Human Chromosomes — Down's Disorder and the Binder's

by Daniel J. Kevles

This article, part of which appeared originally in The New Yorker, *is drawn from Kevles's new book,* In the Name of Eugenics: Genetics and the Uses of Human Heredity, *which was published this month by Alfred A. Knopf, Inc. As the "first book to deal seriously and objectively with the development of human genetics as a scientific and medical discipline," Kevles's account of the application of heredity theories to "improving" the human race also examines the controversial social, moral, and political issues that descended from it — from the origins of eugenics in the late 19th century up to the present.*

In August 1955, Joe-Hin Tjio, a young Indonesian who was then working in Zaragoza, Spain, came to Lund, Sweden, for one of his periodic collaborations with Albert Levan. Both were primarily plant cytologists, but now their attention was turned to the chromosomes in the human cell. The nucleus of the normal human cell contains two sex-determining chromosomes — *XX* for females and *XY* for males — plus 22 pairs of autosomes — that is, chromosomes unrelated to sex. The total comes to 46. That fundamental number of human cytogenetics was established by Tjio and Levan during Tjio's visit in 1955 — long after cytologists had started counting the chromosomes of man in the 1890s.

The very early counts had yielded numbers that varied around 24, which was consistent with those obtained for other mammals. The trouble then was that cytologists made their counts with tissue taken from corpses, often those of executed criminals; upon the death of mammalian cells, the chromosomes tend to clump together rapidly, thus deceiving even the microscope-aided eye into falsely low counts. Recognizing the problem, the Belgian cytologist Hans von Winiwarter used fresh tissue obtained during surgery and immediately fixed with a chemical preparation. In 1912, he reported the human chromosome number to be 47 for males and 48 for females. Von Winiwarter explained the sexual difference by arguing that while the human female had two sex chromosomes — a double *X* — the human male must have only one, a single *X*.

Von Winiwarter's result, neither confirmed nor rejected, was evidently regarded as an anomaly by most cytologists, but at the beginning of the 1920s his use of fresh tissue caught the attention of Theophilus S. Painter, a cytogeneticist at the University of Texas.

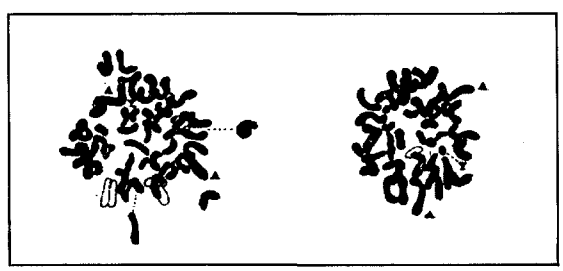
Mistakes

One of Painter's former students happened to be practicing medicine at the state mental institution in Austin. Painter obtained the testes from three patients — one white, two black — all of them castrated, Painter reported, because of "excessive self-abuse coupled with certain phases of insanity." Within a few minutes of their removal from the blood supply, the specimens were slit into multiple sections and dropped into a fixing solution. In mid-1921, Painter reported to a colleague that "my best counts now give me 48 chromosomes for both the Negro and white man. . .and [I] feel confident that this is correct." Perhaps his confidence derived from the fact that the figure squared with von Winiwarter's for females. More important, as in other mammals, the total included the male sex-chromosome combination, *X* and *Y*. It was also consistent with his counts in spermatocytes, which, as the products of sexual division, should have contained half the number in non-sex cells, and, so far as Painter saw, did have 24. After Painter published a full report of his work in 1923, other cytogeneticists confirmed his count. For the next 30 years, just about everyone believed the human chromosome number to be 48, for both sexes.

In retrospect, the reasons for the persistent miscounting are clear enough. Normally, the chromosomes lie in a region of the cell nucleus that takes on a deep color upon staining. In the quiescent cell, the individual chromosomes cannot be visually differentiated from the region. They can only be seen — and counted — in the process of cell division, when they emerge as separate, colored — hence the name — rodlike entities. To obtain a chromosome count, human cells had to be captured and fixed at the moment of division. The more cells in a state of division, the better the prospect for chromosomal observations. Particularly suitable were tissues with rapidly proliferating cells, notably embryos or testes, which are sites of constant cellular division.

Such material, obtained fresh from living bodies, was, to say the least, difficult to come by. Many more human chromosome counts seem to have been done with testes than with ovaries for the simple reason that the taking of ovarian tissue required a major surgical procedure. The human cytogeneticist often had to wait, ready to fix his specimens, outside operating rooms or, in the case of a team that confirmed Painter's count, literally at the foot of the gallows. Once obtained and fixed, the specimens were sliced into thin sections with a fine blade — the blade cutting through the nucleus of a given cell as a knife might cut through an egg in the middle of a meat loaf. Just as successive sections of meat loaf would contain successive slices of egg, successive slices of cell — perhaps two or three — would include serial slices of the complete nucleus. Since the chromosomes were spread through the nucleus, some would wind up in one section, some in the next. The cytologist added the number found in each section to reach the total in the cell. But because of imprecision in where the blade happened to cut, fragments of a chromosome located — and already counted — in one section might turn up as candidates for counting in the next. Then, too, compared to fruit flies, which have four pairs of chromosomes, the human cell nucleus is small and the number of chromosomes large. Even when separated and fixed during cell division, human chromosomes are crowded together. They appeared to cytologists of Painter's era as something like the noodles suspended in a soup — some lying beneath others and difficult to count accurately. It was not easy to decide whether the noodle that resembled an "L" under the microscope was a single bent chromosome or two straight ones.

The cytologist Tao-Chiuh Hsu, who once saw a slide of one of the human testicular sections that Painter had prepared, later wrote:



In the early 1920s Painter found 48 chromosomes in each of these cells — a number that remained unchallenged for 30 years.

"I failed to make any sense of the twisted, crowded, stacked chromosomes. It's amazing that [Painter] even came close!" Every enumeration of human chromosomes required judgment, and judgment left room for conformation to orthodoxy. Human chromosomal counts sometimes suggested a figure different from 48, but most cytologists, expecting to detect Painter's number, virtually always did so. Indeed, the preconception in favor of 48 was so powerful that it operated on Hsu himself when, in 1952, he set off the train of experimental work that led to the revision down to 46.

Hsu had come from Chekiang University, in China, in 1948 to take a PhD at the University of Texas; now a postdoctoral fellow in human cytology at the medical branch of the university in Galveston, he was looking at cell nuclei in preparations of fetal spleen tissue. It was with distinct incredulity, Hsu recalled, that he saw in one of the preparations "some beautifully scattered chromosomes." Similar pretty pictures appeared in other slides, but when he examined additional preparations, the chromosomes "resumed their normal miserable appearance." Hsu guessed that something about the original preparations must have been special. For some months, he sought assiduously to find out what. There was no need for him to hover outside some operating-room door to obtain fresh spleen cells. Plenty were available because the original sample had been subjected to tissue culture — the technique by which cells are kept alive and multiplying in vitro with suitable nutrients. Tissue culture had come into use in cytology laboratories after the Second World War, and it provided a continuous supply of dividing cells. Hsu systematically altered the preparation procedure of one sample after another of the abundant embryonic spleen cells. Nothing worked until April 1952, when he added distilled water to the balanced salt solution commonly used to rinse the tissue specimens before fixation.

This so-called hypotonic solution liberated the chromosomes from the cell spindle — a warp of fibers that form during cell division to guide them on their journey — and it also swelled the cell volume, which allowed the chromosomes more room to separate. Hsu guessed that the preparations in which he had seen the chromosomes so clearly must have been accidentally washed in hypotonic solution before being fixed. Turning accident to advantage, he proceeded to look closely at the human chromosomes — not to check the number but to examine their structure. In many cells, he recalled with some irony, "I had difficulty in getting the count to equal 48." Nevertheless, his vision filtered through the prevailing preconception, Hsu managed to count to Painter's figure. He later confessed to feeling like a football player who returns an interception 40 yards only to find himself "fumbling the ball at the three-yard line."

Hsu's metaphor did him a disservice; at the time, he did not know that he was in a contest with nature for the correct human chromosomal count. Neither, three years later, did Tjio and Levan when they found the right number: Their aim had been to explore in detail the morphology of human chromosomes in lung tissue taken from legally aborted embryos. The difference between their work and that of all previous analysts of human chromosomes was its reliance not only on tissue culture and hypotonic treatment but on two other techniques newly deployed in human cytology. One was the pre-treatment of the cells with colchicine, an alkaloid extracted from the seeds of a crocus-like herb. Colchicine arrests cell division midway through its course, thus providing many more cells to be observed in the process of splitting. It does so in a way that further frees the chromosomes to disperse throughout the cellular volume. And it tends to contract chromosomal size, thus diminishing the likelihood of confusing overlaps. The other was the "squash technique," so named because, instead of being sectioned, the cells to be examined were literally squashed with the thumb under a thin glass plate. With the cell thus flattened into something resembling a pancake, the chromosomes are spread onto a single plane of optical focus. Once Tjio and Levan applied all four techniques in combination to their embryonic lung cells, they immediately saw an unambiguous 46 human chromosomes. Further experiments in the fall and winter of 1955 yielded the same count with high consistency, and in 1956 they published their results, though not without residual anxiety about challenging Painter's much-confirmed number.

Within days of its publication, Tjio and Levan's article was read in England by Charles E. Ford, a cytogeneticist in a radiobiological research unit of the Medical

Research Council located at the British Atomic Energy Research Establishment at Harwell, near Oxford. In connection with studies in leukemia, Ford had worked with mouse and, recently, human cytogenetics. Already adept at the essential techniques of the field, he had in fact helped alert Tjio and Levan to the value of treating specimens with colchicine and hypotonic solution. An Oxford University surgeon, impressed with the clarity of Ford's cytological preparations, had offered to send human testicular material for chromosomal analysis. Ford had passed up the opportunity and, as he read Tjio and Levan, wished he had not. Now Ford and John Hamerton, a colleague at Harwell, swiftly confirmed the count of 46, using fresh human tissue supplied by the Oxford surgeon. The work brought Ford to the attention of the human geneticists in London, where interest in human cytogenetics was rising rapidly.

Among those concerned with the subject was Paul E. Polani, a physician at Guy's Hospital on the south side of the Thames, on a sight line from St. Paul's Cathedral. Polani had started in genetics during his undergraduate days in Italy just before the Second World War, and from 1948 to 1950, while on a fellowship, he had spent part of his time at the Galton Laboratory, which was part of University College London and was one of the leading centers in the world of work in human genetics. In 1954, in the course of his research on the causes of congenital heart disease, Polani came across three women who suffered from an aortal defect usually found among males but who also had Turner's syndrome, a condition found almost exclusively among females. Given the characteristics of Turner's syndrome — a thick, webbed neck, shortness of stature, and, especially, rudimentary ovarian and mammary development — Polani wondered whether the Turner's patients might genetically resemble males. At this time, indications of human genetic sex were beginning to be obtained by using the 1949 discovery of Murray L. Barr, a cytologist at the University of Western Ontario: routine staining revealed a small satellite (eventually called a "Barr body") near the nucleolus in the cells of females but not usually of males. Females were thus classified as "chromatin positive," males as "chromatin negative." Polani tested his Turner's females and found that all three were chromatin negative.

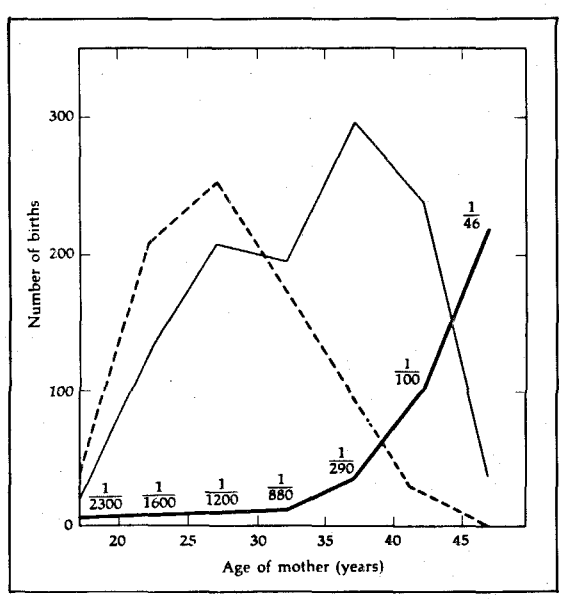
This outcome stimulated Polani to further research into human "intersexes" — people of one sex who displayed some characteristics of the other — and he gathered information on 25 more women, about half with Turner's syndrome and the rest with simply no ovarian development. He found 20 of the 25 to be chromatin negative. There was, however, scientific doubt that chromatin negativity could be taken as a definite sign of genetic maleness, particularly among abnormal human beings. Pondering how alternatively to determine the genetic sex of the women, Polani hit upon the ingenious idea of surveying them for a sex-linked trait. Following a discussion of the matter with Lionel S. Penrose, the head of the Galton Laboratory, he resolved to test them for the predominantly male trait of red-green color blindness. He observed this trait in 4 out of the 25 women — a frequency significantly higher than expectation in such a group of genetic females, but one consistent with expectation in a comparably sized sample of genetic males. In his report of these results in *The Lancet*, in July 1956, Polani suggested that the Turner's women might be chromosomally *XO* — that is, might have only one *X* chromosome, instead of the normal female's two.

Polani enlarged his work on color blindness in the human intersexes to include males with Klinefelter's syndrome — a condition with the symptoms of tallness, minor mammary development, and, often, testicular atrophy and mild mental deficiency. Barr and a colleague had just found that Klinefelter's males were chromatin positive — that is, they displayed the nuclear staining feature characteristic of normal females. In October 1958, Polani reported that color blindness occurred among such Klinefelter's with a frequency characteristically observed among females, and he suggested that, like females, Klinefelter's males must have two *X* chromosomes. The question was whether they had a *Y* chromosome, too. There was no way to determine the answer without looking directly at the karyotypes — the word comes from *karyon*, the Greek for "kernel," and signifies the display of chromosomes in the cell nucleus.

In 1955, Polani had tried to determine the genetic sex of a few of his Turner's patients by looking at their karyotypes with the aid of Gordon Thomas, an anatomist at Guy's Hospital who knew how to do tissue cultures. Inexperienced at working with human chro-

mosomes, they obtained — from three Turner's women and seven normal people used as controls — only a handful of complete cell samples, and none of sufficient quality to assess what sex chromosomes the cells contained. (They did manage to count 45 chromosomes in one of the karyotypes but mistrusted the result, partly because the number did not square with the prevailing belief in a normal total of 48 chromosomes, even if the cell was one *X* chromosome short.) In February 1956, Polani attempted to persuade a practiced cytogeneticist to help him; the man declined because he was unconvinced by Polani's arguments that the Turner's women might be *XO*. But in the fall of 1958, now eager to examine the karyotypes of Klinefelter's males, Polani turned with success to Charles Ford, whom he had met the year before at a conference on sex and the cell nucleus at King's College Hospital, in London.

Ford had recently perfected a method for treating bone marrow — another source of rapidly proliferating cells — in a way that yielded a large number of cells in a state of mitosis within a matter of hours. The method reduced to virtually nil a then-presumed risk of long-term tissue culture: that it could result in chromosomal changes of a misleading kind because they occurred not in the body but in the process of cell division in the culture itself. Early in 1958, Ford had used the bone-marrow technique to scrutinize a Klinefelter's karyotype in collaboration with Lazlo G. Lajtha, a hematologist at the Churchill Hospital, Oxford, and Patricia A. Jacobs, a young cytogeneticist from Edinburgh who had come to Harwell for a few months to learn the techniques of bone-marrow preparation. They had counted 46 chromosomes, including two *X*'s, which was consistent with the chromatin-positive reading characteristic of females. They had not found a *Y* chromosome. Even though the Klinefelter's was an apparent male, this was no surprise at the time. Fruit flies with an *XO* complement of sex chromosomes were males, while those with an *XXY* complement were females. The prevailing extrapolation from these data had it that the *Y* sex chromosome played no role in the determination of maleness, even in human beings. Still, the examination of one Klinefelter's karyotype hardly settled the matter, and late in 1958 Polani sent a sample of Klinefelter's bone marrow for analysis to Ford at Harwell.



Penrose determined in the 1930s that the incidence of Down's syndrome (represented here by the thick line) was a function of the mother's age. The dashed line plots total number of births (in thousands), and the thin solid line represents the number of babies born with Down's syndrome.

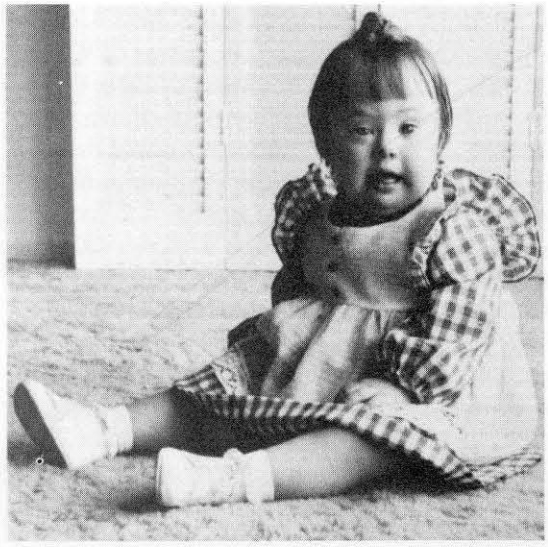
Unknown to Ford, the chromosomes of a Klinefelter's male had been under scrutiny in Edinburgh since the early summer by Patricia Jacobs and John A. Strong, a local physician. Jacobs had returned to her Medical Research Council Unit, which specialized in radiation genetics and where she had been examining the karyotypes of human beings with radiation-induced leukemias. Unable to find more than a few such people, Jacobs had decided to apply her newly mastered bone-marrow techniques in a resumption of the Klinefelter's work she had begun with Ford. Though she did not at first believe what the Klinefelter's karyotype revealed, Jacobs was compelled to the identical conclusion that Ford at Harwell, still ignorant of her investigations, reached when he scrutinized the sample from Polani: The Klinefelter's male karyotype contained not two but three sex chromosomes — two *X*'s plus the *Y* of the normal male. Jacobs and Strong published their results in January 1959. At the time, as Lionel Penrose later wrote to his long-time friend J. B. S. Haldane, the discovery of the extra Klinefelter's chromosome "astonished everyone." Not the least astonishing feature of the new knowledge was that human beings differed from fruit flies in the role played by their sex chromosomes: In *Homo sapiens*, the *Y* determined maleness, even if in *Drosophila* it did not.

The Klinefelter's results set Penrose to thinking about a subject that had long interested him — the disease then termed mongolian imbecility. The first systematic identification of the disease had been made in 1866 by the British physician John Langdon

Haydon Down. Down described a syndrome that, along with severe retardation, included an enlarged head and a prolonged, or epicanthic, fold to the eyelid; often there was also a fissured tongue and the so-called simian crease, a pronounced transverse palmar line. In Down's time, Western physicians had observed the syndrome only in Caucasians. Down supposed that the disease indicated a biological reversion in its victims to the Mongols of Asia, whom he thought they physically resembled, and who he assumed were a surviving example of an earlier human type. Down believed the disease to be congenital rather than hereditary, and he speculated that the reversion might be caused by parental tuberculosis.

The tubercular explanation was, of course, wrong, and so were others of a similar shot-in-the-dark nature advanced in the early 20th century. In the 1930s, Penrose demonstrated conclusively that the probability of the birth of a child with Down's syndrome depended strongly upon the age of the mother, with the probability rising rapidly after the age of 35. However, the physical cause of the disease still remained entirely unknown. Early in the 30s, the Dutch physician P. J. Waardenburg and the St. Louis pediatrician Adrien Bleyer independently suggested that Down's syndrome might be the product of a chromosomal anomaly, and by the end of the decade Penrose had come to embrace the suspicion. In 1952, at his urging, Ursula Mittwoch, a member of the Galton staff, scrutinized the sex-cell karyotype of a Down's male. Though inexperienced at cytology, she managed to count 24 chromosomes, half of the 48 that one would then expect to find in a normal cell after meiotic division — which implied that Down's syndrome was not the result of a chromosomal disorder. For Penrose, the Klinefelter's results reopened the question. Penrose knew of a Klinefelter's Down's at the Harperbury Hospital, identified in a search he had initiated there in the fall of 1958 for chromatin-positive males and chromatin-negative females. In his letter to Haldane a few months later, Penrose recounted, "Naturally, I wanted at once to try our luck with the Klinefelter mongol."

Charles Ford was ready and eager to do the karyotype analysis, but it took time to get the relatives' consent for the removal under anesthetic of the bone-marrow cells. Then, for three weeks or so from late February 1959, a virulent Asian flu epidemic completely tied up the hospital facilities. In the meantime, reports filtered into England that Jérôme Lejeune, a young French human geneticist, had learned something of consequence about Down's syndrome karyotypes.



Characteristic of Down's syndrome are the eye folds that led early researchers to label the condition "Mongolism." (Photo courtesy of the Oregon Health Sciences University.)

LEJEUNE'S CAREER IN genetics started in 1952, when, as a recent graduate in medicine, he returned from military service to work with Raymond Turpin at the Hospital Saint-Louis, in Paris. Turpin, a professor of pediatrics at the University of Paris, was one of the very few people in France at the time interested in human genetics. His hospital practice included a group of Down's syndrome patients, and he turned over responsibility for them to Lejeune. Neither Turpin nor Lejeune believed John Langdon Down's original hypothesis that victims of the condition were throwbacks to some atavistic Mongolian "race." In his clinical work, Lejeune saw a Down's child from Indochina whose appearance differed sharply from that of normal children of the region; the syndrome stood out among Orientals as well as among Caucasians. Lejeune suspected that Down's syndrome had something to do with hereditary mechanisms. Like a number of physicians elsewhere confronted with such inklings, he embarked on a postmedical course of study toward a doctorate in science with emphasis on biochemistry and genetics. Postwar French austerity made the task of research less straightforward: Lejeune had no laboratory, no microscope, only a single room without running water. Pondering what experimental research he might pursue under those conditions, he decided to concentrate on the palm prints of Down's victims.

In 1953, Lejeune scrutinized the configurations of lines on the palms of 93 Down's patients, 246 members of their families, and two large control groups drawn at random — except that one group was evenly divided for sex — from the Parisian population. Lejeune assessed the configurations quantitatively and arrived at a numerical index of the degree to which, on a given palm, they occurred in association with each other. He found that the Down's patients had a strikingly higher associative frequency of abnormal palm lines than did the people in either of the control groups. To Lejeune, this signified that Down's syndrome must involve some deep genetic change from the normal. Lejeune knew very little about primatology, but it occurred to him that a clue to the deep change might be found in the palm configurations of apes and monkeys — especially the lower-order monkeys from which the simian crease, that frequent palmar characteristic of the syndrome, took its name.

At the Natural History Museum in Paris, he measured the configuration of palm lines on the skins of the apes and monkeys preserved there. The palm lines of normal human beings showed no resemblance to those of either the lower-order monkeys or the anthropoid apes — orangutans, gorillas, and chimpanzees. But there were extraordinary similarities between the Down's palms and those of the inferior monkeys — for example, mangabeys and macaques. Lejeune supposed that the distinction between the palm lines of anthropoid apes and those of the lower-order monkeys must have resulted from the accumulation of numerous single-gene changes over evolutionary time. He speculated that the Down's palm lines, too, must arise from a polygenic difference between the Down's victims and normal human beings — occurring, obviously, not over evolutionary time but in one generation, from parent to child. Lejeune reasoned that the necessary change had to involve the only genetic material then known to be large enough to carry a polygenic message — a chromosome.

At this point, Lejeune's mind turned to the haplo-four fruit fly. (Cytogeneticists designate as "haploid" those cells — for example, mammalian gametes — that contain only half the normal number of chromosomes. The haplo-four takes its name from the fact that it possesses only one member of the fourth chromosomal pair found in normal *Drosophila*.) The haplo-four fruit fly has various abnormal characteristics, including thinner bristles, a shortened body, and a prolonged larval stage. No one of these characteristics announces the haplo-four; they declare themselves as an ensemble — a syndrome. Lejeune thought of the haplo-four as a kind of "mongol fly." Just as the "mongol fly" was missing a chromosome, Lejeune came to think, in 1954, that the victims of Down's syndrome must lack a chromosome, too.

Lejeune had by this time moved with Turpin's group to the Hospital Trousseau. He wanted to look at the chromosomes of his Down's patients, but he was not familiar with human cytogenetic techniques and was unable to find anyone in Paris who was. Besides, there was not much money for research and only limited laboratory facilities at the hospital. He therefore turned to various other subjects — mainly radiation genetics, for which Turpin, like many biologists, was able to raise funds in the mid-50s. All the while, however, he had his chromosomal hypothesis in mind and kept hoping to test it, especially after the work of Tjio and Levan was published.

The opportunity arose in 1957, with the arrival in Turpin's clinic of Marthe Gauthier, a cardiologist who had recently learned the technique of tissue culture; Turpin authorized her to use it in collaboration with Lejeune. Sometime about the spring of 1958, Gauthier cultured tissue taken from the fascia lata — the smooth connective tissue that covers muscle — of three Down's patients at the Hospital Trousseau. Lejeune, using the newly developed cytogenetic techniques, prepared karyotypes and examined them through a microscope discarded by the hospital's bacteriology laboratory; it was so worn that he had to stabilize its adjustment gears by inserting between them a piece of tinfoil from a candy wrapper. He photographed the karyotypes with equipment borrowed from the pathology department, expecting them to show, like those of the "mongol fly," the absence of a chromosome. Instead, they showed that the Down's patients had 47 chromosomes rather than the 46.

Lejeune wondered whether the extra chromosome was typical of the Down's patients or an artifact of the tissue culturing. Aging cultures were known to produce chromo-

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Human Chromosomes

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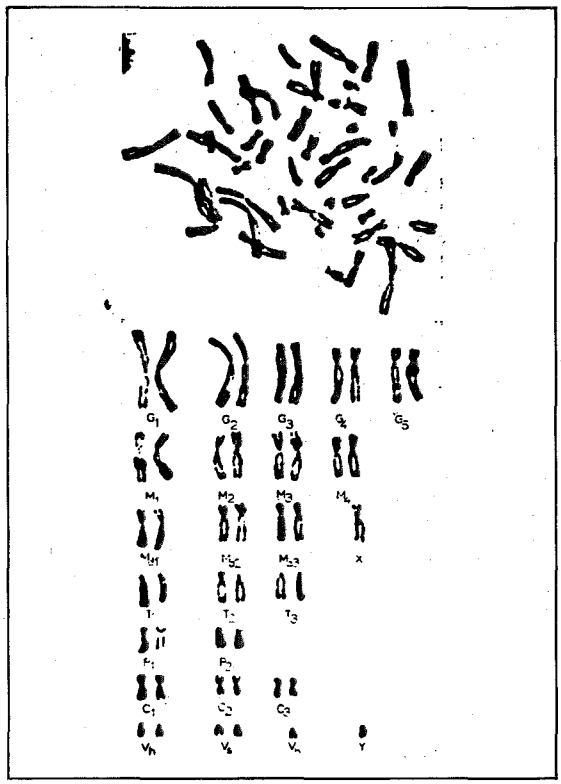

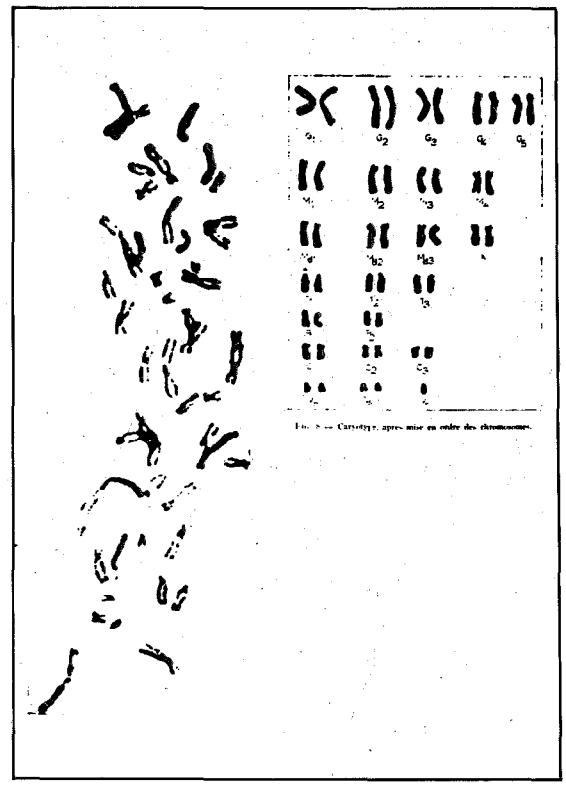

Published in 1959, two of Lejeune's Down's karyotypes (before and after ordering) show the extra chromosome in both the male (left) and female.

somal anomalies. But the cultures had been no more than a month old before he obtained the karyotypes — too short a time, Lejeune thought, for the aging phenomenon to occur. More troubling to him was a recent paper by Masuo Kodani, an American cytogeneticist then working with the Atomic Bomb Casualty Commission in Japan, claiming that in some normal human beings the chromosome number might be 47. If Kodani was correct, then the "extra" chromosome Lejeune had detected in his patients might not be extra at all and might have nothing to do with Down's syndrome. In a lecture at McGill University in September 1958, just after the Tenth International Congress of Genetics, in Montreal, Lejeune swallowed his doubts enough to show the photographs of the three Down's karyotypes and advance his belief that the cause of the syndrome was an extra chromosome. His audience seemed for the most part unconvinced.

After he returned to Paris, Lejeune prepared karyotypes of cells from eight non-Down's patients at the Hospital Trousseau. Each of the karyotypes showed 46 chromosomes. Though still somewhat anxious about putting his Down's results into print, he finally published the work in the *Comptes Rendus* of the French Academy of Sciences in January 1959. In the same journal, in mid-March, he reported the results of an examination of nine Down's karyotypes and argued with greater confidence that the extra chromosome was the cause of the syndrome.

In England by now, the crowding of Harperbury Hospital had eased enough to take the bone-marrow sample from the Klinefelter's Down's (Orlando J. Miller, a young Amercian physician then on a Population Council fellowship at the Galton Laboratory, dates the event between March 19 and March 23, 1959.) Half the sample was sent to Ford at Harwell, who recalls finding the extra Down's chromosome (plus, of course, the extra *X* for the Klinefelter's character) just two days after hearing about Lejeune's results. At the Galton, Miller and Ursula Mittwoch detected the identical chromosomal anomaly in their half of the bone-marrow sample. Additional confirmation came from Edinburgh, where Jacobs and her co-workers, also without knowing about Lejeune, had begun to look at the chromosomes of Down's victims because they tended to suffer from a high incidence of leukemia. News of the Down's results moved the provost at University College London in May to send Penrose a note: "It must be one of the most important things that has happened in genetical studies for a long time." And it was. Penrose remarked some months later that the events of the past year amounted to "a major breakthrough in the

science of human genetics," adding that he found "the photograph of the cell from the man with two extra chromosomes from which the intelligence level, the behavior and sexual characters can be confidently predicted, just about as astonishing as a photograph of the back of the moon."

However, there was still doubt about the nature of the extra Down's chromosome. Penrose thought that it was a member of a trisomy — that is, the occurrence of one of the 22 autosomal chromosomes as a triplet rather than as a pair. Lejeune had not been certain — and neither had the other investigators — whether it was that or a supernumerary chromosomal piece of unknown origin. But within a year the abnormality was demonstrated to be indeed a trisomy — of the chromosome designated No. 21 by agreement at a genetics conference in Denver, Colorado, in April 1960. (The agreement assigned numbers to the chromosomes in order of descending size.)

Also in 1960, investigators in Sweden, in addition to Polani and Ford, and Penrose and others in England, concluded that a particular form of this trisomy accounted for the small number of cases of familial occurrence of Down's syndrome. It arose from the presence in some people of what is called a translocation — in this case, the attachment of one of the 21-chromosomes to the 14-chromosome. If a gamete containing the 14-21 combination plus the other 21-chromosome was passed on to a fetus, the offspring would possess two regular 21-chromosomes plus the 21 on the No. 14. If a gamete transmitted the 21- and 14-chromosomes only in their hybrid form, the child would be normal. But because these chromosomes were attached to each other the child would be a carrier, and his or her children would be at risk for trisomy-21. The detection of the cause of "mongolism" in such cellular accidents finished off — or should have — its vestigial association with some kind of atavism. Lejeune, Penrose, and others publicly urged that the racially tinged nomenclature of the condition be abandoned in favor of different terms, including "Down's syndrome" or "trisomy-21."

The sharp turn of events in human cytogenetics originated in different approaches — particularly in the Cartesian rationalism of Lejeune on the one side of the Channel and British step-by-step empiricism on the other, but they joined incandescently to light up a vast unexplored region on the human cytogenetic map. Charles Ford had analyzed a Turner's bone-marrow sample sent him by Polani and had reported in 1959 that, as Polani suspected, Turner's females were missing a second sex-chromosome. In 1960 other birth defects were shown to result from chromosomal anomalies, and it was demonstrated that lymphocytes in the blood could be cultured for karyotype analysis — a technical advance that put human chromosomal studies within reach of any scientist or physician who wanted to undertake them. Penrose later remarked of the hereditary mechanism that "the instructional errors, when single genes are involved, are too small to be seen. They are like mistakes made by an imaginary printer whereas chromosome aberrations are like the mistakes of a binder." By the early 60s, human geneticists were equipped with the cytogenetic techniques essential to seeing the binder's mistakes. □