A SEX DIFFERENCE IN CHROMOSOME LENGTHS IN THE MAMMALIA[1]

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During the last two years we have had occasion to accurately draw and enlarge human chromosomes. Both male and female, embryonic and adult, tissues have been studied. In surveying the first or longest chromosomes of the series we were early struck by the greater length of this chromosome in the male. Five independent series of measurements have furnished invariable confirmation of the earlier observations.

There are many pitfalls for the investigator who aims at accuracy and comparable results with the mensuration of chromosomes. Prophase chromosomes are invariably slightly longer than those assembled at the equatorial plate after dissolution of the nucleus; varying methods of fixation and dehydration shrink tissues unequally; and finally there are differences in the length and size of chromosomes in various cells especially in embryonic cells as compared with adult ones. It is rather interesting that different types of cells vary somewhat in chromosome length as they undoubtedly do in the size of the cell itself. A striking instance of this is found in the lutein cells in the corpora lutea of the ovary at the time of pregnancy. The cells are greatly enlarged and the chromosomes have a corresponding increase in size, the length and diameter being approximately two or more times those of the chromosomes of the surrounding cells. For all of these reasons we have sought to compare chromosome lengths only in the same phase of mitosis (late prophase) from the same type of tissue (mesenchyme) in embryos of approximately the same age, fixed and dehydrated by identical processes. Many human embryos of both sexes were explored for our work, but an immediate fixation (at the operating table) with resulting clarity of the chromosomes was secured in only two embryos of approximately the same age; a male of G. L. 19.5 to 20.5 mm and a female of G. L. 25 mm.

In both cases we selected ten nuclei of the mesenchyme situated near the ectoderm, displaying clearly all of the forty-eight chromosomes, which were then individually drawn at 3600 diameters. The original

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drawings were enlarged twice photographically. From these records measurements were made of the chromosome lengths, which were determined by measurement of the median axis in every case.

There is a clearly marked sex difference in the length of the first (or longest) pair of chromosomes. The mean length of the two longest chromosomes from the ten male nuclei exceeded by 8.22 mm the mean length of the longest female pair. The probable error of this difference is $+.74$ mm. The difference is thus over ten times the probable error of the difference. It indicates that the longest autosomes in this male embryo

TABLE 1

Individual measurements (×7200) of extreme autosomes (in mm) of ten nuclei from human embryos of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	37.6	37.5	23.7	23.5	7.5	8.3	9.3	9.7
2.	38.9	33.0	35.6	31.0	8.1	9.0	10.3	11.3
3.	39.6	33.5	28.0	25.2	6.5	8.0	10.0	10.6
4.	38.2	36.1	31.1	28.0	7.3	9.0	8.7	9.1
5.	38.5	36.1	34.2	30.2	9.1	9.5	8.7	9.7
6.	38.0	34.7	39.3	27.2	7.8	8.5	9.5	9.5
7.	41.0	40.7	28.7	28.5	10.0	10.0	10.3	10.7
8.	40.4	37.5	36.5	31.0	8.0	9.8	10.0	10.1
9.	45.6	37.7	30.7	29.0	8.1	8.7	9.0	9.0
10.	39.5	38.5	30.0	26.7	8.7	9.0	10.2	11.2
Average	39.7	36.5	31.8	28.0	8.1	9.0	9.6	10.1

TABLE 2

Human embryos (10 nuclei of each sex; measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	$38.13 \pm .41$	$2.73 \pm .29$
Longest pair ♀	$29.91 \pm .62$	$4.08 \pm .44$
Difference ♂ − ♀	$8.22 \pm .74$	$-1.35 \pm .51$
Average of all 46 autosomes ♂	$18.79 \pm .24$	$7.46 \pm .17$
Average of all 46 autosomes ♀	$17.23 \pm .16$	$5.17 \pm .11$
Difference ♂ − ♀	$1.56 \pm .30$	$2.29 \pm .21$
Shortest pair ♂	$8.55 \pm .13$	$.90 \pm .10$
Shortest pair ♀	$9.86 \pm .11$	$.72 \pm .08$
Difference ♂ − ♀	$-1.31 \pm .17$	$.18 \pm .12$

were significantly longer than the longest autosomes in this female embryo. The data are given in table 9, and the constants computed from them, in table 2.

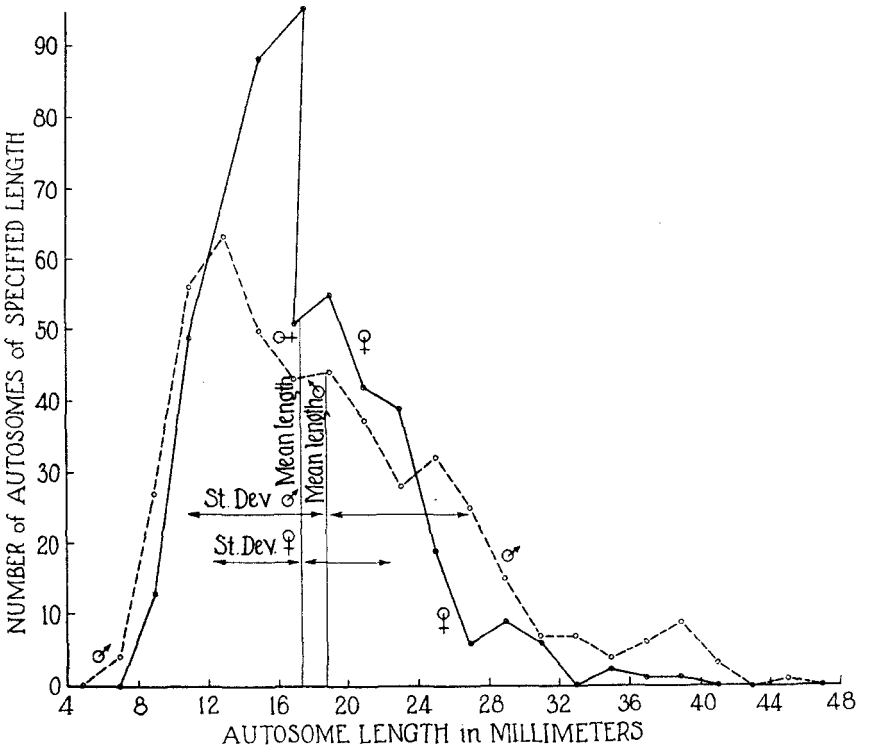

FIGURE 1.—Frequency distribution of autosome measurements in human embryo nuclei; all 46 autosomes in 10 nuclei of each sex.

It will be noted that the standard deviations are not significantly different in the two sexes when only one pair of autosomes is considered. When all twenty-three autosome pairs were studied (neglecting the XX and XY pairs) and the mean autosome lengths computed, the excess in length of male autosomes is seen to be much less than that shown in the case of the longest (first) pair. A difference of only 1.56 mm results. The probable error of the difference is .30 mm. Thus, though this difference is small it is also significant, being over five times its probable error. The frequency distributions of these measurements are given in table 3.

It is interesting to note, both from the frequency distributions and the computed standard deviations, that, when all twenty-three pairs of autosomes are considered, the male autosomes show a much greater variability than the female autosomes. The difference between the standard devia-

TABLE 3

Frequency distributions of autosome measurements

AUTOSOME LENGTH IN MM	ALL THE AUTOSOMES—10 NUCLEI				LONGEST PAIR ONLY			
	Human embryos		Rat embryos		100 nuclei from one individual rat embryo		10 nuclei from each of 10 rat embryos	
	Male	Female	Male	Female	Male	Female	Male	Female
4.0 to 5.9	..	..	2	..	..	..	..	..
6–	4	..	5	1	..	..	..	..
8–	27	13	13	8	..	..	..	..
10–	56	49	41	49	..	..	..	..
12–	63	79	77	71	..	..	..	..
14–	50	88	82	78	..	..	..	..
16–	43	51	82	75	..	..	..	..
18–	44	55	81	71	..	..	..	..
20–	37	42	48	51	..	..	..	..
22–	28	39	52	53	..	2	..	..
24–	32	19	26	44	..	10	..	1
26–	25	6	15	34	4	17	..	3
28–	15	9	13	18	3	28	2	20
30–	7	6	15	19	15	37	8	29
32–	7	..	6	12	22	41	7	56
34–	4	2	9	6	29	25	18	29
36–	6	1	9	3	43	22	32	30
38–	8	1	3	4	22	11	43	17
40–	3	..	2	..	19	4	24	7
42–	..	..	6	..	20	3	24	5
44–	1	..	4	1	9	..	17	2
46–	..	..	3	2	6	..	10	1
48–	..	..	..	..	7	..	3	..
50–	..	..	2	..	..	..	7	..
52–	..	..	2	..	..	..	1	..
54–	..	..	..	..	1	..	1	..
56–	..	..	1	..	..	..	..	..
58–	..	..	1	..	..	..	..	..
60–	..	..	..	..	..	..	3	..
Total	460	460	600	600	200	200	200	200

tions is 2.29 ± .21 mm so that the difference is about ten times the probable error of the difference. The same sex difference in variability is shown by the coefficients of variation, which are, respectively, 39.70 ± 1.02 mm and 30.01 ± .72 mm.

This greater variability is, in fact, so great that the sex difference in mean autosome length is actually reversed when the shortest pair of autosomes is considered. This fact is, of course, indicated by the much

smaller sex difference in mean autosome length which was noted when all twenty-three pairs of autosomes were averaged, as compared with the sex difference when the longest pair only was considered.

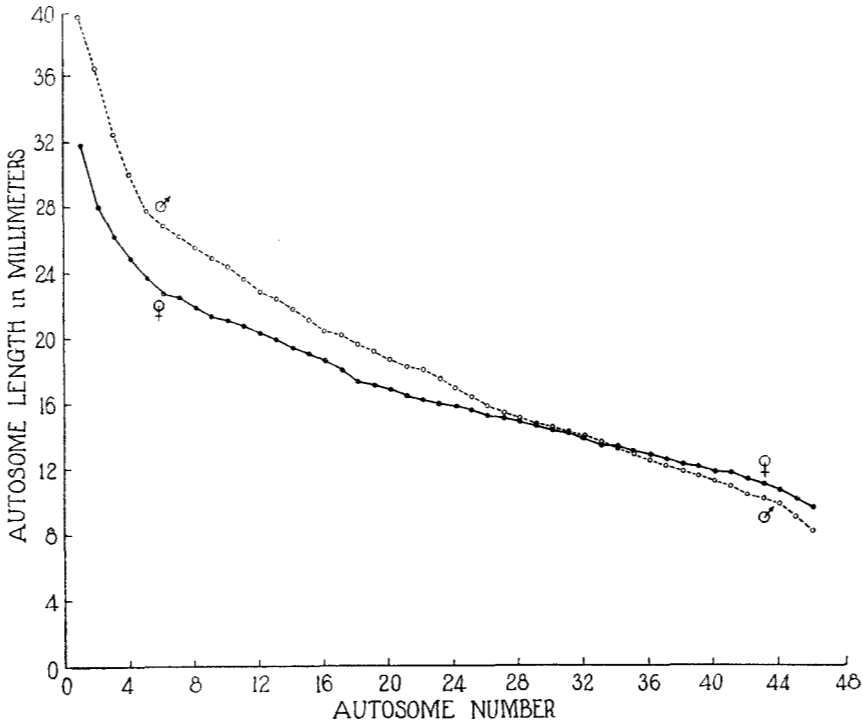

FIGURE 2.—Average lengths of successive autosomes in 10 nuclei of each sex—human embryo.

It thus becomes of interest to consider the smallest pair only. These data also are presented in table 1 and the constants in table 2. In this case the autosome lengths in the female *exceeded* those in the male by 1.31 $\pm$.17 mm. The difference thus indicates that in this male embryo the shortest autosomes were significantly shorter than the shortest autosomes of the female embryo.

Since the standard deviations are again not significantly different, the greater variability of the lengths of the autosomes of the male indicates a greater variation or range in length of the autosomes within a single nucleus, and not a greater variation between lengths of correspon ding autosomes in different nuclei. Table 4, showing the averages and stan dard deviations of the twenty-three pairs in each of the ten nuclei of each sex, is presented to corroborate these points. The standard deviations for the individual nuclei of the male range from 6.64 to 8.06 mm with a

value for the total distribution of 7.46 ± .17 mm; those of the female range from 4.03 to 5.69 mm, with a value for the total distribution of 5.17 ± .11 mm. The difference in standard deviations is thus 2.29 ± .20 mm. The difference in variability is therefore highly significant. Figure 1, plotting the frequency distributions, means, and standard deviations of the autosome lengths and figure 2, plotting the average lengths of the successive autosomes, show these sex differences graphically.

TABLE 4

Average of all 46 autosomes in each individual nucleus (in mm) in ten nuclei of human embryos of each sex.

NUCLEUS	MALE		FEMALE	
	Mean	Standard deviation	Mean	Standard deviation
1.	18.80 ± .69	6.94 ± .49	15.83 ± .40	4.03 ± .28
2.	17.57 ± .70	7.02 ± .49	17.39 ± .54	5.46 ± .38
3.	17.48 ± .76	7.62 ± .54	16.65 ± .49	4.89 ± .34
4.	17.52 ± .66	6.64 ± .47	16.09 ± .51	5.17 ± .36
5.	19.83 ± .70	7.08 ± .50	18.17 ± .55	5.57 ± .39
6.	20.17 ± .73	7.32 ± .51	16.70 ± .55	5.55 ± .39
7.	17.61 ± .80	8.06 ± .57	17.17 ± .47	4.68 ± .33
8.	21.17 ± .80	8.04 ± .57	18.09 ± .57	5.69 ± .40
9.	18.74 ± .68	6.88 ± .48	18.57 ± .54	5.40 ± .38
10.	18.74 ± .79	7.94 ± .56	17.61 ± .42	4.23 ± .30
Average	18.79 ± .23	7.46 ± .17	17.23 ± .16	5.17 ± .11

We were interested in seeing whether this sex difference in chromosome lengths, so evident in embryonic tissue, is demonstrable in the tissue of adults. Unfortunately tissue growth in the male, save in the testis, (spermatogenesis), consists merely in the relatively slight normal reparative processes seen, for instance, in the epithelia of the skin and gut. We hence sought neoplastic growths in boys or men and finally secured a labial carcinoma in a man of 55 years, where mitotic division was rife. The use of this material is unfortunately open to the charge of "embryonic character" of neoplastic growth. In the case of the female, normal reparative processes in the endrometrium of early post menstruum furnished abundant instances and stages of dividing cells.

Measurements were made of chromosome lengths in ten nuclei of each sex. Here again the longest autosome pair of the male exceeded that of the female by ten times the probable error of the difference (table 6). When the lengths of the twenty-three pairs of autosomes were averaged,

TABLE 5

Individual measurements of extreme autosomes (in mm) in ten nuclei of human adults of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	40.4	37.1	27.2	26.3	7.0	10.0	6.7	9.3
2.	47.9	38.1	34.3	33.0	9.0	9.3	10.1	10.3
3.	37.2	37.0	30.1	25.8	10.5	11.0	9.1	9.3
4.	38.0	31.0	28.8	25.3	10.0	10.5	11.1	12.0
5.	39.0	34.5	28.9	28.3	13.1	14.8	10.0	10.3
6.	36.0	30.3	30.6	30.5	10.5	11.5	8.5	9.2
7.	50.0	44.8	31.6	31.5	9.2	10.2	11.0	11.4
8.	42.0	40.5	36.8	32.8	10.0	12.1	8.5	9.5
9.	37.3	33.5	24.0	22.7	11.4	12.0	7.5	7.5
10.	44.3	44.0	32.3	29.2	8.9	10.2	8.3	8.9
Average	41.2	37.1	30.5	28.5	10.0	11.2	9.3	9.8

TABLE 6

Human adult (10 nuclei of each sex, measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	39.15 ± .76	5.07 ± .54
Longest pair ♀	29.49 ± .54	3.58 ± .38
Difference ♂ − ♀	9.66 ± .93	1.49 ± .66
Average of all 46 autosomes ♂	20.12 ± .22	7.15 ± .16
Average ot all 46 autosomes ♀	16.46 ± .16	5.24 ± .12
Difference ♂ − ♀	3.66 ± .27	1.91 ± .20
Shortest pair ♂	10.56 ± .25	1.63 ± .17
Shortest pair ♀	9.53 ± .18	1.21 ± .13
Difference ♂ − ♀	1.03 ± .31	.42 ± .21

the difference between male and female (3.66 mm), though smaller in the case with the longest pair only (9.66 mm), is nevertheless, also ten times its probable error. Even the shortest autosome pair in these adult autosomes was longer in the male than the female, though the sex difference here is by no means as great as with the longest pair. It will be remembered that in the embryonic mitoses the shortest male autosomes were shorter than the female ones. The mensurations of adult chromosomes showed, as did the embryonic, a much greater variability in male autosome lengths within a given nucleus as compared with female, whether measured by standard deviations or by the coefficients of variation. These measure-

ments thus furnish a confirmation of Darwin's belief in a greater variability in the male as a general law.

TABLE 7

Average of all 46 autosomes in each individual nucleus (in mm) in ten nuclei of human adults of each sex.

NUCLEUS	MALE		FEMALE	
	Mean	Standard deviation	Mean	Standard deviation
1.	18.61±.65	6.51±.46	16.39±.46	4.58±.32
2.	19.26±.79	7.97±.56	17.30±.65	6.54±.46
3.	19.87±.61	6.15±.43	15.61±.47	4.77±.34
4.	20.04±.69	6.92±.49	17.30±.43	4.29±.30
5.	20.52±.48	4.85±.34	17.52±.43	4.34±.31
6.	18.96±.55	5.49±.39	14.13±.46	4.60±.32
7.	21.39±.89	8.92±.63	18.09±.48	4.86±.34
8.	21.30±.74	7.47±.53	17.74±.58	5.87±.41
9.	20.96±.68	6.85±.48	14.30±.44	4.38±.31
10.	20.30±.86	8.65±.61	16.17±.58	5.85±.41
Average	20.12±.22	7.15±.16	16.46±.16	5.24±.12

Though we had taken all of the precautions possible to insure reliability in our conclusion of increased length of the longest male autosomes, the number of actually different animals studied was so small that we sought added evidence through the study of another mammalian form (the rat) and through a more rigorous control of all the factors influencing so delicate a task.

A pregnant rat was chloroformed on the twentieth day of gestation. The successive uterine enlargements were rapidly and deftly opened with fine tweezers and scissors, the embryos being instantly plunged under Bouin's fluid at 39°C. In each case the central body wall was cut widely open, enabling an immediate view of the gonads and diagnosis of sex to be made. We took care to secure identity in the fixation, dehydration, and all technical procedures involved in sectioning and staining the male and female litter mate embryos. Here again, in each sex, we delineated all the chromosomes in ten prophase mesenchyme nuclei located near the skin ectoderm and ideally fixed. As one of us has discovered (SWEZY, 1927), the hybrid rat of our colony possesses both sixty-two and forty-two chromosomes instead of the single count of forty-two which, in agreement with PAINTER (1927), we find the albino possesses. The rat chromosomes were all magnified 8400 diameters for measurement. The data are presented in table 8 and the constants in table 9.

TABLE 8

Individual measurements (×8400) of extreme autosomes (in mm) in ten nuclei from rat embryos of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	44.8	42.0	32.7	32.2	8.3	8.5	10.7	10.7
2.	46.7	41.1	44.6	39.6	8.0	8.2	11.0	11.1
3.	39.5	39.3	35.2	33.7	7.3	7.3	8.5	9.5
4.	57.8	45.8	46.5	37.8	6.5	10.7	8.2	10.0
5.	59.3	51.6	31.3	30.6	10.7	11.2	9.7	10.0
6.	52.1	46.5	32.8	32.5	10.7	10.8	7.5	8.8
7.	44.0	42.2	36.1	33.6	10.1	10.6	10.7	10.8
8.	42.3	36.9	33.1	34.1	4.5	7.6	10.6	10.6
9.	45.3	43.9	47.5	38.3	5.0	9.0	10.0	10.0
10.	53.2	42.0	36.0	35.8	6.0	9.4	8.7	10.2
Average	48.5	43.1	37.6	34.8	7.1	9.4	9.5	10.2

TABLE 9

Rat embryo (10 nuclei, measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	45.81± .89	5.92± .63
Longest pair ♀	36.20± .72	4.80± .51
Difference ♂ − ♀	9.61±1.14	1.12± .81
Average of all 60 autosomes ♂	19.71± .22	8.16± .16
Average of all 60 autosomes ♀	19.61± .18	6.38± .12
Difference ♂ − ♀	.10± .28	1.78± .20
Shortest pair ♂	8.24± .45	2.98± .32
Shortest pair ♀	9.84± .18	1.22± .13
Difference ♂ − ♀	1 .60± .49	1.76± .34
	Rat Embryo (100 nuclei from one individual)	
Longest pair ♂	37.87± .24	5.03± .17
Longest pair ♀	32.39± .20	4.11± .14
Difference ♂ − ♀	5.48± .31	.92± .22
	Rat Embryo (10 nuclei from each of ten different individuals)	
Longest pair ♂	40.30± .26	5.45± .16
Longest pair ♀	34.02± .18	3.82± .13
Difference ♂ − ♀	6.28± .32	1.63± .21

Here again a clear difference exists in the case of males and females in the length of the first autosome pair, the difference between the means being 9.61 mm with a probable error of 1.14 mm. The difference is thus approximately nine times the probable error of the difference. The difference between the standard deviations is insignificant just as in the human nuclei when only one pair of autosomes is considered.

In the case of the rat, when the entire sixty or forty chromosomes were studied (omitting the XX and XY pairs) and a grand mean thus established, the superiority in length of the male autosomes practically completely disappears. The difference between the means is then merely .10 mm, with a probable error of .28. Thus, in the rat, as in man, the sex difference in autosome length is reversed in the smaller pairs of autosomes. The difference between the standard deviations is $1.78 \pm .20$ mm. The autosomes of the male rat, like those of man, thus show a greater variability than those of the female. The coefficients of variation are here $41.40 \pm .93$ mm and $32.53 \pm .69$ mm.

Computing the constants for the shortest pair only, the actual excess in length of the *female* autosomes is found to be $1.60 \pm .49$ mm. The difference in standard deviations is in this case significant, the first case in which a significant sex difference in variability had been found when only one pair of homologous autosomes in different nuclei is considered. The difference is $1.76 \pm .34$ mm.

To dissipate any lurking suspicion that we had consciously or unconsciously *selected* male prophase nuclei with longer autosomes than in the female, we decided to study a hundred nuclei from each sex, considering the two longest autosomes only. It does not seem to us remotely possible that biased choice could have been exercised by us in such an extensive record. The results of these measurements gave us a difference between the average length of the male and the female first autosome pair of 5.48 mm, with a probable error of only .31 mm. The difference between these means is highly significant, being approximately eighteen times its probable error. The frequency distributions of these measurements are given in table 3. The standard deviations are here again significantly different.

As a fifth test of this sex difference, it was thought desirable to secure corresponding measurements in a large number of different individuals. Therefore the longest pair of autosomes was measured in 10 nuclei from each of 10 different rat embryos of each sex. The averages for the different individuals are listed in table 10; the constants computed from the entire series, in table 9; and the frequency distributions of the individual measurements in table 3. This series of measurements again shows the

same sex difference, the longest autosomes of the male being significantly longer than the longest autosomes of the female. The difference in this case is $6.28 \pm .32$ mm, which is nearly twenty times the probable error of the difference. The difference is nearly ten times the probable error of the difference when the average for each embryo is treated as a single observation. (It will be noted that the individuals from which the 100

TABLE 10

Average lengths of longest pair of autosomes in ten different rat embryos of each sex.

EMBRYO NUMBER	AVERAGE OF 10 NUCLEI OF EACH EMBRYO			
	Male		Female	
1	39.8	35.4	39.4	34.9
2	50.1	44.5	40.0	35.1
3	39.6	37.4	35.8	32.5
4	41.7	38.1	35.0	32.1
5	39.3	36.7	33.1	31.2
6	43.1	38.4	35.0	30.9
7	46.0	42.6	34.0	31.3
8	39.8	35.2	35.0	32.8
9	41.9	38.2	32.9	30.9
10	41.7	36.2	35.8	32.8
Grand Average	42.3	38.3	35.6	32.5

TABLE 11

Rat Embryo (10 nuclei, measurements ($\times 8400$) in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	$45.81 \pm .89$	$5.92 \pm .63$
Longest pair ♀	$36.20 \pm .72$	$4.70 \pm .51$
Difference ♂ − ♀	9.61 ± 1.14	$1.12 \pm .81$
Average of all 60 autosomes ♂	$19.71 \pm .22$	$8.16 \pm .16$
Average of all 60 autosomes ♀	$19.61 \pm .18$	$6.38 \pm .12$
Difference ♂ − ♀	$.10 \pm .28$	$1.78 \pm .20$
Shortest pair ♂	$8.24 \pm .45$	$2.98 \pm .32$
Shortest pair ♀	$9.84 \pm .18$	$1.22 \pm .13$
Difference ♂ − ♀	$1.60 \pm .49$	$1.76 \pm .34$
	Rat Embryo (100 nuclei)	
Longest pair ♂	$37.87 \pm .24$	$5.03 \pm .17$
Longest pair ♀	$32.39 \pm .20$	$4.11 \pm .14$
Difference ♂ − ♀	$5.48 \pm .31$	$.92 \pm .22$

nuclei were measured happened to be near the lower end of the range of variation displayed by the 10 different embryos in this last series, while the individuals from which the first 10 nuclei were measured happened to be near the upper end of the range.)

Thus in five independent series of measurements, comprising the measurements of all the autosomes of ten nuclei of one individual of each sex in human embryo, human adults, and rat embryos, and the measurements of the longest pair of autosomes in one hundred nuclei from one rat embryo of each sex, and from ten nuclei from each of ten rat embryos of each sex, consistent and highly significant sex differences were found. The same sex difference in length of the longest autosomes is shown but not commented upon in part of the figures in PAINTER'S (1924) plate of the chromosomes of the monkey.

A study is being made of the reduction divisions in spermatogenesis to see whether this sex difference is due to a selective segregation of the chromosomes in the spermatids. This will be reported upon at a later date.

SUMMARY

1. In man and in the rat a significant and consistent sex difference occurs in the length of the autosomes of the somatic cells of both the embryo and adult.

2. The longest pair of autosomes in the male is longer than the longest pair in the female. This difference may be related to the greater body size generally found in the male.

3. Males also show a greater variation in the length of the autosomes within an individual nucleus. This difference could not be predicted from the greater body size of the male.

4. Thus, in addition to their possession of different chromosomes—the so-called sex determining chromosomes, the XX and XY pairs— male and females differ in the character (length) of the autosomes. This difference occurs in the early embryo and persists in the adult. It probably appears at the time of fertilization and can be looked upon as a "secondary" sex character inpressed from the beginning upon every cell in the body

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