CHROMOSOME NUMBERS IN SOMATIC TISSUES OF MOUSE AND MAN

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IT has long been accepted as a valid generalization, subject to certain recognized exceptions, that the number of chromosomes is the same in all somatic cells of an organism. The most commonly recognized types of exceptions are polyploidy and polyteny, which have been found to occur in the cells of differentiated tissues in a variety of animal and plant species. Since these types of variation in chromosome number involve whole genomes, however, they represent balanced systems and are not difficult to fit into the general theories on the genetic control of development.

Direct evidence on chromosome numbers in differentiated tissue is often not available, because of the lack of mitoses, but some indirect evidence supports the contention that the number of chromosomes is constant. BOIVIN, VENDRELY and VENDRELY (1948) believe that the desoxyribose nucleic acid (DNA) is constant in the cells of a species, a view supported by MIRSKY and RIS (1949). SWIFT (1950), studying the amount of Feulgen-positive material in individual nuclei of various tissues of the mouse, found the quantity of DNA to be constant or to be some multiple of the basic amount. This was taken to mean that the amount of chromatin was constant from cell to cell except for cases of polyploidy.

Within the last few years the question of the constancy of the chromosome number has once again come under review (cf. HUSKINS 1947) with reports of somatic reduction in plants (HUSKINS 1948; HUSKINS and CHENG 1950; HUSKINS and CHOUINARD 1950; WILSON, HAWTHORNE and TSOU 1951) and of aneuploidy in mammals (MELANDER 1950; TIMONEN 1950; TIMONEN and THERMAN 1950; THERMAN and TIMONEN 1951) which are difficult to reconcile with classical theory. MELANDER's case of aneuploidy in a "triploid" rabbit might be passed over as being due to the unusual nature of the material, but TIMONEN's (1950) report of a high frequency of aneuploidy in man cannot be so considered.

TIMONEN (1950) counted 1000 chromosome groups in Feulgen squash preparations of human endometrium, 100 from each of ten individuals, and found a range of chromosome numbers from 4 to more than 100. The distribution was found to be bimodal, with "the highest peak lying at 20–25 chromosomes and a much lower one at 45–50 chromosomes" (THERMAN and TIMONEN 1951). Measurements of 2000 nuclei gave a distribution of volumes which was similar to the distribution of chromosome numbers. Estimations based on the size of the metaphase plates in various human embryonic tissues, as well as some actual counts, likewise showed "that most of the cells were hypoploid." These findings have led THERMAN and TIMONEN to suggest that a

high frequency of aneuploidy, with most cells hypoploid, may be common in higher animals. The fact that most studies of chromosome numbers in higher animals have been made on germinal tissue is advanced to explain why such a high frequency of aneuploidy has been overlooked in the past. They suggest that gene action in these forms may take place largely through the various glands, so that the lack of some of the chromosomes in cells of differentiated tissues may be relatively unimportant.

Because of the broad theoretical implications of THERMAN and TIMONEN's findings, should they be confirmed and also found to hold true for other warm-blooded animals, it was felt that a similar study should be made on another mammal. Consequently, the present investigation was undertaken, using the mouse, *Mus musculus,* as the experimental material. Human endometrium was also used for direct comparison with THERMAN and TIMONEN's results. Preliminary results have been published elsewhere (BOOTHROYD and WALKER 1952).

MATERIALS AND METHODS

All materials in this study were fixed in 95% alcohol–glacial acetic acid (3:1). Semi-permanent preparations of material stained and mounted in aceto-carmine were made by tilting the slide and allowing WILSON's (1945) Venetian turpentine medium to run under the cover glass to displace the stain. The cover glass was then pressed down and its edges sealed with nail polish.

The four methods used for preparing mouse tissue were (1) placing pieces of intestine from day-old mice under a cover glass and spreading them by tapping the cover glass; (2) dissecting off a thin membrane from the outer circumference of the intestine of day-old mice; (3) dissecting off the epithelial layer from the cornea of adult mice (GAY and KAUFMANN 1950); (4) placing pieces of adult mouse intestine in fixative and mechanically stirring the fixative (the "spun tissue method"). The tissues in methods (1) and (4) were stained with aceto-carmine just before the cover glass was added, and in methods (2) and (3) the aceto-carmine was allowed to flow under the cover glass after the membranes were laid on the slide in fixative in an unfolded condition. The mechanical stirring referred to was achieved by spinning the tissue for 4 minutes in 1 cc of fixative with a gear wheel seven millimeters in diameter powered by a "Tiny Atom" motor with two 1½ volt flashlight batteries in series. Human endometrium, obtained during dilatation and curettage for metrorrhagia, was prepared by the "spinning method" and by the Feulgen squash technique.

All mitotic figures of sufficient clarity were drawn, and counts were made from the drawings. No revision of an initial count was made, but some of the figures were drawn separately by both authors to get some indication of the reliability of the counts.

RESULTS

Aceto-carmine squashes of the intestine of day-old mice (method 1) provided an abundance of easily-counted mitoses. Results (table 1 and fig. 1) ap-

TABLE 1

Chromosome counts from mouse and human tissues.

Number of chromosomes per group	Frequency of groups						
	Day-old mouse intestine			Adult mouse tissues			Human endometrium "spun" (4)
	Squash (1)[1]	Membrane (2)		Corneal epithelium (3)	Intestine, "spun" (4)		
		Original	Check[2]		Original	Check[2]	
1	5						
2	1						
3	1						
4	2						
5	3						
6	1						
7	1						
8	2						
9	3						
10	5						
11	3						
12	6						
13	3						
14	2						
15	3						
16	4						
17	4						
18	5						
19	4						
20	2						
21	1						
22	3						
23	3						
24							
25	1						
26							
27	1						
28	2						
29	3						
30	1	1					
31	1						
32		1					
33	3						
34	6	2				1	
35	9	2	1			1	
36	7	6	3	6	1		
37	4	9	2	5	6	1	
38	7	5	2	6	6	12	
39	4	11	6	7	8	6	
40	6	9	1	10	4	7	
41	3	7	3	2	5	4	
42	6	3	1	3	5	1	1
43		2					3
44	2	2	1		3	1	1
45		1		1	1		3
46	1	1			1		2
47							4
48		1					4
49							4
50							5
51							1
52							3
53							3
54							
55							1
56							1

[1]Numbers in brackets refer to methods as listed under Materials and Methods.

[2]Check counts by the junior author to give an estimate of the amount of variation which might be attributed to counting error (not included in graphs).

TABLE 1 (*continued*)

Number of chromosomes per group	Frequency of groups						
	Day-old mouse intestine			Adult mouse tissues			Human endometrium "spun" (4)
	Squash (1)[1]	Membrane (2)		Corneal epithelium (3)	Intestine, "spun" (4)		
		Original	Check[2]		Original	Check[2]	
57							
58							2
59							1
60							
61							
62							1
....							
75			1[3]				
Mean ± S.E.	24.5 ± 1.14	38.9 ± .39	38.8 ± .51	38.8 ± .31	39.7 ± .57	39.0 ± .32	49.5 ± .75
S.D.	13.2	3.1	2.2	2.0	3.6	1.9	4.7

[1]Numbers in brackets refer to methods as listed under Materials and Methods.
[2]Check counts by the junior author to give an estimate of the amount of variation which might be attributed to counting error (not included in graphs).
[3]Probable tetraploid cell; omitted from calculations.

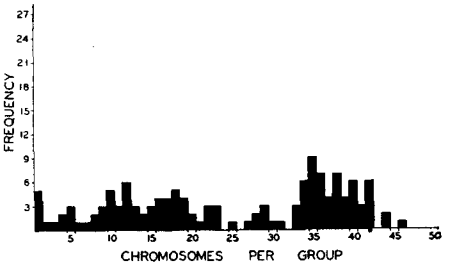

FIGURE 1.—Counts from day-old mouse intestine prepared by the squash method.

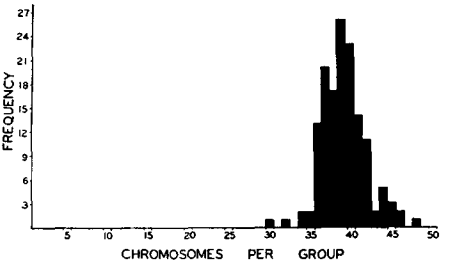

FIGURE 2.—Graph combining figs. 3, 4, and 5.

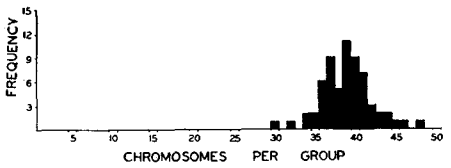

FIGURE 3.—Counts from mounts of day-old mouse intestinal membrane.

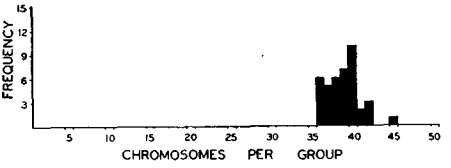

FIGURE 4.—Counts from mounts of adult mouse corneal epithelium.

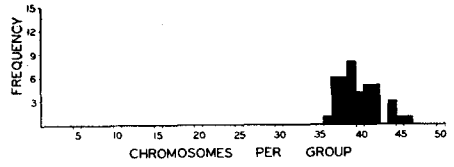

FIGURE 5.—Counts from adult mouse intestine prepared by the spinning method.

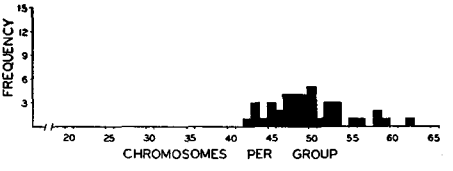

FIGURE 6.—Counts from human endometrium prepared by the spinning method.

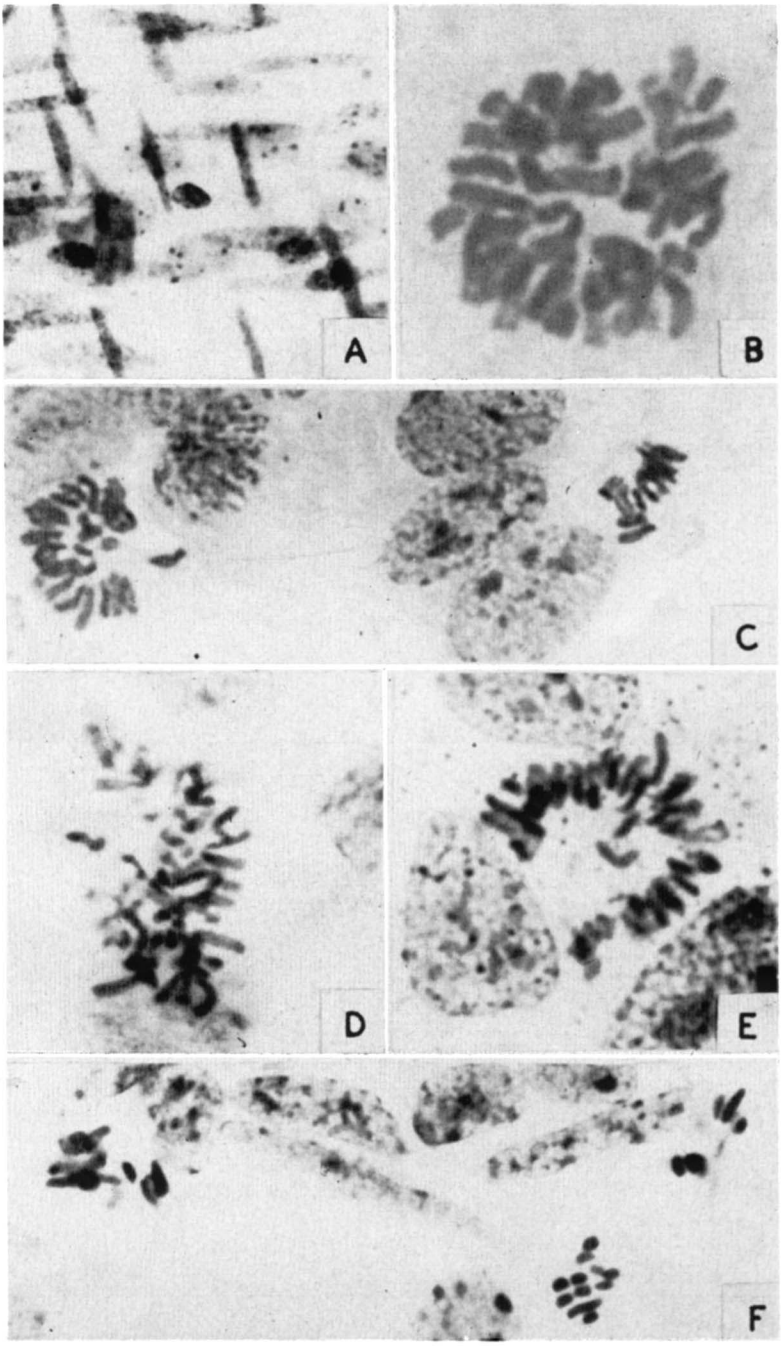

PLATE I

peared to indicate that a condition of aneuploidy existed in somatic tissues of mice similar to that reported for human tissue by THERMAN and TIMONEN.

However, as a precautionary measure, it seemed desirable to use some other method in which the possible factor of chromosome groups breaking apart during treatment could be better controlled. Consequently, flattened membranes were used. In the pieces of membrane taken from newborn mouse intestine (method 2), the cells away from the edges are intact, as can be deduced from the orientation of nuclei in plate I A. Counts of chromosome numbers made independently by the two authors in tissue prepared in this way gave results which differed strikingly from those obtained by the squash method (table 1 and fig. 3). In the membranes no figures were found with less than 30 chromosomes. Extending the study to adult mouse tissue, counts were made on sheets of corneal epithelium (method 3), and the deviation from diploid was found to be even less, with the counts ranging from 36 to 45 chromosomes per group (fig. 4).

The technique of dissecting off membranes cannot be applied to very many tissues, so a more widely applicable method was sought for preparing tissues without causing widespread cell breakage. The procedure of fragmenting tissue by agitation in fluid (method 4) produced remarkably little breakage of chromosome groups (plate I B) if treatment was stopped when small, but macroscopic, bits of tissue were abundant. These bits of tissue tend to flatten on the slide like miniature membranes, although this tendency varies with the type of tissue used. Among the 80 figures counted in " spun " material only three showed evidence of having been broken (plate I C). The counts obtained from spun adult mouse intestine were closely grouped around 40 (table 1 and fig. 5).

Because methods involving membranes and spun material gave different results from those obtained by squashing, it was decided that human endometrium should be investigated using the spinning method. The distribution of counts from endometrium is shown in table 1 and figure 6. There was a complete absence of small chromosome groups, and among the figures clear enough to count, (e.g., plate I D) none fell below 42.

PLATE I

A. Small portion of a mount of day-old mouse intestinal membrane showing the regular arrangement of oblong muscle nuclei. A few rounded mesothelial nuclei can also be seen. ca. 700 ×.

B. A group of approximately 39 chromosomes found in a spun preparation of adult mouse intestine. ca. 2600 ×.

C. Adjacent chromosome groups in adult mouse intestine prepared by the spinning method. The two chromosome groups (approximately 29 and 13 chromosomes) in this field are considered to have originated from one metaphase plate. ca. 1600 ×.

D. A chromosome group from human endometrium prepared by the spinning method. ca. 2000 ×.

E. A broken-ring metaphase plate from a squash preparation of day-old mouse intestine. ca. 2100 ×.

F. Small aggregations of chromosomes in close association in a squash preparation of day-old mouse intestine. ca. 1300 ×.

As a further check on the chromosome numbers in human tissue, endometrium was prepared by the Feulgen squash method as used by TIMONEN, with the difference that less pressure was used, to avoid scattering the chromosomes. This made accurate counts impossible, except in a few cases, but good estimates could usually be made. The minimum number of chromosomes was estimated for each of the first 50 metaphases found, classified simply as more than 40, more than 35, and less than 35 chromosomes. The following results were obtained: more than 40, 44 cells; more than 35, 4 cells; less than 35, 2 cells. Of the two which had less than 35 chromosomes, one cell was obviously broken and in the other the chromosomes were strung out. In twelve cells which were clear enough for counting, the counts ranged from 44 to 48 chromosomes per cell.

DISCUSSION

The squash technique, when used on mammalian somatic tissues, causes rupturing of chromosome groups, the percentage of cells ruptured being dependent in part on the particular squash technique used. The first evidence for dispersion was seen in the squash preparation itself. Table 2 shows counts on all closely associated chromosome groups, first on the assumption that they are true hypoploid groups and then assuming closely associated groups to be a single group which had been broken apart (e.g., plate I F). It is interesting to note how the latter assumption leads in most cases to counts approximating forty. TIMONEN (1950) said that, in their work, all obviously broken groups were bypassed, but they did not offer any criteria for identifying these obviously broken groups. The arrangement of the chromosomes immediately comes to mind as a possible method of determining the completeness of the group. A solid ring as compared to a broken ring in a metaphase plate would be an example. However, late prophases contribute many of the counts and they cannot be identified in this manner, nor can the metaphase itself if it is not oriented in a polar view. It is easy to understand why chromosome arrangement is not widely discussed in relation to making counts, since figures that are well enough spread to provide ease of counting are generally pushed out of shape, or broken open as with a broken-ring metaphase plate (e.g., plate I E). The new methods used in this study give a minimum of figure distortion, but do so at the expense of easy counting. The choice had to be made

TABLE 2

All closely associated chromosome groups from squashes of baby mouse intestine.

Counted as separate groups[1]	9	6	11	5	5	10	19	18	29	22	12
	2	35	12	4	4	15	1	16	13	20	16
	27		16	20	9	11	15	8			
				13	18	3	1				
							10				
Counted as one group	38	41	39	42	36	39	46	42	42	42	28

[1]**The vertical columns represent closely associated groups.**

between unbroken groups, and groups that were well enough spread to provide high accuracy of counting. It was decided that the risk of counting error was more acceptable than the risk of error from breakage. Independent counts on the same cells by both authors show that in most cases the error was small, usually less than three chromosomes.

The main evidence against the reliability of the squashing method comes from a comparison of the squash method with the other three methods used. The most accurate comparison would be between intestinal squash preparations on the one hand and intestinal membrane plus spun intestine on the other hand, since spun intestine included mainly the mucosa which separated more easily than the muscle layers. However, since no significant differences exist between the three new methods (see P values in table 3), they can be considered together. Table 3 shows that the mean from squash counts is very significantly different from the mean of the combined counts from the three other methods. The difference in results is quite apparent without any need to refer to statistical analysis, as a glance at figure 1 and figure 2 will show. Upon finding that 58 counts out of 134 fell below 20 with one method and that none of the 143 counts made with other methods fell below 30, the conclusion was reached

TABLE 3

Significance of the difference between means.

Items compared	d.f.	t	P
Squashed intestine with membranes and spun	275	11.6	<.001
Intestinal membrane with spun intestine	101	1.116	.3
Spun intestine with corneal epithelium	78	1.308	.2
Intestinal membrane with corneal epithelium	101	0.163	.9

that the squash technique is an unsatisfactory method for investigating variations in chromosome numbers. It can safely be concluded that there is no large and frequent deviation in chromosome number in the following mouse tissues: intestinal mesothelium, longitudinal and circular muscle layers of the intestine, intestinal mucosa and corneal epithelium.

Evidence now accumulated indicates that the aneuploidy reported by THERMAN and TIMONEN to occur in human endometrium was an artifact produced by their technique. Although they did not give actual frequencies, it was determined by measuring TIMONEN's (1950) frequency histograms that approximately 791 of his 1,000 counts were below 40 chromosomes (the intermode of their distribution) and 209 above. In the present study no counts of less than 40 were obtained in "spun" tissue. Comparing the results of the present investigation with those of THERMAN and TIMONEN on the basis of frequencies above and below 40 chromosomes, the chi square test gives a probability of $< .001$ ($\chi^2 = 124$, 1 d.f.) that the two samples originate from one population. The most reasonable conclusion, therefore, is that THERMAN and TIMONEN were counting broken chromosome groups. Their interpretation of variations in nuclear volume as evidence of corresponding variations in chromosome number also appears open to question, especially in the light of SCHRADER and

LEUCHTENBERGER's (1950) demonstration that in Arvelius different sized nuclei contain protein proportional to their volumes, but their desoxyribose nucleic acid content and chromosome number remain constant.

Although the high frequency of low hypoploid numbers obtained by the squash technique can be considered a product of the method, even the counts obtained from " spun " tissue and membranes were not uniformly diploid. The problem remains, therefore, of deciding whether the variation found represents nothing more than counting errors, or whether some variation in chromosome number actually exists. That counting errors occurred is certain. Counts made independently by the two authors on 35 of the same cells from " spun " mouse intestine differed, on the average, by 2.3 chromosomes per group. However, some real variation may also occur. HSU (1952), in his study of human cells in tissue culture, found some aneuploidy, although deviations from the diploid were much less frequent and less extreme than those reported by THERMAN and TIMONEN. Of 124 cells from spleen cultures which HSU studied, 91 were diploid, one was hypotetraploid, and the remaining 32 contained 44 to 47 chromosomes. His remarkably fine preparations leave little room for counting error, so that it may safely be stated that about a quarter of the cells deviated from the diploid by one to four chromosomes. HOWARD and SANDER (G. F. SANDER personal communication) found similar minor fluctuations in chromosome number in mouse cells.

Limitations of the squash technique should be recognized by workers using this method, and if the cells they are working on are tightly cemented together, possible fracturing of mitotic figures should be anticipated. For studies where this dispersion could be misleading, methods used here might provide successful alternatives. Membrane mounts give the most controlled results, but it is only occasionally that suitable membranes are available. The spinning method is undoubtedly more widely applicable.

SUMMARY

Chromosome counts performed on somatic tissue in the mouse showed wide deviations from diploid in the direction of hypoploidy when the tissue was prepared by the squash technique, but only slight deviations when the tissue was prepared by certain other methods. It was thus shown that the squash technique had broken apart many of the mitotic figures during the mounting of the tissue.

Hypoploidy from squashed human endometrium, as reported in the literature, was studied by another method, and again a discrepancy was found which indicated that the squash technique tended to break up chromosome groups.

It was concluded that in the mammalian somatic tissues studied by the more controlled methods, most of the deviations from diploid were due to counting error, although some of the minor deviations might have been due to aneuploidy.

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LITERATURE CITED

BOIVIN, A., R. VENDRELY and C. VENDRELY, 1948 L'Acide désoxyribonucléique du noyau cellulaire, dépositaire des caractéres héréditaires; arguments d'ordre analytique. Compt. rend. Acad. sci. **226:** 1061–1063.

BOOTHROYD, E. R., and B. E. WALKER, 1952 Somatic chromosome numbers in mice. Genetics **37:** 567.

GAY, HELEN, and B. P. KAUFMANN, 1950 The corneal epithelium as a source of mammalian somatic mitoses. Stain Tech. **25:** 209–216.

HSU, T. C., 1952 Mammalian chromosomes in vitro. I. The karyotype of man. J. Hered. **43:** 167–172.

HUSKINS, C. L., 1947 The subdivision of the chromosomes and their multiplication in non-dividing tissues: possible interpretations in terms of gene structure and gene action. Am. Nat. **81:** 401–434.

1948 Segregation and reduction in somatic tissues. I. Initial observations on *Allium cepa*. J. Hered. **39:** 311–325.

HUSKINS, C. L., and K. C. CHENG, 1950 Segregation and reduction in somatic tissues. IV. Reductional groupings induced in *Allium cepa* by low temperature. J. Hered. **41:** 13–18.

HUSKINS, C. L., and L. CHOUINARD, 1950 Somatic reduction: diploid and triploid roots and a diploid shoot from a tetraploid Rhoeo. Genetics **35:** 115.

MELANDER, Y., 1950 Chromosome behavior of a triploid adult rabbit. Hereditas **36:** 335–341.

MIRSKY, A. E., and H. RIS, 1949 Variable and constant components of chromosomes. Nature **163:** 666–667.

SCHRADER, F., and CECILIE LEUCHTENBERGER, 1950 A cytochemical analysis of the functional interrelations of various cell structures in *Arvelius albopunctatus* (De Geer). Exptl. Cell Research **1:** 421–452.

SWIFT, H. H., 1950 The desoxyribose nucleic acid content of animal nuclei. Physiol. Zool. **23:** 169–198.

THERMAN, EEVA, and S. TIMONEN, 1951 Inconstancy of the human somatic chromosome complement. Hereditas **37:** 266–279.

TIMONEN, S., 1950 Mitosis in normal endometrium and genital cancer. Acta obst. et gynec. Scandinav. **31** (Suppl. 2): 1–88.

TIMONEN, S., and EEVA THERMAN, 1950 Variation of the somatic chromosome number in man. Nature **166:** 995.

WILSON, G. B., 1945 The Venetian turpentine mounting medium. Stain Tech. **20:** 133–135.

WILSON, G. B., MARY E. HAWTHORNE and TEMAY TSOU, 1951 Spontaneous and induced variations in mitosis. J. Hered. **42:** 183–189.