susceptible to hemolysis by certain drugs suggests that the activity of this enzyme may be an important factor in the maintenance of the integrity of these cells.

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Three Chromosome Numbers in Whites and Japanese

Since Painter (*1*) reported in 1923 that the chromosome number in man is 48, this has been confirmed by a number of authors (*2*). This number (48) has had general acceptance except by the Japanese school, which has followed de Winiwarter and Oguma's report (*3*) that the Y-chromosome does not exist in man and that the total number of chromosomes in the male is 47. Recently a different number, 46, has been found by Tjio and Levan (*4*) in cultured tissues of four Swedish embryos. The same number of chromosomes (46) was discovered by Ford and Hamerton (*5*) in the testes of three Whites in England and by me and my coworkers (*6*) in the testes of Japanese. There is no doubt that this number (46) exists in man, but this is not the only possible number in the species; we (*6*) found, besides individuals with 46 chromosomes, some with 47 and others with 48 among Japanese.

A new group of Japanese was studied recently to extend the earlier investigation (*7*). A group of Whites was also studied to determine whether the same chromosomal variation exists in this ethnic group (*8*). The material examined consisted of tissue from testes of 15 Japanese (epididymitis patients), secured by biopsy, and testes from eight American Whites (prostate cancer patients), secured by total orchidectomy. Immediately upon removal, the specimens were pretreated with a mixture of equal volumes of 1-percent chromic acid and 3-percent potassium bichromate solutions for about 1½ hours. Specimens were then fixed with a mixture of equal volumes of 4.5-percent chromic acid and 1.5-percent potassium bichromate solutions for 17 to 20 hours. After being washed thoroughly in running water, they were stained by Feulgen's method and squashed. The pretreatment in this procedure facilitates the dispersion of the chromosomes in squashed metaphase cells. Some of the White testes showed slight fibrosis, but otherwise no testes showed indications of pathologic changes. At least 15 first meiotic metaphases and three or four spermatogonial metaphases, in which the chromosomes were dispersed well in the cell and could be observed clearly, were selected in each specimen. The number of chromosomes as well as the structure of individual chromosomes was carefully studied.

In nine of the 15 Japanese testes the spermatogonial metaphases showed 46 chromosomes, and the first meiotic metaphases showed consistently the heteromorphic X-Y pair and 22 paired autosomes. While the autosomal pairs always formed tetrads, the sex chromosomes were separate from each other in 40 percent of the first metaphases. The pairing irregularity of the X-Y pair was found to occur also in the other six Japanese and the eight White testes.

In one of the 15 Japanese testes, the number of chromosomes was found to be consistently 47 in the spermatogonia and first meiotic metaphases. In the latter metaphases a small univalent was always present in addition to the X-Y and the 22 autosomal bivalents. There was no indication that the univalent chromosome was produced by fragmentation of one of the sex chromosomes or of one of the autosomes. It is an intact chromosome with its own centromere. The X-Y and the 22 autosomal bivalents in this testis were compared with those in the nine testes with 46 chromosomes, and all chromosomes were found to match well in size and shape. This indicated that the univalent chromosome in the testis with 47 chromosomes is an extra element present in addition to the 23 pairs that constitute the regular complement in 46- and 47-chromosome individuals.

In the remaining five of the 15 Japanese testes, 48 chromosomes were found in spermatogonial metaphases, and the heteromorphic X-Y pair and the 23 homomorphic bivalents were found in all first meiotic metaphases. The matching of these bivalents with those of the 47-chromosome individuals indicated that one of the 23 bivalents corresponds to the univalent chromosome of the latter individual. Evidently the extra chromosome present singly in the 47-chromosome individual is duplicated in individuals of the 48-chromosome type. Thus, the Japanese comprise individuals of three different chromosomal constitutions, and the differentiating factor is the chromosome which occurs singly in some individuals but as a duplicate in others. This chromosome appears to be a supernumerary chromosome.

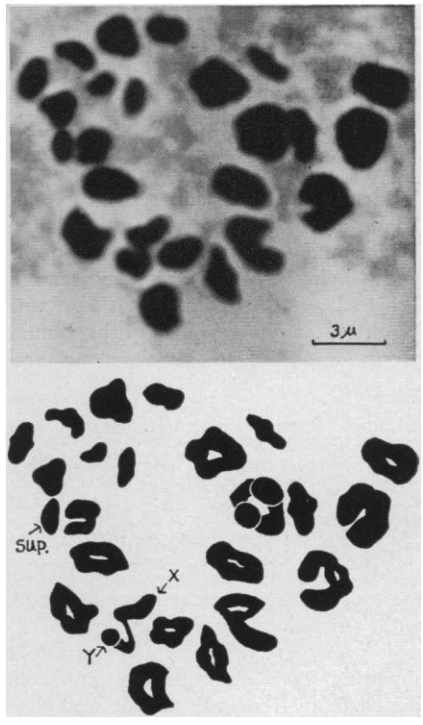

Fig. 1. A first meiotic metaphase of a White male with 48 chromosomes. Note the X-Y pair, the 22 autosomal pairs, and a bivalent supernumerary chromosome (*sup.*).

In seven of the eight White testes, 46 chromosomes were present consistently in all spermatogonial and primary spermatocyte metaphases. In size and shape the individual chromosomes were essentially the same as the 46 chromosomes in Japanese of the same type. In the remaining one of the White testes, 48 chromosomes were found in spermatogonial metaphases and the X-Y and the 23 bivalents were found in all first meiotic metaphases (Fig. 1). In Fig. 1 (top). the supernumerary bivalent appears to be connected to the adjacent bivalents. However, extensive observations of both Japanese and White materials have never indicated any tendency for the super-

numeraries to associate with any particular chromosome. In this case, then, the apparent connection is probably an artifact. These meiotic chromosomes were found to match well with those of Japanese of the 48-chromosome type. Evidently the same supernumerary and 23 regular pairs are present in these Whites and Japanese. The finding of 46- and 48-chromosome individuals among Whites leaves little doubt that men with 47 chromosomes exist also in this human group. Thus, presumably the same three chromosomal constitutions exist in the two ethnic groups.

The present study has shown that the human supernumerary chromosome has the following characteristics: (i) It is a metacentric chromosome with the centromere located near its middle; (ii) its size is approximately that of the Y-chromosome; (iii) it never pairs or associates with any other chromosome except its own homolog; (iv) at metaphase I, two supernumerary chromosomes are conjoined more frequently at one arm than at two arms. In either case the attachment of the arms is always completely terminal, and the attachment region is sometimes strikingly attenuated. A similar manner of pairing is observed in the X-Y pair but not in other autosomes.

Since 1956, 15 individuals have been reported to have 46 chromosomes, including the recent one studied by Bender (*9*) and the seven described here. The White individual with 48 chromosomes in our sample is the only one with this number established since 1956. The ratio of the frequency of the 46- and 48-chromosome types in the present Japanese sample is 9:5 (a previously reported ratio, 4:16, was not based on a random sample). The numbers of Whites and Japanese studied so far are too small to provide the basis of reliable estimates of the frequencies of the three karyotypes in the two ethnic groups.

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Continuous Measurement of the Depth of Sleep

Electroencephalographic records characterizing levels of natural sleep in human beings (*1, 2*) appear to be marked by a diminution of the number of brain-wave peaks during the passage from wakefulness to deepest sleep. The present report deals with the validation of this observation.

Bickford (*3*) has used the integrated energy of the electroencephalographic output to control the level of anesthesia, the energy decreasing with the depth of narcosis. Forbes *et al.* (*4*) have measured the depth of barbiturate narcosis, in animals already lightly anesthetized, by counting manually the number of brain waves that exceed one-third the maximum amplitude in successive 40-second intervals. This brain-wave count appeared to measure the absolute depth of narcosis more reliably than did the Bickford energy record.

The measurement described in the present report differs from that of Forbes *et al.* in employing nonnarcotized human beings; in producing a continuous 12 in./hr record of the electroencephalographic frequency during sleep; and in obviating the need for knowledge of the eventual maximum amplitude of the brain-wave potentials. The use of the energy-output method would probably fail during natural sleep because such electroencephalographic records do not reveal a proportionality between energy output and depth of sleep.

The brain-wave potentials during sleep are obtained from two needle electrodes, placed ipsilaterally over the occipital and frontal areas of the scalp, with a third silver disc electrode attached to the ear lobe on the same side and grounded. This electrode placement gives maximum alpha and delta waves.

The potentials were amplified by a Type 122 Tektronix low-level preamplifier, the low impedance output being fed into an electrocardiograph, whose "voltage gain" was set so that the maximum amplitude of the alpha rhythm in the waking state was 7 volts. This amplified signal then passed into a Schmidt trigger circuit set to pass only positive-going pulses greater than 2 volts. The square-wave output of the Schmidt circuit actuated an electronic counter and rate transducer (*5*) to produce, finally, a record of frequency versus time on a Leeds and Northrup Speedomax recorder. The frequency scale was set to cover the range from 3 to 16 cycles per second on the 10 in. recorder paper; the relationship between millivolts and the reciprocal of frequency is linear. The electronic counter keyed a relay every 32 counts; the transducer thus produced a rate based on the mean of successive trains of 32 positive-going waves of greater than 2 volts amplitude.

If the amplified output of the alpha rhythm is set lower than 7 volts or if the trigger threshold is set higher than 2 volts, the so-called transition or "B" stage of sleep (*2*), a relatively fast rhythm of low amplitude, will appear to be of low frequency and will consequently be interpreted as a deeper stage of sleep. There is no reason to believe that marked differences will occur if this ratio is varied between 3:1 and 4:1.

The use of the electroencephalographic frequency as a continuous measure of the depth of sleep was validated as follows: At intervals of 8 to 10 minutes throughout the night a 30-second written record of the electroencephalographic potentials was obtained on the strip chart of the electrocardiograph; the time at which this record was taken was signal-marked on the continuous electroencephalographic frequency recording. This record was then cut in half, and each 15-second strip was randomly numbered; at the end of the night, 100 such strips had been accumulated. These strips were then shuffled so that their subsequent order would differ from the order in which they had been obtained. Three judges then independently evaluated each record on a scale of 4, zero being the waking state and 1, 2, and 3 being light, moderate, and deep sleep, respectively. Prior to classifying the records, each judge was shown sample sleep records from the electroencephalographic literature. After 24 hours or more had elapsed the judges again evaluated the same records after they had been shuffled into a different order. Thus, each 30-second record received six scores on each of two occasions. The phi correlation coefficient was computed for the means of the first and second judgments and in three different subjects was found to be 0.88, 0.88 and 1.00, respectively; in the first two cases this particular correlation coefficient probably indicated too low a degree of reliability of the judgments. The phi correlation coefficient was then computed as a validity coefficient for the correspondence between the mean of the 12 judgments of each record and the corresponding electroencephalographic frequency. For the two males and one female of this study, this coefficient was 0.76, 0.75, and 0.91, respectively. Although highly significant, this coefficient probably indicates too low a degree of reliability.