes.

The question next arises, what would be the distribution of sperm lengths on these assumptions when, to be conservative, the ratio of 3:2 is used in our calculations? Since, on the present assumptions, the Oregon-R sperm is quantitatively homogeneous in its chromatin content, we may use its distri-

bution to represent that of each of the component distributions of the NOVITSKI stock sperm. However, the means of these two components will be symmetrically situated about the Oregon-R mean, with a distance between them equal to 40% of the Oregon-R mean (since on the scale of 3:2 for the NOVITSKI means the Oregon-R mean would be 2.5, and the difference 3 – 2, or 1, is 40% of 2.5). Hence to construct our artificial curve we first construct two curves, each having the same spread and shape as the Oregon-R curve but only half its height at each point, and place them with their means respectively at 80% and 120% as compared with the Oregon-R mean. We then combine them into a single curve by adding their heights wherever they overlap with one another, and finally join the ordinates thus established by a continuous line. In this way the curve has been constructed which is shown as a dot-dash line in figure 2. Its standard deviation may be calculated to be $\sqrt{20^2 + 8.2^2}$, or $\pm 21.6(\%)$.

It is immediately apparent that this hypothetical curve, with its extreme bimodality, is quite irreconcilable with the observed curve for the NOVITSKI stock. Moreover, the difference between the enormous standard deviation of the hypothetical curve and that ($10.07 \pm 0.43\%$) observed for the NOVITSKI stock is many times its own statistical (standard) error. It must therefore be concluded either that the sperm chromosomes are in straight end-to-end alignment, or that their heterochromatin blocks are substantially smaller than at mitosis, or that both our previous assumptions are wrong at once.

Let us see if we can save the assumption of straight end-to-end arrangement by assuming the heterochromatin blocks to be relatively smaller than at mitosis. We may first try out the most extreme assumption regarding them, namely, that they are of negligible size as in interphase and salivary chromosomes. Here, however, we come up against two objections. First, as earlier noted, the significantly greater dispersion of the NOVITSKI than of the Oregon-R stock distribution shows that the heterochromatin blocks are of appreciable size. Second, if the blocks were inappreciable there would be a considerable dimorphism among the Oregon-R sperm. On the assumption that the euchromatin in the X is only 0.8 as long as in the arms of the major autosomes, the two classes of sperm would have means approximately 91.5% and 108.5% of the mean of the mixed lot of sperm. Thus, even if all sperm of a given class were exactly equal in length, as measured, the standard deviation of the mixed lot would be 8.5%, since all sperm would have this amount of deviation from the common mean. The resultant "curve" would then consist of two vertical lines separated by an interval of 17%. But only $8.2 \pm 0.4\%$ has been observed as the standard deviation, so that nothing is left for deviation about the means of the component distributions. Moreover, as the shape of the observed curve shows, much (if not all) of its dispersion must in fact be caused by differences of real or apparent length among sperm of the same sex-chromosome content. Hence the premise of straight end-to-end arrangement of chromosomes completely lacking blocks is ruled out.

A little calculation and plotting of graphs was then carried out to ascertain

how much dimorphism the Oregon-R stock might conceal. The procedure followed was based on the principle that the standard deviation-squared of a distribution resulting from a combination of two distributions with the same dispersions, but means differing by d, is equal to d^2 plus the standard deviation-squared of the component distributions. This procedure showed that if the Oregon-R distribution is a combination of two equal component distributions of identical shape, the means of these components cannot differ by more than 8 or 10% (i.e., they will stand at not more than $\pm$ 4%, or possibly $\pm$ 5%, from the mean of the mixed distribution). For if they differ more than this they will result either in too sharply bimodal a combined curve to be reconciled with the observed Oregon-R curve, or else in a curve having a standard deviation significantly greater than that observed for the Oregon-R sperm.

If, now, we take the extreme assumption of a 10% difference in means for Oregon-R, we arrive at a contradiction with observation when we calculate the result for the NOVITSKI stock. To calculate the NOVITSKI stock curve, we must first determine the difference in means which its two components would have on the given assumptions. This is derived as follows. The difference in means in the Oregon-R stock if the blocks had been negligible would, as noted previously, have been at least 17%, but on the present assumptions it is at most 10%. Therefore in this case a differential effect on the length of at least 7%, acting in an opposite direction to the 17% euchromatically caused difference, is being exerted by heterochromatin. It is exerted by the heterochromatin-block *excess* carried by the Y as compared with the X, and this is approximately one half of all the heterochromatin-block material carried by both the X and Y, considered together (since that in the Y is to that in the X approximately as 3 : 1, the excess of Y over X thus being $3-1$, or 2, while the total is $3+1$, or 4, which is double the excess, 2). That is, the total sex-chromosome heterochromatin would occupy a length of twice 7%, or 14%, of the mean sperm length. (This is approximately half of its observed length at mitosis.) Hence, in the NOVITSKI stock sperm, in which the mean of one component differs from that of the other both by reason of the 17% difference caused by the euchromatin of the X, and also of the 14% minimum difference just calculated to arise from the blocks, the means would differ by 31% from each other, and would accordingly occupy places at $\pm$ 15.5% from the mean of the whole lot of sperm. The separation of means, then, is only moderately less in this case than that shown in the hypothetical bimodal curve of figure 2.

Yet despite this wide separation between the two means the dispersion about each of them has become less than in the curve of figure 2. For the present assumptions, according to which the Oregon-R curve has two components, with means differing by $\pm$5% from the common mean, have reduced the residual variability allowed to each of these components to a standard deviation of 6.5%, in order to meet at the same time the condition that the standard deviation of the total remain only 8.2% (by the calculation

that, approximately, $8.2 = \sqrt{(6.5)^2 + 5^2}$). Now, when the dispersion of each component of the NOVITSKI-stock curve is reduced to this standard deviation of 6.5%, the bimodality is sharpened and becomes even more pronounced than that in the hypothetical curve of figure 2, and is even less reconcilable than before with the observed NOVITSKI stock curve of figure 2. At the same time, the standard deviation of the hypothetical curve becomes $\sqrt{6.5^2 + 15.5^2}$, or $\pm$ 16.8, instead of the observed 10.07 $\pm$ 0.43 or the value of 21.6 calculated on the assumption of heterochromatin like that at mitosis.

We have now examined the effects, on the hypothetical NOVITSKI-stock distribution, of supposing the total sex-chromosome heterochromatin to have the least size consistent with the Oregon-R data, namely, 14% of all the chromatin, and at the other extreme, a size as great as in mitotic chromosomes, namely, 27% or more. We have seen that in both cases, so long as a straight end-to-end arrangement is assumed, the hypothetical curve is glaringly at variance with the observed one. Any assumption regarding heterochromatin sizes intermediate between these extremes must give a similarly impossible result. This will be better realized in consideration of the following course of reasoning. It has just been seen that the assumption of a minimum of sex-chromosome heterochromatin (14%) results in a curve with far too marked a bimodality and too great a standard deviation to fit the observed results for the NOVITSKI stock. If, now, the total heterochromatin of the sex-chromosomes of the sperm is assumed to be any greater than this, by an amount termed h, this amount h will be added to the amount of separation between the means of the two component curves of the hypothetical NOVITSKI stock which has just been calculated, thus *increasing* its standard deviation to a corresponding (and almost equal) extent. At the same time, the dimorphism in the Oregon-R stock will be reduced only by $\frac{h}{2}$, since in that stock only half of this heterochromatin constitutes the excess of Y over X, and the dispersion allowed the component curves will only be slightly increased thereby, so that the bimodality will remain marked. Hence there are no allowable assumptions regarding heterochromatin size in the sperm whereby a straight end-to-end arrangement can give a distribution even distantly reconcilable with that observed for the NOVITSKI stock.

Having thus abandoned the hypothesis of a straight end-to-end arrangement, let us see whether there is some other hypothesis which will fit the data adequately. In choosing it, we should be guided by the information, provided by the significantly greater dispersion found for the NOVITSKI stock than for the Oregon-R stock, that the heterochromatin blocks remain developed to a perceptible degree in the spermatozoon stage. This being the case, the simplest assumption is to consider them as being of the same size, relative to the euchromatin, as they are at mitosis, a premise which also agrees best with the observations of COOPER (1952) on the chromosomes of not quite mature spermatozoa. Adopting this assumption, and also taking, as before, the figure 3:2, i.e., 1.5, as the ratio between the bulks of the two classes of sperm of

the NOVITSKI stock, it is evident that the ratio of their lengths would be $\sqrt[3]{1.5}$, or 1.145, if the sperm of both classes had the same shape and therefore had lengths proportionate to the cube root of their volumes. This would give, as the mean lengths of the two classes, $1.0725 \pm .0725$, or, on the scale which takes the mean of the combined distribution as 100%, it would give $100 \pm 6.75\%$. To get the standard deviation of the combined distribution, we must take into account not only this but also the standard deviation of the component curves, $\pm 8.21\%$, obtained from the Oregon-R results. We then arrive at $\sqrt{6.75^2 + 8.21^2}\%$, or 10.6%, as the resultant standard deviation. This agrees excellently with the observed value of $10.07 \pm 0.43\%$ for the standard deviation of the NOVITSKI stock, being removed from it by only 1.2 times the standard error of the latter value, and by less than the error of the difference itself.

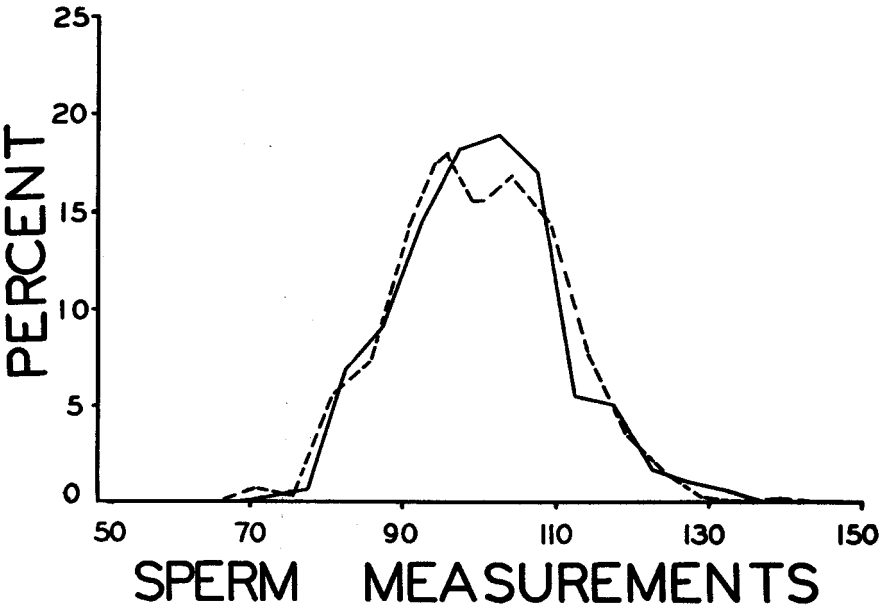

FIGURE 3.—Superposition of observed NOVITSKI stock graph (continuous line) with hypothetical graph (dashed line) based on two combined graphs of same shape as that of Oregon-R but with means related as $\sqrt[3]{3/2}$.

In order to compare the shape of the resulting curve with that observed for the NOVITSKI stock, we have plotted the two together in figure 3. The hypothetical (dashed-line) curve of this figure was constructed in the same way as the hypothetical curve of figure 2, except that in the present case the means of the two component curves by the summation of which it was built were taken as being in the positions $100 \pm 6.75\%$, in accordance with the above calculation. It is to be observed that the hypothetical and observational curves fit each other very satisfactorily throughout, when the errors of sampling to which they are subject are taken into consideration.

Despite the good fit of the calculated to the observed values, the correct-

ness of the premises on which the calculations were based cannot yet be considered as actually proved, but only made probable. It might still be held that the lengths of sperm do not tend to be proportional to the cube root of their volume. In that case, however, the agreement of theory with observation would have to be ascribed in part at least to coincidence. It would have to be supposed that technical errors and/or complicating influences acting on the length of sperm heads had so counterbalanced the effects of the operation of a rule different from that of proportionality of lengths to the cube root of volumes (a rule according to which the shapes of sperm heads of different sizes do *not* tend to remain constant) as to mask the main principle at work. It would be idle to discuss these other possibilities in detail here. However, attention may be called to the possibility of an adaptive mechanism which tended to hold sperm heads to a given, functionally advantageous length, even in the face of differences in their chromatin content.

Light on the questions here at issue might be thrown by further studies of the same type as the present ones, to determine whether their results agreed with those here presented, especially in cases in which other chromosome differences than those here studied were concerned. In the meantime, however, the lack of discrepancy between our observations and the calculated consequences of our assumptions, considered in connection with the simplicity of these assumptions, argues strongly for the validity of the latter. And, in any case, the hypothesis of a straight end-to-end arrangement of chromosomes in the sperm head of *Drosophila melanogaster* can no longer be considered as admissible for the majority of spermatozoa, even though, conceivably, that arrangement might occur in occasional cells.

If, as seems probable, the rule of the proportionality of the chromatin length in the sperm to the cube root of the chromatin mass is valid, even if only approximately, the question arises, in what way is this rule brought about? As previously mentioned, the rule would imply that the sperm heads of different sizes have substantially the same shape. This would have to be true despite the fact that when the chromosome contents of the sperm of different sizes were laid out in any fixed arrangement (such as end-to-end in one row or in two rows, etc.), and then subjected to any proportionate deformation, the resulting chromatin masses would *not* have lengths proportional to the cube roots of these masses. The production of this relationship is more likely to follow, in case the chromosomes involved are of mitotic shapes to start with, if their mutually repulsive forces were reduced to a minimum or overcome, and they were pressed into a compact mass of a given shape, perhaps more or less ovoid, as though they were so much modelling clay, and if after that they became squeezed out into a very elongated shape of fairly constant proportions. In that case there might not be a substantial change in the arrangements of the parts with regard to one another, aside from this lengthwise deformation, and as a result there might be considerable overlapping of the chromosomes and of their parts in most of the sperm. Indeed,

to explain the results with neutrons previously referred to, taken together with the occasional production of highly complex mutual (" cyclical ") interchanges of parts, several different chromosomes, and chromosome arms, must not infrequently be present in the same cross-section of a sperm head.

It is of course conceivable that the chromosomes of the mature sperm, instead of being, primarily, in more or less discrete masses as at mitosis, have a conformation resembling, in respect to interpenetration of parts (but not in respect to amount of non-chromatin material), that of interphase. However, the evidence derived from our present results, indicating the existence of blocks at this stage, as well as the cytological observations earlier referred to, make it more likely that the chromosomes are more like those of mitosis, except in regard to their crowding and deformation.

SUMMARY

1. The lengths of the chromatin masses were measured in spermatozoa of a normal stock and in those of a stock (NOVITSKI's attached X.Y) in which half of them contained both X and Y and the rest contained neither. The frequency distribution of lengths had a significantly greater dispersion in the latter stock than in the former.

2. It is inferred that this difference in distribution of lengths was caused by the presence in the spermatozoa of heterochromatin blocks, similar to those in mitotic chromosomes, and that these blocks were differently distributed in the two stocks among their two classes of spermatozoa.

3. The relatively small dispersion and the effective unimodality of distribution of chromatin-lengths found in both stocks are facts irreconcilable with the hypothesis of a straight end-to-end arrangement of chromosomes in the sperm head of *Drosophila melanogaster*.

4. When the chromosomes of the spermatozoa are assumed to have the same proportions of parts, including blocks, as those of mitotic and meiotic divisions, and when they are assumed to become squeezed together into masses of substantially the same shape in the sperm head, regardless of the amount of chromatin present, so that the lengths of these masses are proportional to the cube roots of their volumes, the resulting distributions of lengths, calculated for the two stocks, are found to be in excellent statistical agreement with the distributions observed by us.

5. It is to be expected that a method of formation of sperm heads of the type above suggested would entail considerable overlapping of chromosomes and of chromosome parts, along the length of the sperm heads. This type of arrangement had been inferred earlier, on the basis of genetic data concerning the relations between neutron dosage and the frequency of induced translocations. If, on the contrary, a straight single-file arrangement of chromosomes had been established for the sperm head, a radical revision of current theories regarding the way in which structural changes of chromosomes are produced would have been required.

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