THE SOMATIC CHROMOSOMAL CONSTITUTION OF SOME HUMAN SUBJECTS WITH GENETIC DEFECTS

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Development of techniques for reliable cultivation *in vitro* of cells from any individual under conditions of constancy of karyotype, made possible routine establishment of normal diploid cell strains taken from human subjects with known or suspected genetic aberrations.[1] The availability of a simple, precise method for delineation of the chromosomes of such cells grown as monolayers on glass,[2] and completion of the morphological characterization of all the human chromosomes.[3] also made possible analysis of such cells for cytogenetic abnormalities. Several individuals with genetic diseases have now been screened for relatively gross chromosomal abnormalities. Aberrations of the sex chromosomes offer particularly simple applications of such analysis because of the great difference in size between the human X and Y chromosomes.[3] An independent program of somatic chromosomal analysis has also been undertaken by several British investigators, utilizing bone marrow biopsies, in which mitotic figures of dividing cells are examined directly with few or no divisions occurring *in vitro*.[7, 10a]

Methods.—Stable, *in vitro* cultures were initiated from skin biopsies of about 10–30 mgs taken from the forearm or back of the neck, as previously described. The cells were dispersed by trypsin, plated, and incubated, in the standard manner.[1] Human cord serum, rather than fetal calf serum, was employed as a nutritional supplement, since it appeared to give somewhat better results than those obtained with the particular batches of fetal calf serum available to us during the preceding winter. As an economy measure, the concentration of N16 solution in the farming medium was dropped from 40 per cent to 20 per cent, with no adverse effect on the results. Within 3 weeks of cultivation, cells form a confluent layer on the petri dish, and may be trypsinized, transferred to culture bottles, and farmed continuously, as indicated.[1] After sufficient cells had been grown for chromosomal analysis, the cultures were frozen and stored at −70°C by the procedure of Swim *et al.*[4] Such cultures, on re-thawing after periods of several months, have grown excellently and revealed an unchanged chromosome constitution. Samples taken for chromosome delineation were treated as described earlier,[2] except that after incubation for 1 hr with colchicine (0.15 γ/cm^3) the cells were placed at room temperature for 15 min, then flooded with 0.064 per cent NaCl (adjusted to pH with 10^{-3} *M* phosphate buffer) at room temperature in which solution they remained for 10 min for the hypotonic swelling step originally introduced by Hsu.[5] Fixation was accomplished by consecutive 10-sec immersion of the slides into dilutions of the standard fixative (alcohol:acetic acid:formalin = 6:2:1) of 1:2000, 1:1000, 1:50, followed by a 30-sec immersion into full strength fixative. The slides were then rinsed in water, air-dried and stained, exactly as previously described.[2] The same fixation and staining procedure was employed for the Barr sex chromatin determination on both tissue culture cells or buccal smears, except that treatment with colchicine and hypotonic saline was omitted. Photographs were taken with a Leitz Ortholux Aristophot camera-microscope.

Results.—Earlier reports have demonstrated the highly uniform chromosomal constitution which is regularly obtained by the methods here employed from the somatic cells of different normal human subjects, regardless of the organ which is sampled.[2, 3] For comparison with the new karyotypes here to be presented, the typical karyotype of the human male cell, as obtained from normal subjects, is

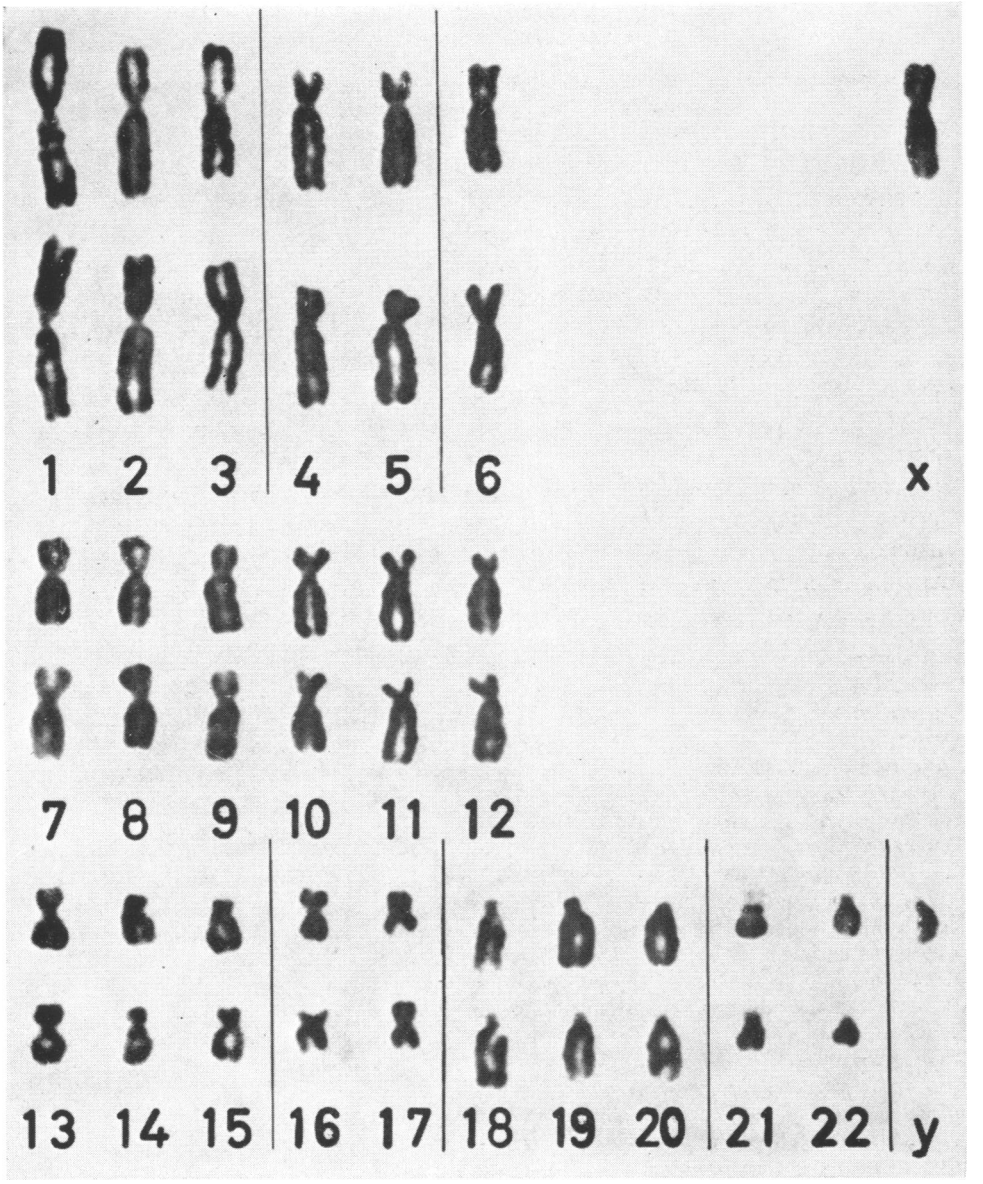

FIG. 1.—The ideogram of a normal human male. The normal female is the same, except for its possession of an additional X and no Y chromosome (3).

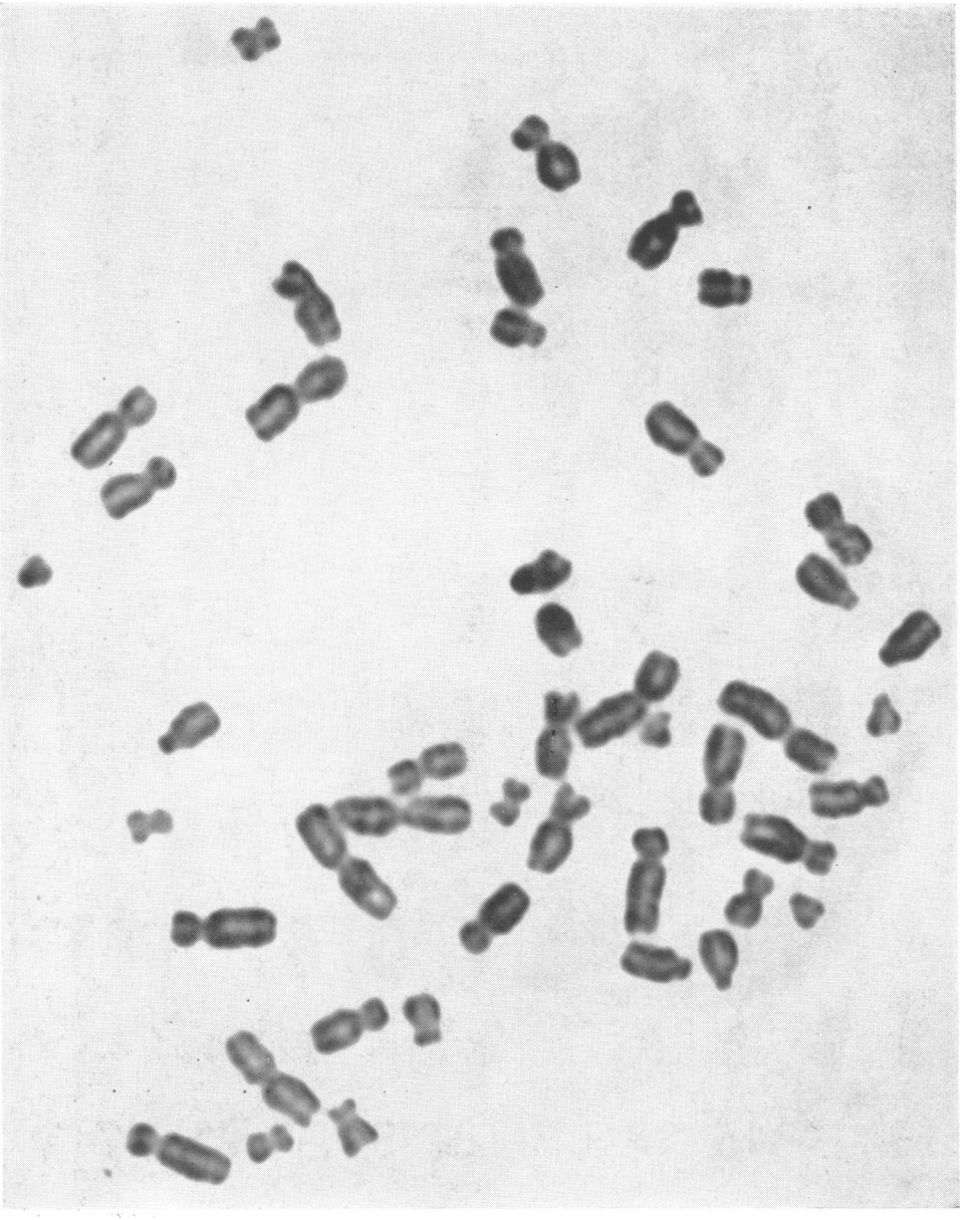

FIG. 2.—A typical chromosome set from the patient with ovarian dysgenesis. Only 45 chromosomes are present.

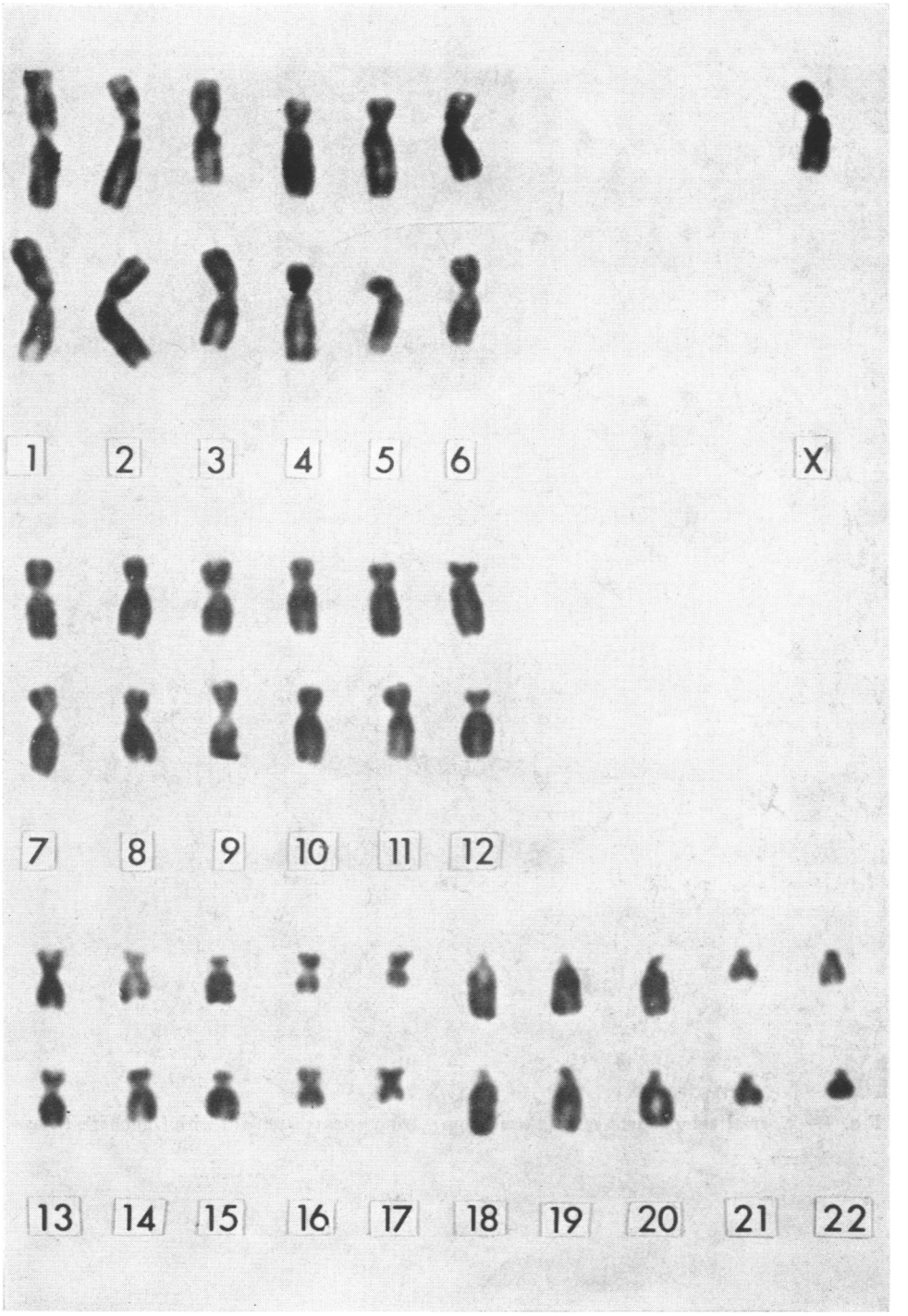

FIG. 3.—Identification of the chromosomes from the patient with ovarian dysgenesis. The numbers refer to the Tjio and Puck[3] classification of the human chromosomes.

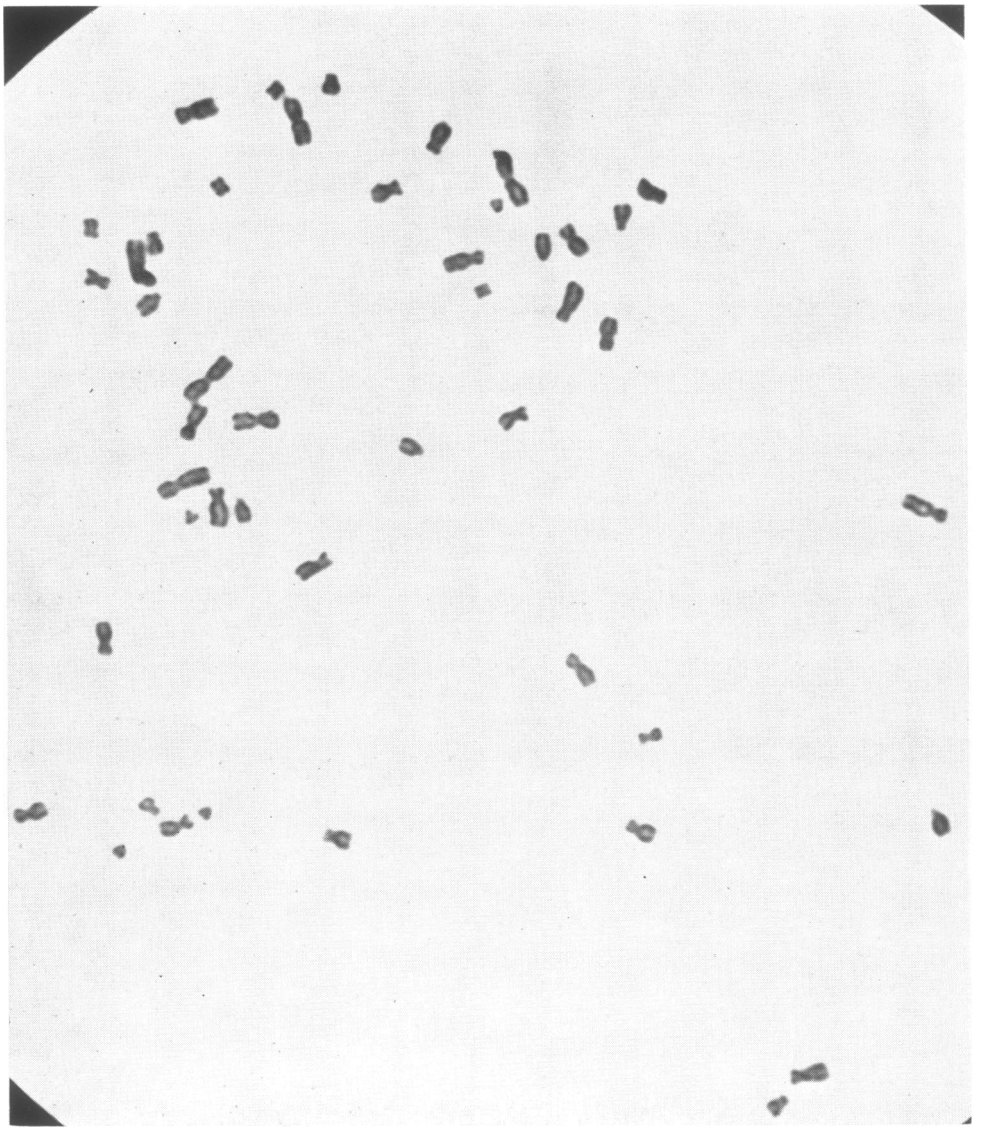

FIG. 4.—Normal complement of 46 chromosomes from a female patient with Gaucher's Disease.

reprinted in Figure 1. The normal female karyotype is identical, except for the possession of an additional X and no Y chromosome.

Chromosomal analysis of the somatic cells of a female patient with gonadal dysgenesis was carried out.† Diagnosis of gonadal dysgenesis of this 17-year-old female was made on the basis of shortness of stature; primary amenorrhea; impalpable condition of the ovaries; female genitalia; mammary hypoplasia; small uterus; negative Barr test for female sex chromatin; and the characteristic, elevated urinary excretion of gonadotropins. Webbing of the neck was absent, but the typical reverse direction of the whorls of the hair on the back of the neck was present. Other abnormalities like heart defects, cubitus valgus, and mental retardation sometimes associated with this disease were absent.

In Figure 2 is presented a typical set of chromosomes from this patient, revealing the presence of only 45 members. Analysis of more than 150 clearly delineated mitotic figures revealed no other chromosome number, except for an occasional tetraploid cell with 90 chromosomes. In Figure 3, the chromosomes have been identified and labeled in accordance with the classification system of Tjio and Puck.[3] It is evident that the autosomes are normal, but only one sex chromosome, the X, is present.

Single cells from the culture of the XO individual, when plated by the standard technique,[1] readily formed colonies in the medium here employed, with plating efficiencies like those of other human cell lines. The growth rate is approximately the same as that of normally diploid male or female human cells. Hence, in our medium, no growth hindrance is caused by the missing chromosome. Other biochemical studies are in progress.

In contrast to these findings, the chromosomes of skin cells of patients with Marfan's syndrome, phenyl ketonuria, and female pseudohermaphroditism‡ and of the bone marrow cells of a patient with Gaucher's disease have been found to be normal with respect to number and gross morphology. Figure 4 presents a picture of the chromosomes of the female patient with Gaucher's disease.

Detailed measurement of individual chromosome arms in all these cases, to determine whether minute deletions or other abnormalities exist, is in progress and will be reported elsewhere.

Discussion.—Study of several thousand randomly selected mitotic figures obtained by the technique here employed from somatic cells of presumably normal human subjects has yielded the normal chromosome number (46 or, in an occasional cell, the tetraploid 92) with a constancy better than 99.9 per cent. Though far fewer cells of normal persons have as yet been accurately measured for chromosomal morphology, our data have shown remarkable anatomic constancy of the individual chromosomes in the cells of presumedly normal persons except for occasional individuals with more pronounced satellites in 1 or more chromosomes of the pairs 18 or 21.[2] A similar degree of constancy of human chromosomal morphology has been reported by Chu and Giles.[6] Thus, application of these methodologies to cells of persons with known or suspected genetic defects can be carried out with considerable confidence.

The present results demonstrate that the absence of the Barr sex chromatin reaction is not sufficient grounds for concluding a male chromosomal constitution to exist in the given cell type. While the simplicity of the Barr test is a great ad-

vantage, it does not afford as much information as does delineation of the entire karyotype.

These findings make it evident that a human subject with an XO sex chromosome constitution is female rather than male, though femaleness is not complete, since the ovulation function is absent. The present data demonstrates that in Man the Y chromosome contains genes needed for development of both the primary and secondary male characteristics, a condition very different from that in *Drosophila*. Similarly, the fact that a human XO subject is not possessed of the complete apparatus for ovulation suggests that the double complement of X chromosomal genes is necessary in an autosomally diploid individual for full expression of normal female reproductive power. Since the determinants for maleness resides to so large an extent in the Y chromosome, it is conceivable that true human hermaphroditism might arise as a result of exchange between the X and Y chromosomes, or from simple deletion in the Y chromosome, as well as from the production of the genetic constitution XXY, as has been recently reported as in a case of Klinefelter's syndrome.[7] In this case, however, the occurrence of XXY human females inferred by Lewit and Serebrowsky[8] from the data of Cunier[9] would require re-interpretation.

It is evident that the chromosome loss associated with ovarian dysgenesis in the patient here studied could have resulted from non-disjunction in the stemline of the egg, which caused loss of its X chromosome, or in that of the fertilizing sperm, causing loss of either its X or its Y chromosome. The possibility of a mosaicism developing after fertilization cannot yet be excluded, although it seems unlikely and could be checked by sampling cells of tissues with maximally divergent embryologic development. Of particular interest will be the study of cells of individuals with ovarian dysgenesis accompanied by a positive sex chromatin reaction, a condition which could arise from a deletion in one X chromosome.

Non-disjunction in the sex-chromosomes of the sperm could lead to individuals with either XXY or XO constitution, while if it occurred in the egg, it would lead on fertilization to XXX, XXY, XO or YO constitution of which the last is probably lethal. Thus, if non-disjunction in gametogenesis is the principle cause of these conditions, Turner's and Klinefelter's syndromes should occur with equal frequencies, while the XXX condition, not yet described for man, should occur at a lower rate. Study of these frequencies and search for the superfemale, XXX condition among precociously pubescent females is in progress.

As the present paper was being written, an independent series of papers[10] appeared in the *Lancet* by Ford, Jacobs and their co-workers, describing further developments in their highly productive program, analyzing the chromosomal constitution of somatic (bone marrow) cells from human patients with various genetic diseases, and including a study of the chromosomes of a case of Turner's syndrome. The chromosomal analysis of that patient is identical to the one presented here, in revealing an XO constitution, despite some differences in some of the clinical aspects, as in the subnormal mentality of the English patient, in contrast to the far-above-average intelligence of our own.

In another study which has also just appeared,[11] Russell and his co-workers report the occurrence in mice of individuals with sex chromosome constitution XO, which possess the anatomy of a female. Such animals were able to carry out normal female reproduction. However, the presence of a particular sex-linked gene

in some such individuals made reproduction possible only when their ovaries were transplanted to a normal female mouse. These investigators concluded that, for the mouse, maleness requires the Y chromosome. These findings lead to the expectation that in human females too, the XO chromosome condition may display different kinds of reproductive behavior depending on the individual's genic constitution. Moreover, the fact that in the mouse, at least some XO karyotypes can ovulate normally leads to the speculation that XO human females might be induced to develop more completely if the molecular (and presumably hormonal) environment were properly adjusted during prenatal or possibly postnatal development. In principle, at least, it is possible by means of the present technique to diagnose such conditions in the foetus. While the many different kinds of complexities involved are fully appreciated, it does not seem impossible that, as in one or two other conditions, ultimately it may be possible to prevent some of the pathologic consequences of this genetic defect (though not the defect itself) in humans.

The differences in the frequency of occurrence of the associated clinical symptoms in patients with ovarian dysgenesis who display the same chromosomal constitution may involve: the existence of allelic differences in the genes of the remaining X chromosome, (such genes, however, would normally have to be present on the Y chromosome also, since these manifestations are not frequent in males); the development of complex interactions of biochemical imbalance due to the XO condition with subtle gene defects on other chromosomes; or, if mosaicism is involved, differences in the time and site at which the abnormal chromosomal condition arose.

It is obvious that the XO cells here described furnish a genetically labeled strain which offers possibilities for a variety of experimental operations.

The methods here employed for chromosomal analysis of the somatic cells require more time than those utilizing bone marrow biopsy. However, they are less painful for the patient and permit even repeated sampling of patients because of the ease of removal of the tiny amounts of skin required. Moreover, they also make possible establishment of the cells in stable, continuous, *in vitro* growth for further genetic and metabolic studies.

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† A report on this case was presented at the Meeting of the Society for Pediatric Research, at Buck Hill Falls, Pa., May 6, 1959.

‡ This patient is an infant, 2 months of age, with male-appearing genitalia consisting of a phallus with first degree hypospodias, no scrotal masses, hirsutism, and a positive sex chromatin test. The child developed Addisonian-like crises which responded to steroids, DOCA, and salt. An older sibling died in infancy with a similar condition.

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[10] (*a*) Ford, C. E., *et al.*, *Lancet*, 709 (1959); (*b*) Jacobs, P. A., *et al.*, *Lancet*, 710 (1959); (*c*) Ford, C. E., *et al.*, *Lancet*, 711 (1959).

[11] (*a*) Welshons, W. J. and L. B. Russell, these PROCEEDINGS, **45**, 560 (1959); (*b*) Russell, W. L., L. B. Russell and J. S. Gower, *Ibid.*, **45**, 554 (1959).

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*THE EFFECTS OF DELETIONS, POINT MUTATIONS, REVERSIONS AND SUPPRESSOR MUTATIONS ON THE TWO COMPONENTS OF THE TRYPTOPHAN SYNTHETASE OF ESCHERICHIA COLI**

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Introduction.—In previous investigations with the tryptophan synthetase (TSase) of *Neurospora crassa*[1] and *Escherichia coli*[2] it was observed that gene mutations affecting the formation of this enzyme may result in the production of a protein (designated CRM) which is immunologically similar to the enzyme. In other mutants lacking TSase, in both organisms, this protein was not detected. Subsequent studies have shown that the TSase of *E. coli*[3, 4] and *N. crassa*[5] catalyzes two reactions (2 and 3) in addition to the previously studied reaction, (1).

(1) indole + *L*-serine → *L*-tryptophan
(2) indoleglycerol phosphate ⇌ indole + triose phosphate
(3) indoleglycerol phosphate + *L*-serine → *L*-tryptophan + triose phosphate

It was also concluded that reaction (3) is not the sum of reactions (1) and (2) since free indole is not formed during the course of this reaction. It appears likely therefore that reaction (3) constitutes the actual physiological mechanism of tryptophan synthesis in these organisms. Investigations on the identity of the CRM's formed by several of the TSase-lacking mutants of *E. coli* which were previously studied revealed that these proteins were enzymatically active in reaction (2), but not in the other reactions.[6] Thus the CRM's in these strains appear to be altered proteins which have retained both an enzymatic activity and an immunological identity characteristic of wild type TSase. More recent studies[4] with the TSase of *E. coli* have led to the finding that this enzyme system consists of two separable protein components (designated A and B), both of which are required for the catalysis of any of the above reactions at maximal rates. Each of the components, when tested separately, appears to have slight activity in one of the three reactions—component A in reaction (2), and component B in reaction (1). However, in neither case is the activity more than ten per cent of that obtained with a mixture of the two components.