Proc. Natl. Acad. Sci. USA
Vol. 78, No. 12, pp. 7670–7673, December 1981
Genetics

Down syndrome fibroblasts are hyperresponsive to β-adrenergic stimulation

(adenylate cyclase/chromosome 21/cyclic AMP)

JOHN D. MCSWIGAN*, DANIEL R. HANSON*, A. LUBINIECKI†, LEONARD L. HESTON‡, AND J. R. SHEPPARD*§

*Dight Institute for Human Genetics and ‡Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota 55455; and †Meloy Laboratories, Springfield, Virginia 22151

Communicated by John W. Littlefield, July 27, 1981

ABSTRACT **The hormonal response of human skin fibroblasts after exposure to β-adrenergic agonists, prostaglandin E_1 (PGE_1), and cholera toxin was monitored by intracellular cyclic AMP accumulation. Down syndrome (DS; trisomy 21) cells had an approximately 10-fold greater response to β-adrenergic agonists than did either normal diploid skin fibroblasts or other aneusomic fibroblast strains (trisomy 13, 18, and 22). The altered response in DS fibroblasts was specific for β-adrenergic agonists, because treatment of DS or control cells with PGE_1 or cholera toxin resulted in the same degree of cyclic AMP accumulation. Experiments with 3-isobutyl-1-methylxanthine, a cyclic nucleotide phosphodiesterase inhibitor, indicated that the increased response of DS fibroblasts was not primarily a function of altered cyclic AMP degradation. Monosomy 21 cells responded less than normal diploid fibroblasts to stimulation by the β-adrenergic agonist isoproterenol. These findings suggest that genetic information on chromosome 21 participates in regulating the β-adrenergic response of human fibroblasts.**

Down syndrome (DS), described by Langdon Down in 1866, is today recognized as mankind's most prevalent viable chromosomal anomaly, with a frequency of approximately 1 in 1000 live births. Despite the fact that Lejeune *et al.* (1) in 1959 reported the presence of an extra chromosome 21 in the cells of these patients, thereby identifying the cause of the disease at a cytological level, there is no effective treatment for the disorder and the molecular basis for expression of the DS phenotype remains unknown. Any such explanation would have to account for the diffuse symptoms affecting many organ systems in DS patients, of which the most distressing feature is severe mental retardation.

In addition to the mental deficiency, however, DS is also characterized by retarded physical development (2). *In vitro* studies of DS cells indicate that the presence of the extra chromosome 21 is associated with reduced cellular proliferation. These studies indicate that the time for cell cycle traverse is increased in the DS cell (3) and cultured DS fibroblasts have fewer cell doublings (4).

Physiological defects concerned with changes in membrane function are known to exist in DS cells. The well-documented increased sensitivity of DS cells to interferon is most likely due to an increased density of membrane-associated interferon receptors (5). On the other hand, platelets from trisomy 21 individuals have a decreased binding of serotonin (6), and Melman *et al.* (7) reported that trisomy 21 lymphocytes have a reduced response to the mitogen phytohemagglutinin. Rappaport and Bach (8) compared the antigens on lymphocytes from DS children with their normal siblings and found a significant proportion of unusual DS-associated antigens. More recently, changes in lymphocyte capping induced by concanavalin A and IgG were reported in DS lymphocytes (9). In addition to these membrane alterations, major differences exist in the catecholamine metabolism of DS patients. Dopamine β-hydroxylase activity, which catalyzes the production of norepinephrine, is decreased in DS plasma (10) and catechol *O*-methyltransferase activity is increased (11). Such membrane and metabolic changes could contribute to the well-known sensitivity of DS individuals to certain drugs (12).

The alterations in cell growth, membrane function, and catecholamine metabolism suggest that the cellular response to hormones could be perturbed in DS. To test this possibility, we examined the β-adrenergic, prostaglandin E_1 (PGE_1), and cholera toxin response of normal human diploid fibroblasts, trisomy 21 fibroblasts, monosomy 21 fibroblasts, and a variety of other human aneusomic fibroblast strains. The experimental results indicate that chromosome 21 influences the β-adrenergic responsiveness of human fibroblasts.

MATERIALS AND METHODS

DS skin fibroblasts purchased from the Human Genetic Mutant Cell Repository (Camden, NJ) were cell strain numbers GM 2067 and GM 2504. In addition, DS cell strains from Meloy Laboratories (ML 1199, ML 1252, and ML 2475) were also used in these studies. Normal diploid human skin fibroblasts (NSF) and non-DS aneusomic (NDA) strains (trisomy 13, 18, and 22) were initiated and cytologically verified by the cytogenetic laboratories at the University of Minnesota. Monosomy 21 skin fibroblast strains were from the Human Genetic Mutant Cell Repository (GM 230) and Meloy Laboratories (ML 468 and ML 2287). ^{3}H-Labeled cyclic AMP (cAMP) was purchased from New England Nuclear. PGE_1 was the gift of John Pike, Upjohn. 3-Isobutyl-1-methylxanthine (iBuMeX), an inhibitor of cyclic nucleotide phosphodiesterase, was purchased from Sigma.

The fibroblast cultures were maintained on Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum in a 37°C incubator with 5% CO_2. For testing, cells were plated at approximately 1.2×10^5 cells per 60-mm plate (Falcon or Nunc) and were examined at confluency unless noted otherwise. Medium was changed every 3 days and always 24 hr before an experiment. The cell lines used in all experiments were maintained for less than 20 passages in culture.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: DS, Down syndrome; cAMP, adenosine 3′,5′-cyclic monophosphate; iBuMeX, 3-isobutyl-1-methylxanthine; PGE_1, prostaglandin E_1; NSF, normal skin fibroblasts; NDA, non-DS aneusomic skin fibroblasts.

§ To whom reprint requests should be addressed.

Table 1. Hormonal responsiveness of human fibroblast strains

Type of cell	Response, nmol cAMP/mg protein: Basal	1 μM isoproterenol (10 min)	1 μM isoproterenol + 1 mM iBuMeX (10 min)	1 μM PGE_1 (10 min)	10 μg/ml cholera toxin (60 min)
NSF	0.040 ± 0.017	0.100 ± 0.039	0.368 ± 0.039	1.87 ± 0.563	2.77 ± 0.442
Non-DS trisomy	0.022 ± 0.009	0.070 ± 0.024	0.274 ± 0.197	1.43 ± 0.251	1.37 ± 0.225
DS	0.026 ± 0.016	0.746 ± 0.170	1.832 ± 0.284	1.74 ± 0.034	2.02 ± 0.454

These are data obtained from four separate experiments (performed with triplicate assays) using three NSF strains, three non-DS trisomy strains (trisomy 13, 18, and 22) and three DS strains (GM 2504, ML 1252, and ML 2475). Data are given as mean ± SEM. The cells were plated at approximately 4.0 μg of protein per cm^2 and medium was changed every third day. The cell cultures were confluent at the time of assays, which were performed 24 hr after the last medium change.

Hormone response experiments were performed with intact cells in 60-mm plates. The growth medium was aspirated and cells were washed twice with phosphate-buffered saline (pH 7.3) and 2 ml of this buffer at 37°C was added to the plate. Test agents were added at the concentration indicated and the cells were incubated for the times indicated. Reactions were terminated by aspiration of the test medium and then flooding of the dish with 1 ml of ice-cold 5% trichloroacetic acid. The trichloroacetic acid was extracted with HCl-acidified ether, and cAMP was measured by the isotope dilution method of Brown *et al.* (13). Protein on the plates was solubilized with 0.5 M NaOH and aliquots of solubilized protein were assessed by the method of Lowry *et al.* (14).

RESULTS

β-Adrenergic Response in DS Skin Fibroblasts. The cellular response of NSF or NDA fibroblasts to 1 μM isoproterenol was a 3- to 10-fold increase in the cellular cAMP level after 10 min (Table 1). This was in agreement with previously published results (15, 16). In contrast, DS skin fibroblasts showed a 30- to 80-fold increase in the basal cAMP level after exposure to this drug.

The range of the β-adrenergic response in DS cells was large, when all known relevant variables were controlled, but the response was always greater than that of controls. This increase in response was not a nonspecific trisomy effect, because cell strains trisomic for chromosomes other than 21 responded to a lesser degree than the trisomy 21 strains. Others have reported that cell culture density can exert a large effect on hormonal response (15, 17). The β-adrenergic response of the DS cells was consistently higher than that of NSF when tested as a function of time after subculture (Fig. 1). Both DS and NSF responded maximally at 2–3 days after subculture. Moreover, when cells were plated at different densities and tested 2 days later, the same biphasic effect of cell density on DS and NSF hormonal response was observed (Table 2). Cell culture density did not attenuate the increased β-adrenergic response exhibited by the DS fibroblasts.

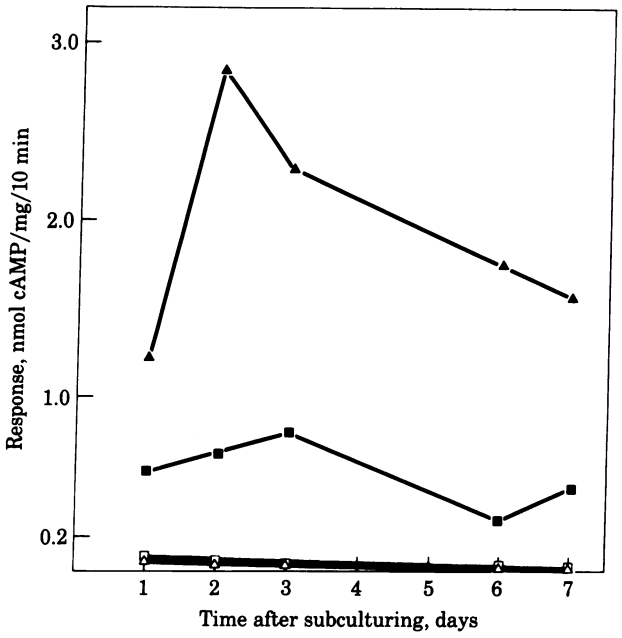

FIG. 1. Effect of time after subculturing on response to isoproterenol. △ and ▲, DS (GM 2504); □ and ■, NSF; open symbols, 1 mM iBuMeX; filled symbols, presence of 1 mM iBuMeX and 1 μM isoproterenol. Cell cultures were confluent at day 6.

Specificity of Increased β-Adrenergic Response in DS Fibroblasts. To determine the hormonal specificity of the increased response in DS cells for β-adrenergic agonists, we examined activators of adenylate cyclase that were independent of the β-adrenergic system. The NSF, NDA, and DS cell strains responded to PGE_1 or cholera toxin with accumulation of cAMP that did not differ significantly among the three classes of fi-

Table 2. β-Adrenergic responsiveness of human fibroblast strains at various cell densities

Cell strain	Incubation conditions	Cell density, μg protein/cm^2	Cell cAMP, nmol/μg protein
Control NSF	A	3.6 ± 0.85	0.081 ± 0.020
	B		0.312 ± 0.059
	A	5.4 ± 0.70	0.070 ± 0.026
	B		0.675 ± 0.028
	A	7.3 ± 0.15	0.038 ± 0.007
	B		0.385 ± 0.014
	A	14.6 ± 0.18	0.037 ± 0.020
	B		0.179 ± 0.026
DS (ML 1252)	A	4.4 ± 0.04	0.084 ± 0.017
	B		2.263 ± 0.151
	A	6.3 ± 0.13	0.050 ± 0.012
	B		3.151 ± 0.030
	A	9.1 ± 0.15	0.051 ± 0.005
	B		2.291
	A	16.9 ± 0.22	0.033 ± 0.004
	B		1.334 ± 0.027

NSF and DS skin fibroblasts were seeded at various densities and the cells were tested for β-adrenergic response 2 days later. The conditions of incubation were: A, 10-min exposure to 1 mM iBuMeX; B, 10-min exposure to 1 mM iBuMeX + 1 μM isoproterenol. The methods and conditions of the cAMP and protein measurements are described in the text. The data (shown as mean ± SEM) are derived from quadruplicate determinations.

broblasts (Table 1). Moreover, the response to PGE_1 and cholera toxin indicated that NSF and NDA cell strains were capable of accumulating very high levels of cAMP.

The increased hormonal response of DS cells to β-agonists could be due to an altered degradation of cAMP by its phosphodiesterase. The data presented in columns 2 and 3 of Table 1 show that iBuMeX potentiated the hormonal response of the NSF, DS, and NDA strains, indicating that each of these fibroblast cultures had a phosphodiesterase activity that was inhibited by iBuMeX. The similarity of NSF, NDA, and DS responses to PGE_1 also argues against phosphodiesterase involvement in the DS β-adrenergic response.

Further evidence for an association of the cellular response to β-adrenergic agonists with chromosome 21 came from a study of monosomy 21 fibroblasts. The response of monosomy 21 cells to the β-agonist isoproterenol (Fig. 2) was approximately half of that of a NSF control (in the presence or absence of iBuMeX). This fibroblast strain was karyotyped at the same time as the experiment was performed because of the known instability of monosomic cells in culture and was verified as having a single chromosome 21. Two other monosomy 21 strains showed a similar reduction in β-adrenergic responsiveness.

Pharmacology of β-Adrenergic Response in DS. Analysis of the β-adrenergic receptor subtype yielded results consistent with a β_2 receptor (Fig. 3) for both DS and NSF. Dose–response analysis for isoproterenol (Fig. 4*A*) and PGE_1 (Fig. 4*B*) indicated that the concentrations at which 50% of the activation occurred for both activators were similar in the two cell types (50 nM and 700 nM, respectively). Furthermore, the consistent similarity of response of the two classes of cells with increasing concentrations of PGE_1 was in sharp contrast to the differential effect of isoproterenol on NSF and DS fibroblasts.

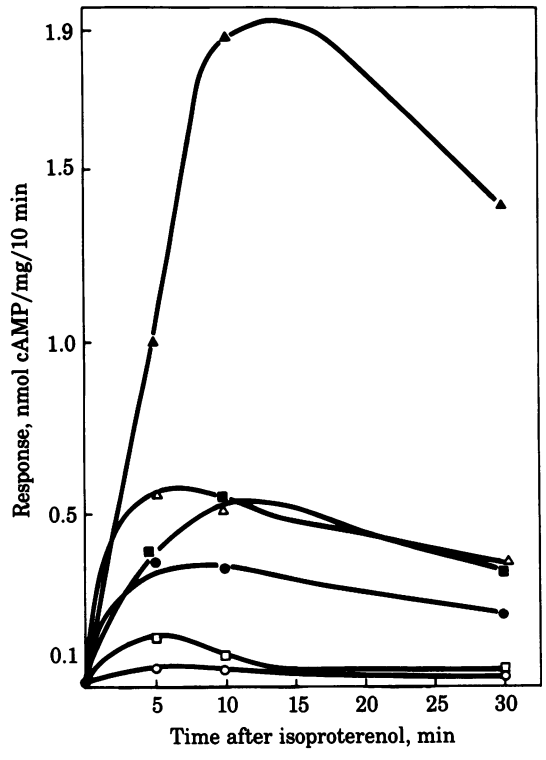

FIG. 2. Response to 1 μM isoproterenol of cells monosomic, diploid, and trisomic for chromosome 21. Response was measured as in Fig. 1. △ and ▲, DS (GM 2067); □ and ■, normal diploid; ○ and ●, monosomy 21 (ML 2287); open symbols, 1 μM isoproterenol; filled symbols, 1 μM isoproterenol and 1 mM iBuMeX. Cell cultures were confluent at the time of analysis.

DISCUSSION

These results describe an altered response of trisomy and monosomy 21 fibroblasts to β-adrenergic hormones. The altered β-adrenergic response was not the result of simply a chromosome imbalance, which has been reported for alkaline phosphatase (18). Other explanations that could account for the increased hormonal response in DS fibroblasts were also examined. An increased maximal catalytic capacity of the adenylate cyclase enzyme was not the cause, because PGE_1 and cholera toxin showed the same degree of enzyme activation in both DS and NSF cells. The possibility that the DS cells lacked cAMP phosphodiesterase activity was eliminated by the similarity of DS and NSF response to PGE_1 and cholera toxin as well as the similarity of effect by iBuMeX (a phosphodiesterase inhibitor) in the different cell strains. Cell culture density had a substantial effect on the hormonal response of both DS and NSF cell strains, as others had previously reported for NSF (15, 17), but did not change the relative difference between DS and NSF. DS fibroblasts did not exhibit a different affinity for the β-adrenergic agonist isoproterenol, because the concentration for 50% activation of the adenylate cyclase in DS and NSF fibroblasts was similar. Other explanations remain to be examined—e.g., superoxide dismutase activity is increased in trisomy 21 cells (19, 20) and has been closely linked to expression of the DS phenotype (21). It is possible this enzyme may participate in catecholamine regulation of adenylate cyclase activity.

Changes in β-adrenergic response have been noted in skin fibroblast cultures derived from individuals with cystic fibrosis, but these changes have not been consistent. Buchwald, who initially reported a 2- to 5-fold increase in the sensitivity of cystic fibrosis fibroblasts to β-adrenergic agonists (22), subsequently retracted the claim as being unreliable when a larger number of cystic fibrosis strains were compared to normal human fibroblasts (23).

Other changes in membrane structure and function have been associated with the extra chromosome 21 found in DS cells. Response to interferon (probably mediated by an inter-

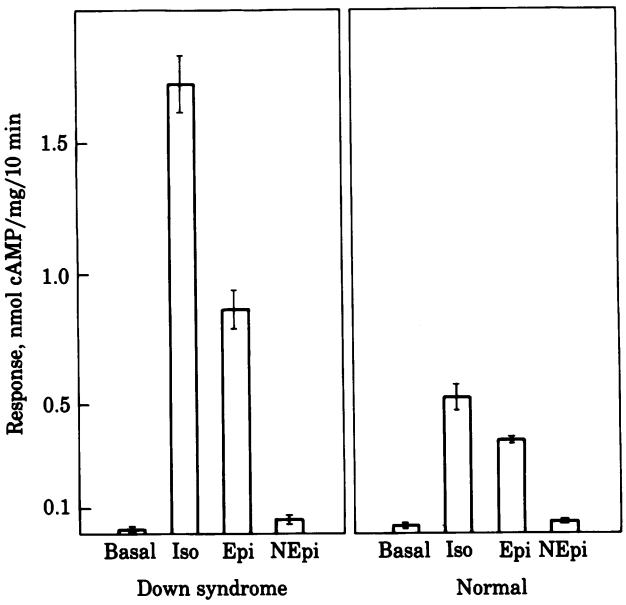

FIG. 3. Order of potency for β-adrenergic agonists. All β-adrenergic agonists were present at 1 μM concentration and in the presence of 1 mM iBuMeX. The DS fibroblasts (GM 2504) and NSF were confluent at the time of analysis. Error bars indicate SEM. Iso, isoproterenol; Epi, epinephrine; NEpi, norepinephrine.

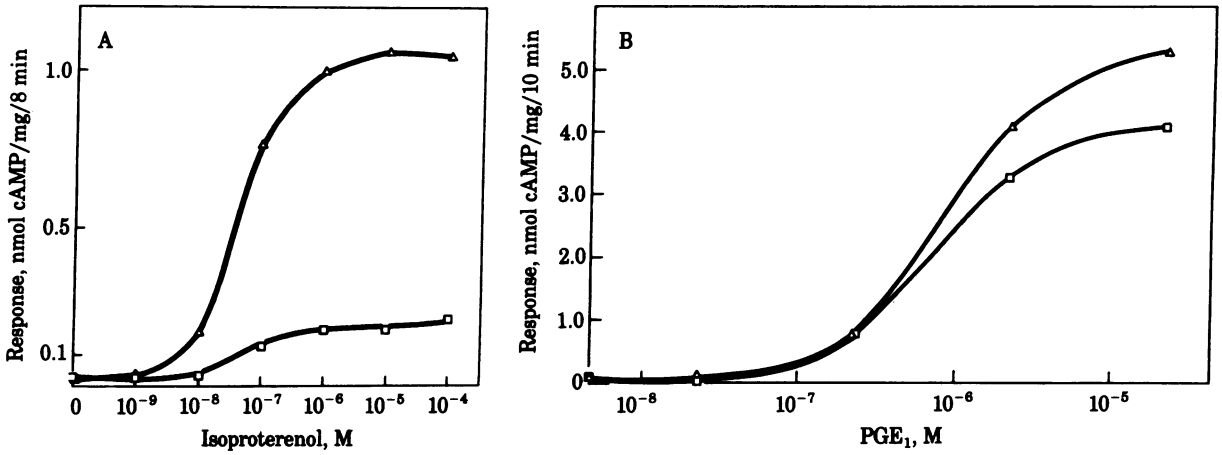

FIG. 4. Dose–response for isoproterenol and PGE_1. (*A*) Response to indicated isoproterenol concentrations at 2 min for DS fibroblasts (ML 1199) (△) and NSF (□). (*B*) Response to indicated PGE_1 concentrations at 10 min; symbols are the same as for *A*. The cell cultures were confluent at the time of analysis.

feron membrane receptor) has been assigned to chromosome 21 by somatic cell hybridization (24), gene dosage (5), and competition (25) experiments. Changes in immunosensitivity (26) and lectin capping (9) have also been reported for DS cells. These membrane changes or the previously observed changes in catecholamine metabolism (10, 11) may be related to the increased β-adrenergic hormone response. Direct involvement of the membrane in the pathophysiology of human genetic disorders has been previously observed in diabetes (27), familial hypercholesterolemia (28), and pseudohypoparathyroidism (29, 30). In contrast to DS, however, these disorders were characterized by an attenuated cellular response to a specific biological signal (e.g., insulin refractoriness in diabetes).

The DS phenotype is characterized by profound mental deficiency, by growth retardation (31), and, interestingly, by a high incidence of leukemia (32). It is conceivable that a change in the β-adrenergic response could significantly alter the mental and physical developmental patterns of the DS individual. Further examination of the trisomy 21 (and monosomy 21) fibroblast's response to β-adrenergic agonists may be useful not only for understanding the pathogenesis of the DS phenotype but also for studying the molecular regulation of adenylate cyclase enzyme activity by catecholamine hormones.

We thank Elizabeth Anton for excellent technical assistance and Karen Larson for her help in typing the manuscript. This work was supported by U.S. Public Health Service Grant NS 14436, The Leukemia Task Force, and National Institute of Mental Health Grant 1-T1 MH 10679.

1. Lejeune, J., Gautier, M. & Turpin, M. R. (1959) *C. R. Hebd. Seances Acad. Sci., Ser. D* **248**, 1721–1722.
2. Shapiro, B. L. (1975) *Ann. Hum. Genet.* **38**, 429–437.
3. Patton, C. R., Silver, M. F. & Allison, A. C. (1974) *Hum. Genet.* **23**, 173–182.
4. Schneider, E. & Epstein, C. (1972) *Proc. Soc. Exp. Biol. Med.* **141**, 1092–1094.
5. Tan, Y. H., Schneider, E. L., Tischfield, J., Epstein, C. J. & Ruddle, F. H. (1974) *Science* **186**, 61–63.
6. Jerome, D. M. & Kamoun, D. M. (1970) *Ann. N.Y. Acad. Sci.* **171**, 543–550.
7. Melman, W. J., Younkin, L. H. & Baker, D. (1970) *Ann. N.Y. Acad. Sci.* **171**, 537–542.
8. Rappaport, F. T. & Bach, F. H. (1973) *Transplant Proc.* **5**, 1139–1143.
9. Naeim, F. & Walford, R. L. (1980) *J. Gerontol.* **35**, 650–655.
10. Lake, C. R., Ziegler, M. C., Coleman, M. & Kopin, I. J. (1979) *Psychiatry Res.* **15**, 1–6.
11. Gustavson, K. H., Wetterberg, L., Backstrom, H. & Ross, S. B. (1973) *Clin. Genet.* **4**, 279–280.
12. Harris, W. S. & Goodman, R. M. (1968) *N. Engl. J. Med.* **279**, 407–410.
13. Brown, B. L., Ekins, R. P. & Albano, J. D. M. (1972) *Adv. Cyclic Nucleotide Res.* **2**, 25–39.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
15. Kelly, L. A. & Butcher, R. W. (1974) *J. Biol. Chem.* **249**, 3098–3102.
16. Franklin, T. J. & Foster, S. J. (1973) *Nature (London) New Biol.* **246**, 119–120.
17. Harden, T. K., Foster, S. J. & Perkins, J. P. (1979) *J. Biol. Chem.* **254**, 4416–4422.
18. Hosli, P. & Vogt, E. (1979) *Clin. Genet.* **15**, 487–494.
19. Sinet, P. M., Lejeune, J. & Jerome, H. (1979) *Life Sci.* **24**, 29–34.
20. Feaster, W. W., Kwok, L. W. & Epstein, C. J. (1977) *Am. J. Hum. Genet.* **29**, 563–570.
21. Hagemeijer, A. & Smit, E. M. E. (1977) *Hum. Genet.* **38**, 15–23.
22. Buchwald, M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2899–2903.
23. Buchwald, M. & Mapleson, J. L. (1977) *Mod. Probl. Paediatr.* **19**, 165–174.
24. Tan, Y. H., Tischfield, J. & Ruddle, F. H. (1973) *J. Exp. Med.* **37**, 317–330.
25. Slate, D. L. & Ruddle, F. H. (1979) *Pharmacol. Ther., Part B* **4**, 221–230.
26. Wisniewski, K., Cobill, J. M., Wilcox, C. B., Caspary, E. A., Williams, D. C. & Wisniewski, H. M. (1979) *Biol. Psychiatry* **14**, 463–471.
27. Roth, J., Lesniak, M. A., Bar, R. S., Muggeo, M., Megyesi, K., Harrison, L. C., Flier, J. S., Wachslicht-Rodbard, H. & Gordon, P. (1979) *Proc. Soc. Exp. Biol. Med.* **162**, 3–12.
28. Brown, M. S. & Goldstein, J. L. (1976) *Science* **191**, 150–153.
29. Drezner, M. K. & Burch, W. M. (1978) *J. Clin. Invest.* **62**, 1221–1227.
30. Farfel, Z., Brickman, A. S., Kaslow, H. R., Brothers, V. M. & Bourne, H. R. (1980) *N. Engl. J. Med.* **303**, 237–242.
31. Segal, J. D. & McCoy, E. H. (1974) *J. Cell. Physiol.* **83**, 85–90.
32. Krivit, W. & Good, R. (1957) *Am. J. Dis. Child.* **94**, 289–293.