sult is observed in the data of Fig. 2. A second possibility, which is also completely consistent with the data of Fig. 2, is that families of repeated DNA sequences in both human and chimpanzee consist of related base sequences having the same proportion of their bases changed and that these changes occur randomly at any site in the sequence. Again for this case the repetitive DNA heteroduplex of chimpanzee and human should have a thermal stability profile identical to that of renatured repetitive human DNA. Both of these possibilities should be contrasted to the very different case of renatured satellite DNA's from *Drosophila* (*10*). In these sequences specific mutations are located at specific sites in the sequence. As a result, the renaturation product of two different *Drosophila* satellite DNA's has a much lower thermal stability than the self-renaturation product of an isolated satellite.

A complication in these experiments is the existence of a zero-time binding fraction which binds to hydroxylapatite without undergoing second-order renaturation with a second DNA strand (*3*, *11*). At least part of this fraction results from inverted repeated DNA sequences. This sequence class could alter the melting profiles of the repetitious sequence classes, especially since short DNA strands containing this fraction have a high thermal stability (*11*). As a control, we have removed this fraction (*3*) from the isotope-labeled DNA preparations from the human and chimpanzee, 4 and 7 percent, respectively, and we have repeated the experiments described above. Again, the melting profiles of the repetitive labeled human DNA and chimpanzee DNA hybrids were indistinguishable.

A second complication in these experiments is the possible self-renaturation of chimpanzee DNA. To test this possibility labeled chimpanzee DNA was renatured exactly as described for the renaturation of the admixed DNA's to C_0t of 21, except that the placental human DNA was omitted. On binding to hydroxylapatite 11.5 percent of this DNA behaved as duplex so that, excluding the zero-time binding fraction, only 4.5 percent (11.5 − 7 percent) of the chimpanzee DNA would self-renature (with other chimpanzee DNA sequences) during the C_0t 21 incubation. This is, in principle, an upper limit to the amount of chimpanzee DNA which self-renatures since some fraction of this 4.5 percent may also share homology with human DNA sequences. The small extent of self-renaturation of the chimpanzee DNA would not influence the results of the thermal stability study.

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References and Notes

1. E. H. Davidson, G. A. Galau, R. C. Angerer, R. J. Britten, *Chromosoma* **51**, 253 (1975).
2. J. E. Manning, C. W. Schmid, N. Davidson, *Cell* **4**, 141 (1975).
3. C. W. Schmid and P. L. Deininger, *ibid.* **6**, 345 (1975).
4. M. E. Chamberlain, R. J. Britten, E. H. Davidson, *J. Mol. Biol.* **96**, 317 (1975).
5. D. E. Kohne, J. A. Chiscon, B. H. Hoyer, *J. Hum. Evol.* **1**, 627 (1972); B. H. Hoyer, N. W. van de Velde, M. Goodman, R. B. Roberts, *ibid.*, p. 645.
6. T. I. Bonner, D. J. Brenner, B. R. Neufeld, R. J. Britten, *J. Mol. Biol.* **81**, 123 (1973).
7. N. R. Rice, in *Evolution of Genetic Systems*, H. H. Smith, Ed. (Gordon & Breach, New York, 1972), p. 44.
8. M. King and A. C. Wilson, *Science* **188**, 107 (1975).
9. The Pt 1 Lu chimpanzee cells were provided by contract E-73-2001-N01 within the Special Virus-Cancer Program NIH, PHS through the courtesy of Dr. Walter A. Nelson-Rees.
10. J. G. Gall, E. H. Cohen, D. Datherton, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 417 (1973); M. Blumenfeld, *ibid.*, p. 423.
11. D. A. Wilson and C. A. Thomas, Jr., *J. Mol. Biol.* **84**, 115 (1974); C. W. Schmid, J. E. Manning, N. Davidson, *Cell* **5**, 159 (1975).
12. Supported by PHS grant GM 21346.

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A Specific Population of Gonadotrophs Purified from Immature Female Rat Pituitary

Abstract. *When dispersed pituitary cells from 14-day-old female rats were sedimented in a bovine serum albumin gradient, a fraction was isolated which consisted of almost 90 percent of large cells that stained purple in the periodic acid–Schiff (PAS) reaction. Immunostaining indicated that over 85 percent of these PAS-purple cells were gonadotrophs containing both follicle stimulating and luteinizing hormone. Reproducible cell cultures could be obtained on poly-L-lysine coated dishes. As early as the second day in culture, the secretion of both follicle stimulating and luteinizing hormone was highly stimulated by luteinizing hormone–releasing hormone. The effect on FSH was as marked as that on LH. The data suggest that the isolated gonadotrophs are a specific functional subtype.*

Gonadotrophs are specific cell types of the anterior pituitary gland which secrete the gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH). There are morphologically distinct subtypes among these cells (*1*). For many years it was thought that a separate gonadotroph subtype secreted LH while another subtype secreted FSH, but recent studies have shown that part of the FSH-type gonadotrophs also contain LH (*2*, *3*). Moreover, hormone distribution over the two types of gonadotrophs has been found to vary with sex, age, and certain physiological conditions, indicating that in addition to morphological heterogeneity there may also be functional heterogeneity (*4*).

Because the occurrence of functional variants of gonadotrophs appears to be directly related to the mechanisms that control gonadotrophin secretion, the characterization of these variants is most important. Most investigators have studied these cells by electron microscopy of fixed tissue. In the study described here, functional heterogeneity was explored in living cells. From dispersed pituitary cells we isolated gonadotrophs to a high degree of purity and with specific functional characteristics. Cells were separated by velocity sedimentation at unit gravity. Using this technique with pituitaries from adult rats, other investigators had already succeeded in obtaining marked enrichments in populations of several pituitary cell types (*5*, *6*). Instead of using adult rats, we used female rats at the age of 14 days, since female rats at this age secrete large amounts of FSH (*7*). We supposed that because of this hypersecretion the gonadotrophs or a certain number among them might be advanced in development and might therefore be larger than other pituitary cells. If so, it should be relatively easy to isolate them by velocity sedimentation.

Monodispersed cells from 14-day-old female Wistar rats were prepared according to a method slightly modified from Hopkins and Farquhar (*8*). The distribution of cell types was monitored by light microscopy after the cells were stained with Alcian blue–periodic acid–Schiff (PAS)–orange G (*9*). Gonadotrophs and thyrotrophs were specifically identified by immunostaining with antiserums to rat LH, FSH, and thyroid stimulating hormone (TSH) prepared in rabbits (*10*). As expected, a considerable number of PAS-purple cells (presumably gonadotrophs) were markedly larger in size than were cells of all other types. Table 1 shows the distribution of cell types after

the cells were sedimented at unit gravity. The PAS-purple cells increased in percentage from the top to the bottom of the gradient, constituting almost 90 percent of the cells in fraction 8. The major contaminants in this fraction were small clumps of "chromophobe" cells. Up to 78 percent of the cells from fraction 8 reacted positively upon immunostaining with antiserum to rat FSH, while 72 percent reacted with antiserum to LH. Only a small percentage of the cells stained positively with antiserum to TSH. Typical photographs of the stained cells are shown in Fig. 1. The staining was weakened to an acceptable background level when antiserum was replaced by normal rabbit serum, and was eliminated when the peroxidase-labeled second antibody was omitted. As shown in Fig. 1, staining also diminished after prior absorption of the antiserum to rat FSH for 3 to 4 days with highly purified rat FSH or of antiserum to rat LH with highly purified rat LH, but was not diminished when absorption was done with the opposite hormone. These data indicate that the staining for FSH and for LH was specific and that the great majority of gonadotrophs isolated in fraction 8 contain both FSH and LH. This finding is supported by the fact that the percentage of positively stained cells did not increase (whereas staining intensity did) when the cells were reacted with both antiserum to FSH and antiserum to LH at the same time (not shown in Table 1). It is interesting that the sum of the percentages of FSH- and TSH-positive cells equaled the percentage of the PAS-purple cells in the fraction (Table 1). From this it can be calculated that about 88 percent of the PAS-purple cells are gonadotrophs while the others are thyrotrophs (*11*).

In order to determine the relative amounts of FSH and LH present in these cells, these hormones were extracted by homogenization of the cells in water and measured by radioimmunoassay (*12*). As shown in Table 1, both hormones were immunoassayable. As far as they were expressed in terms of their respective reference preparations [FSH-RP-1 and LH-RP-1 (*12*)], the amounts measured were comparable. Both FSH and LH were also present in other gradient fractions and their amounts increased with the number of PAS-purple cells or with the number of FSH- or LH-containing cells as identified by immunostaining.

The functional characteristics of the purified gonadotrophs from fraction 8 were explored by studying FSH and LH secretion in vitro and the response of this secretion to synthetic luteinizing hormone–releasing hormone (LH-RH). Since pituitary cells tested within a short period after dispersion respond poorly to LH–RH, these tests were done in culture. Successful and highly reproducible monolayer cultures could be obtained when dishes were coated with poly-L-lysine (*13*). Virtually all cells firmly attached within minutes and subsequent cell loss as evaluated daily under an inverted microscope was minimal. The advantage of this technique was that hormone secretion could be accurately followed from the first day in culture, without the danger of losing cells during removal of the medium.

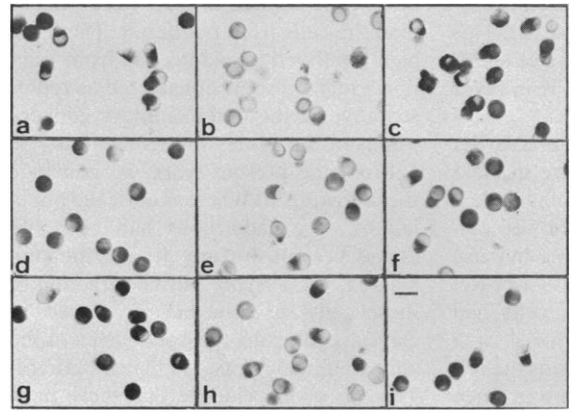

Fig. 1. Immunostaining of pituitary cells of gradient fraction 8. The antiserums and their dilutions were: (a) antiserum to FSH 1 : 50; (b) normal rabbit serum 1 : 30; (c) antiserum to LH 1 : 100; (d) antiserum to FSH 1 : 80; (e) antiserum to FSH 1 : 80 absorbed with 4 μg of rat FSH; (f) antiserum to FSH absorbed with 4 μg of rat LH; (g) antiserum to LH 1 : 200; (h) antiserum to LH 1 : 200 absorbed with 2 μg of rat LH; (i) antiserum to LH 1 : 200 absorbed with 2 μg of rat FSH. These experiments were repeated five times with similar results. The spreading of the cells is caused by poly-L-lysine (*13*). Bar is 20 μm.

Figure 2 shows that both FSH and LH were secreted spontaneously at day 2, 3, or 4. The level of FSH secretion was significantly correlated with the level of LH secretion ($r = 0.95$; $P < .001$). Addition of $2 \times 10^{-8} M$ LH-RH resulted in a sharp increase of both FSH and LH secretion (paired t-test: FSH, $P < .01$; LH, $P < .05$) and this was so as early as day 2. Most interesting was the finding that the effect on FSH secretion was as marked as that on LH secretion. The mean relative increase was 12.4 ± 3.2 for FSH and 8.1 ± 2.3 for LH (the values for cultures 4 and 5, which were less than 5 ng/ml, are not included), while there was a high correlation between stimulated FSH and LH values ($r = 0.99$; $P < .001$). Furthermore, in contrast to endogenous or spontaneously secreted FSH and LH, which in terms of RP-1 (*12*) were measured in almost equal

Table 1. Distribution of pituitary cell types and of cellular FSH and LH in gradient fractions 3, 5, and 8. Isolated cells from 16 pituitaries were sedimented at room temperature through a 500-ml gradient of 0.3 to 2.4 percent bovine serum albumin (BSA) in Eagle's minimum essential medium (MEM) with Earle's salts. The sedimentation chamber and formation of the gradient were as described by Hymer *et al.* (*6*). Sedimentation time (including sedimentation during gradient formation and extraction) was 4 hours. Fractions (50 ml) were collected and the cells sedimented by centrifugation at 70*g* for 10 minutes and resuspended in 0.3 percent BSA in MEM. All procedures were done under sterile conditions. Cells were counted in a hemocytometer. Final recovery ranged from 55 to 70 percent (8.5 ± 0.6) × 10^6 cells). Going from top to bottom, no cells or only debris were found in the first 100 ml of the gradient. Subsequent 50-ml fractions were numbered from 1 to 8. The number of cells recovered in fraction 3, 5, and 8 was (1.67 ± 0.2) × 10^6, (5.4 ± 0.3) × 10^5, and (2.5 ± 0.2) × 10^5, respectively. Cell viability, as estimated by the trypan-blue exclusion test, was better than 95 percent. Histochemical (*9*) and immunocytochemical staining (*10*) were done on samples from the same cell preparation. Values are means ± standard error of the mean from five separate experiments; FSH and LH content are expressed in terms of the NIAMDD reference preparations, FSH-RP-1 and LH-RP-1 (*12*).

Gradient fraction	Histochemical staining (%)			Immunostaining (%)			Hormone content (ng/10^4 cells)	
	PAS purple cells	Somatotrophs	Chromophobes	FSH	LH	TSH	FSH	LH
8	88.0 ± 1.9	3.6 ± 0.3	7.8 ± 1.7	77.9 ± 2.5	71.7 ± 3.1	9.6 ± 0.9	2341 ± 904	2753 ± 853
5	48.9 ± 0.9	37.6 ± 2.1	12.1 ± 2.0	36.9 ± 3.3	31.3 ± 5.8	9.9 ± 2.1	67 ± 9	74 ± 12
3	19.9 ± 2.5	37.1 ± 1.1	39.9 ± 2.2	18.8 ± 3.2	9.6 ± 1.1	0.1 ± 0.1	2.9 ± 0.4	3.8 ± 0.6

amounts, the values of stimulated FSH secretion significantly exceeded those of stimulated LH secretion by a factor ranging between 6.6 and 15.7 ($P < .02$). This indicates that under conditions of short-term secretion, the secretion is in favor of FSH.

Our results on the effects of LH-RH on monolayer cell cultures are in clear contrast to those obtained by other investigators using isolated cells or cell cultures of adult rat pituitaries (*14*). In these earlier studies full response to LH-RH was regained only after the cells had been maintained in culture for 4 to 5 days. Also, in the earlier systems the relative increase in LH secretion was much faster and higher than the increase in FSH secretion, whereas the endogenous amount of FSH relative to that of LH was higher than in the present cell system.

We also explored the gonadotrophs present in gradient fraction 5 (not shown in Fig. 2). Spontaneous secretion and the response to LH-RH were comparable to what was observed with fraction 8. Absolute amounts of FSH or LH secretion, however, were lower but this was also the case with the hormone contents of these cells, which, in fact, were smaller than the cells from fraction 8. Thus, the highly purified gonadotrophs from fraction 8 might also be considered as representative of the other pituitary gonadotrophs of the 14-day-old female rats.

From the present work we conclude the following: (i) Whereas enriched populations of gonadotrophs had been obtained previously only from adult animals (*5, 6*), a highly purified fraction of these cells has now been prepared by carefully selecting the age and sex of the animal (female rats, 14 days old). (ii) There is good evidence that these purified gonadotrophs are a specific functional subtype. Based on immunocytochemical examination the vast majority of the cells contained both FSH and LH. As measured by radioimmunoassay both FSH and LH were present in substantial amounts. In culture, both hormones were spontaneously secreted, while in response to LH-RH, the cells secreted remarkably high amounts of FSH compared to LH. (iii) The characteristics of FSH secretion by the isolated gonadotrophs are compatible with the patterns of gonadotrophin secretion in vivo in 14-day-old female rats, such rats being known to secrete unusually high levels of FSH (*7*). (iv) Finally, the finding that LH-RH can stimulate the secretion of large amounts of FSH supports the theory that this gonadotrophin releasing factor is the major physiological releasing hormone for both FSH and LH (*15*).

The gonadotroph preparation described herein may provide a model system for the further exploration of the functional diversity of pituitary gonadotrophs and for testing the hypothesis that the differentiation of the gonadotroph itself determines its response to LH-RH.

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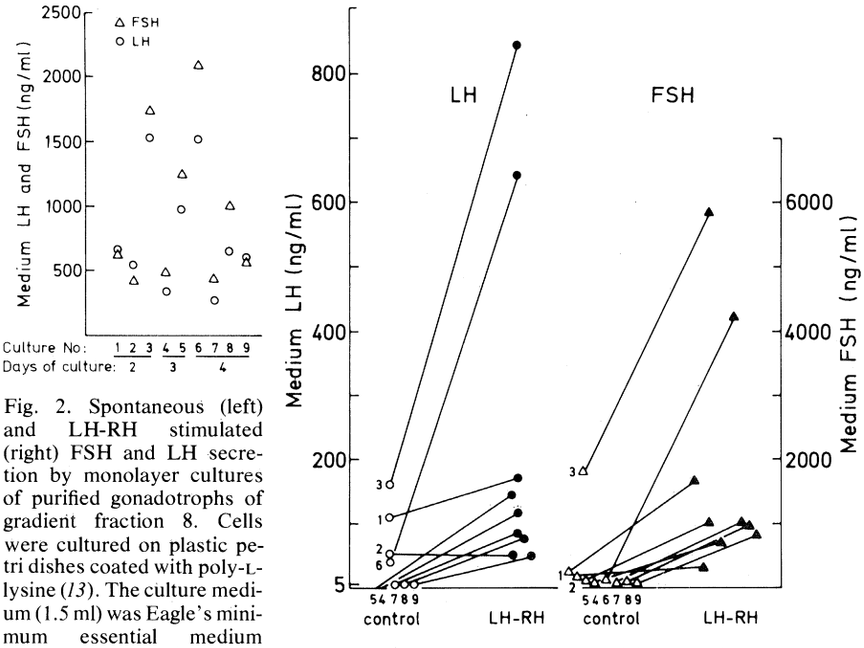

Fig. 2. Spontaneous (left) and LH-RH stimulated (right) FSH and LH secretion by monolayer cultures of purified gonadotrophs of gradient fraction 8. Cells were cultured on plastic petri dishes coated with poly-L-lysine (*13*). The culture medium (1.5 ml) was Eagle's minimum essential medium (MEM) with Earle's salts supplemented with 2.5 percent fetal calf serum, 10 percent horse serum, 1 percent MEM nonessential amino acids, 1 m*M* pyruvate (all obtained from Gibco), and 34 μg of penicillin and 50 μg of streptomycin per milliliter. Cells were plated at a density of 40,000 cells per 10 μl in MEM after which (1 hour) the culture medium was added. The figure illustrates individual results of nine cultures, numbered from 1 to 9 and, as indicated, tested at 2, 3, or 4 days. For each experiment, the cells were divided over two dishes, one being used as a control and one for LH-RH stimulation. The number of cells (in thousands) originally applied to each of the two dishes for cultures 1 to 9 was, respectively, 160, 55, 140, 160, 130, 180, 120, 120, and 140. For LH-RH stimulation the dishes were washed twice with MEM after which 1 ml of 2×10^{-8}*M* LH-RH in MEM was added to one dish and 1 ml of MEM alone was added to the control dish. Incubation was for 4 hours at 37°C in a humidified (CO_2 and air) incubator. Media for assay were centrifuged at 2000 rev/min for 10 minutes and the supernatants stored at − 25°C until tested. Spontaneous secretion was measured as the amount of hormones secreted in the culture medium during 20 hours preceding the LH-RH test. Values are expressed in terms of the NIAMDD reference preparations, FSH-RP-1 and LH-RP-1. The detection limits for LH and FSH were 5 ng/ml and 25 ng/ml, respectively.

References and Notes

1. M. G. Farquhar, E. H. Skutelsky, C. R. Hopkins, in *The Anterior Pituitary*, A. Tixier-Vidal and M. G. Farquhar, Eds. (Academic Press, New York, 1975), p. 83.
2. P. K. Nakane, *Acta Endocrinol. (Copenhagen) Suppl.* **153**, 190 (1971).
3. G. C. Moriarty, *J. Histochem. Cytochem.* **21**, 855 (1973). R. F. Phifer, A. R. Midgley, S. S. Spicer, *J. Clin. Endocrinol. Metab.* **36**, 125 (1973).
4. G. C. Moriarty, *Endocrinology* **97**, 1215 (1975).
5. H. Ishikawa, *Endocrinol. Jpn.* **16**, 517 (1969); R. V. Lloyd and W. H. McShan, *Endocrinology* **92**, 1639 (1973).
6. W. C. Hymer, W. H. Evans, J. Kraicer, A. Mastro, J. Davis, E. Griswold, *Endocrinology* **92**, 275 (1973).
7. S. R. Ojeda and V. D. Ramirez, *ibid.* **90**, 466 (1972).
8. C. R. Hopkins and M. G. Farquhar, *J. Cell Biol.* **59**, 276 (1973). The modifications were as follows: 0.5 percent trypsin (twice crystallized, Sigma) in Eagle's minimum essential medium was used instead of 0.1 percent trypsin in Krebs-Ringer-amino acid medium. The lower trypsin concentration gave lower cell yields, decreased viability, and caused marked clumping of cells. The 15-minute incubation period with neuraminidase (Worthington) did not further improve cell yield and was replaced by a 15-minute incubation period in the regular Ca^{2+},Mg^{2+}-free (1 m*M*) EDTA medium (ethylenediaminetetraacetic acid). Cell yield was 50 percent based on DNA recovery. Viability was better than 95 percent (trypan-blue test).
9. M. Herlant, *Bull. Microsc. Appl.* **10**, 37 (1960).
10. Cells were attached to glass slides coated with poly-L-lysine and fixed in Zamboni solution [see Nakane (*2*)] for 4 to 5 hours at room temperature. Immunostaining was done according to the method of Weir *et al.* [E. E. Weir, T. G. Pretlow, A. Pitts, E. E. Williams, *J. Histochem. Cytochem.* **22**, 1135 (1974)]. Antiserums to rat LH, FSH, and TSH were prepared in rabbits (*12*). The cells were incubated with antiserum at room temperature for 2 hours with new applications of antiserum every 20 minutes. The dilution used was that just above the dilution that decreases stainability. Goat peroxidase-labeled γ-immunoglobulin (Miles Laboratories) was applied undiluted for 30 minutes at room temperature. The staining reaction was with 4-chloro-1-naphthol.
11. Both thyrotrophs and gonadotrophs belong to the basophilic (PAS-positive) cell population of the pituitary.
12. Radioimmunoassay kits [obtained from the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD)] contained rat FSH (No. I_3) and rat LH (No. I_4) for radioactive labeling with iodine-125, the standard reference preparations FSH-RP-1 and LH-RP-1, rat antise-

rums to FSH (FSH-antiserum S6) and rat antiserum to LH (LH-antiserum S3). Assays were done according to the NIAMDD prescriptions. Each sample was assayed in duplicate or triplicate at different dilutions. Influence of cell suspension medium or culture medium was negligible. The measured FSH and LH values are only relative values; they are expressed in terms of the NIAMDD standard preparations FSH-RP-1 and LH-RP-1 which differ in degree of purity among each other.
13. D. Mazie, G. Schatten, W. Sale, *J. Cell Biol.* **66**, 198 (1975).
14. W. Vale, G. Grant, M. Amoss, R. Blackwell, R. Guillemin, *Endocrinology* **91**, 562 (1972); R. Blackwell, M. Amoss, Jr., W. Vale, R. Burgus, J. Rivier, M. Monahan, N. Ling, R. Guillemin, *Am. J. Physiol.* **224**, 170 (1972); A. Tixier-Vidal, in *The Anterior Pituitary*, A. Tixier-Vidal and M. G. Farquhar, Eds. (Academic Press, New York, 1975), p. 181; L. K. L. Tang and H. G. Spies, *Endocrinology* **96**, 349 (1975); H. Nakano, C. P. Fawcett, S. M. McCann, *ibid.* **98**, 278 (1976).
15. A. V. Schally, A. Arimura, T. W. Redding, L. Debeljuk, W. Carter, A. Dupont, J. A. Vilchez-Martinez, *Endocrinology* **98**, 380 (1976).
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Gene Dose Effect: Regional Mapping of Human Nucleoside Phosphorylase on Chromosome 14

Abstract. *Quantitative analyses of erythrocyte nucleoside phosphorylase in four unrelated cases of partial trisomy 14 indicate that the structural gene for this enzyme maps in the chromosome region 14q11→14q21.*

Determination of the subregional location and linear order of genes on human chromosomes is a first step toward the formulation of a comprehensive human gene map. One approach to intrachromosomal gene mapping utilizes the expected correlation between the number of copies of a given gene (allele) and the amount or activity of gene product detected. Quantitative analyses on cells carrying a well-defined duplication or deletion of a chromosome segment permit the localization of genes within the affected region. Such gene-dose studies have also been used successfully for the detection of carriers for many autosomal recessive diseases (*1*). To date, however, only a few genes have been regionally assigned on the basis of a gene-dose relationship (*2*). Here, we report the use of this approach to define more precisely the intrachromosomal location of the gene for nucleoside phosphorylase (NP). A report concerning a child with a severe defect in T-cell–mediated immunity, whose erythrocytes lack NP activity (*3*), has led to clinical interest in this enzyme.

The enzyme NP (purine-nucleoside: orthophosphate ribosyltransferase, E.C. 2.4.2.1) catalyzes the phosphorolytic cleavage of inosine to hypoxanthine, and has been assigned to chromosome 14 (*4*). Recently, Francke *et al.* (*5*), have presented evidence for the localization of NP to region 14pter→14q21 [that is, the region between the terminus of the short arm (p) and band 21 on the long arm (q)] (*6*) by interspecific hybridization of cells containing a balanced reciprocal X-autosome translocation t(X;14)(p22;q21). In order to obtain additional evidence for this assignment, to define more precisely the chromosomal location of NP, and to get a better understanding of the gene-dose relationship for this enzyme, we have measured red blood cell NP activity in four individuals with a duplication of nonidentical segments of chromosome 14. Partial karyotypes of balanced translocation carriers and affected individuals from four different families are shown in Fig. 1. The extent of the duplicated segment in each case is illustrated in Fig. 2.

Blood collected from 61 males and females, aged 1 day to 52 years, served as the control group. The controls included 39 individuals with a normal karyotype, 8 with a balanced translocation, and 14 with a chromosomal imbalance not involving chromosome 14. The activity of NP was stable in blood samples stored at 4°C in 1 ml of acid citrate dextrose for at least 3 weeks, but most samples were assayed within 7 days of collection. After removal of plasma and buffy coat by centrifugation, the red cells were washed twice in isotonic saline, and lysed by the addition of ten volumes of distilled water; the red cell debris was removed from the hemolyzate by centrifugation at 18,000 rev/min for 20 minutes. For quantitative measurements of NP activity we used a slight modification of the method of Hopkinson *et al.* (*7*). The assay mixture contained 0.05*M* sodium phosphate buffer

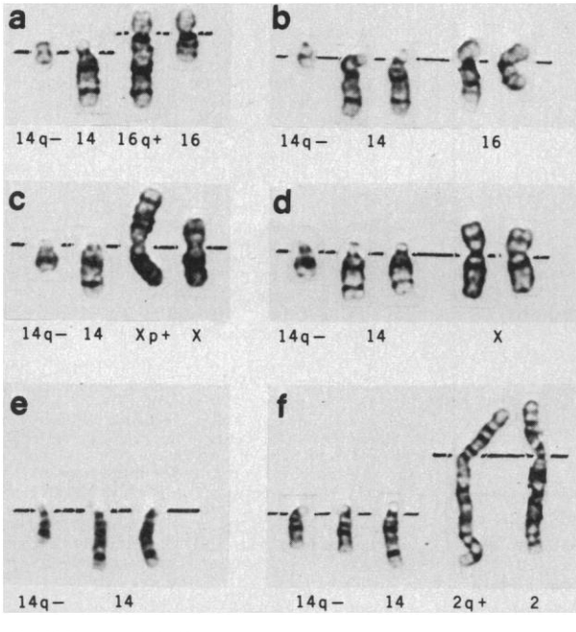

Fig. 1. (a) Balanced reciprocal translocation between long arms of chromosomes 14 and 16, designated t(14;16)(q11;q24) in phenotypically normal mother of subject B.R. (b) Partial karyotype of B.R., a 3-year-old girl with developmental delay and unusual phenotype, who has 47 chromosomes. The extra small metacentric chromosome consists of short arm, centromere, and a minute region of the long arm of chromosome 14 with the telomere band from the long arm of chromosome 16 attached. (c) Balanced reciprocal translocation t(X;14)(p22;q21) between the long arm of chromosome 14 and the short arm of an X chromosome in the phenotypically normal mother of C.M. (d) Partial karyotype of C.M., a 6-year-old girl with severe mental retardation and phenotypic abnormalities, who has 47 chromosomes. The extra small acrocentric chromosome consists of short arm, centromere, and the proximal third of the long arm of chromosome 14 as well as of the telomere region of the short arm of the X chromosome. (e) Partial karyotype of a reported patient (V.J.) with a 47,XX,+(14q−) karyotype. The extra 14q− chromosome is inherited from the t(14;20)(q22;q13) carrier mother and contains a slightly longer portion of the long arm of chromosome 14 than in patient C.M. (*12*). (f) Partial karyotype of S.C., a 5-year-old male who also has the "trisomy 14q− syndrome" with developmental retardation. He has inherited from his father the balanced reciprocal translocation t(2;14)(q37;q24). Due to nondisjunction during meiosis, he has received an additional copy of the 14q− chromosome. Therefore, he has 47 chromosomes with the extra acrocentric chromosome consisting of short arm, centromere, and slightly more than half the proximal long arm of chromosome 14, with the telomere region of the long arm of chromosome 2 attached.