Abnormal regulation of monocyte insulin-binding affinity after glucose ingestion in patients with myotonic dystrophy

(autosomal dominant disorder/membrane disease/insulin receptor/insulin insensitivity)

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ABSTRACT   Insulin insensitivity of uncertain etiology often exists in myotonic muscular dystrophy, a multitissue, autosomal dominant disorder hypothesized to be a hereditary membrane disease. The present studies show that monocytes from patients with myotonic dystrophy fail to demonstrate the normally observed qualitative increase in insulin-binding affinity after oral glucose loading. Monocytes from 10 normal volunteers developed a significantly increased insulin-binding affinity by 2 hr after glucose ingestion (mean ± SEM, 11.7 ± 2.7 ng/ml compared to basal 50% insulin displacement value of 23.3 ± 2.2 ng/ml, $P < 0.005$). This increase was maintained at 5 hr (13.5 ± 2.7 ng/ml, $P < 0.05$). In contrast, no significant increase in monocyte insulin-binding affinity occurred in cells from nine myotonic dystrophy patients at 2 and 5 hr after glucose loading (50% insulin displacement values: basal, 14.2 ± 2.8 ng/ml; 2 hr, 16.7 ± 1.7 ng/ml; 5 hr, 10.8 ± 2.1 ng/ml). These alterations document the presence of abnormalities in the insulin receptor or receptor-associated processes that modulate insulin binding. A hereditary plasma membrane defect may underlie these findings. This abnormality may have an etiologic role in the decreased insulin sensitivity that frequently afflicts patients with myotonic dystrophy.

Myotonic dystrophy is an autosomal dominant, multisystem disease (1); patients frequently exhibit insulin insensitivity (2–5). The major impact of this disease is on skeletal muscle, but recent evidence has demonstrated abnormalities associated with the plasma membrane of many tissues [skeletal muscle (6–8), erythrocyte (9–11), platelet (12), neutrophil (13), and lens (14)]. The etiology of the decreased insulin insensitivity and the muscle wasting that afflicts these patients is unknown. However, both problems could result from a hereditary plasma membrane defect that has compromised both the binding of insulin to its target tissues and the normal anabolic effects of this hormone.

To search for a possible alteration in insulin receptor binding in myotonic dystrophy, we have performed insulin binding studies in circulating monocytes obtained before and after glucose ingestion. Monocytes have been selected for study because they are a readily accessible tissue and have been shown to have specific insulin receptors whose binding characteristics may reflect the state of insulin sensitivity of other target tissues (15–17). Evaluation of insulin binding in skeletal muscle of patients is not technically feasible in view of the small yield and impurity of sarcolemma isolated from muscle biopsy.

This study used an oral glucose challenge because previous work demonstrated an increase in monocyte insulin receptor affinity 2 and 5 hr after carbohydrate loading (18, 19). The use of this test in patients with myotonic dystrophy allows exami-

nation of the acute regulation of receptor binding after a physiologic stimulus. This approach provides an opportunity to search for insulin binding abnormalities that are present when insulin secretion is occurring at both basal and increased rates. The present study demonstrates a failure of monocytes from myotonic dystrophy patients to develop the normal qualitative increase in their insulin binding affinity 2 and 5 hr after glucose ingestion.

METHODS

Glucose Tolerance and Patient Data. Oral glucose tolerance testing after specific dietary preparation was performed as described (2). All subjects (9 myotonic dystrophy, 10 normal volunteers, and 2 myotonia congenita) were less than 120% of ideal body weight (Metropolitan Life Tables, 1959). Each had normal glucose tolerance (20). Myotonic dystrophy patients displayed normal fasting insulin levels [mean ± SEM, 21 ± 4 microunits/ml (normal, 16 ± 2)]; however, after glucose ingestion they manifested hyperinsulinemia of varying degrees similar to that found in other investigations (1–5).

Monocyte Separation. Heparinized blood (120 ml) was collected after an overnight fast and 2 and 5 hr after glucose ingestion, and monocytes were separated according to the method of Boyum (21). Blood was diluted 1:1 with phosphate-buffered saline (pH 7.4), and 20-ml aliquots were layered over 10 ml of the Ficoll/Hypaque gradient and centrifuged ($500 \times g$) at 18°C for 30 min. The mononuclear fraction was removed with siliconized Pasteur pipettes and was washed and resuspended in 25 mM Tris/1% albumin, pH 7.6. The percentage of monocytes in the final preparation was determined by cell size (22) and esterase staining (23). No significant differences in percentages or sizes of monocytes were seen on comparing the 0-, 2-, and 5-hr samples between or within groups. The mean (±SEM) percentage of monocytes in samples from both the myotonic dystrophy and normal groups fell in the range 21.3 ± 2.1% to 23.5 ± 2.4%. Cell viability as assessed by trypan blue exclusion was greater than 98%.

Insulin Binding. ^{125}I-Labeled insulin (125I-insulin; 100–200 Ci/g; 1 Ci = 3.7×10^{10} becquerels) at a concentration of 0.5 nM was incubated with the mononuclear mixture containing 10^6 monocytes. Cells were incubated in 25 mM Tris/1% albumin, pH 7.6, in the absence or presence of unlabeled insulin over a concentration range of 0.5 nM to 0.83 µM in a final volume of 0.35 ml for 3 hr at 22°C. After incubation, the mixture was added to Microfuge tubes containing 100 µl of *n*-butyl phthalate and centrifuged. The tube tips containing the cell pellets plus bound insulin were cut off and assayed for radioactivity in a

Abbreviations: B/F, bound/free; B_{50}, concentration of unlabeled insulin required to displace 50% of bound 125I-insulin.

gamma counter. The relatively low number of monocytes used in the binding assay was dictated by the limited amounts of blood that could be collected at 0, 2, and 5 hr after the oral glucose tolerance test. We considered an alternative approach of drawing 300–400 ml of blood at each sampling interval, which would have allowed the use of larger numbers of monocytes and hence a lower tracer concentration. However, this would also have required separate retransfusion of erythrocytes and plasma into each experimental subject, complicating the logistics of the clinical study and introducing a major maneuver which by itself might alter insulin binding. This would have necessitated additional control investigations. We therefore chose higher tracer concentrations which still allow unambiguous comparison of results with cells of normal subjects and with those of the patients.

All binding assays were performed in triplicate. Insulin degradation as assessed by trichloroacetic acid precipitability during the 3-hr incubation was less than 7% for all experiments and was not significantly different between the study groups. Nonspecifically bound insulin was determined as reported (17) and was 0.2–0.8% of total radioactivity at an insulin concentration of 5 μg/ml. pH was stable during incubation and fell no more than 0.16 unit. All group data are presented as means ± SEM.

RESULTS

^{125}I-Insulin Binding to Monocytes Before and After Glucose Ingestion. ^{125}I-Insulin displacement curves in monocytes ob-

tained from normals and from patients with myotonic dystrophy before and at 2 and 5 hr after glucose ingestion are shown in Fig. 1. The basal curves show a more rapidly falling specifically bound-to-free (B/F) insulin ratio in cells from patients with myotonic dystrophy compared to normals. This increase in insulin binding affinity in monocytes from overnight-fasted myotonic dystrophy patients is emphasized by the significantly lower concentration of unlabeled insulin required to displace 50% of the specifically bound ^{125}I-insulin (B_{50}) compared to cells from normals (myotonic dystrophy, 14.2 ± 2.8 ng/ml; normal, 23.3 ± 2.3 ng/ml; $P < 0.05$). Neither the basal nor the 2- or 5-hr glucose B/F ratio in the presence of tracer alone revealed any significant differences between the normal and myotonic dystrophy groups.

^{125}I-Insulin displacement curves for monocytes obtained from normals 2 and 5 hr after glucose ingestion show a much greater rate of fall in the B/F ratio compared to their 0 time curve. This contrasts with the 2- and 5-hr insulin displacement curves seen in cells from the myotonic dystrophy patients, which showed no change from basal at 2 hr after glucose and only a slightly increased rate of fall at 5 hr. Cells from the two patients with myotonia congenita, unlike those from patients with myotonic dystrophy, showed the same increased rate of decline in B/F ratio in the 2- and 5-hr curves as seen in normals. These findings suggest that hereditary myotonia alone does not account for the failure of monocytes from myotonic dystrophy patients to develop a normal postprandial increase in insulin-binding affinity.
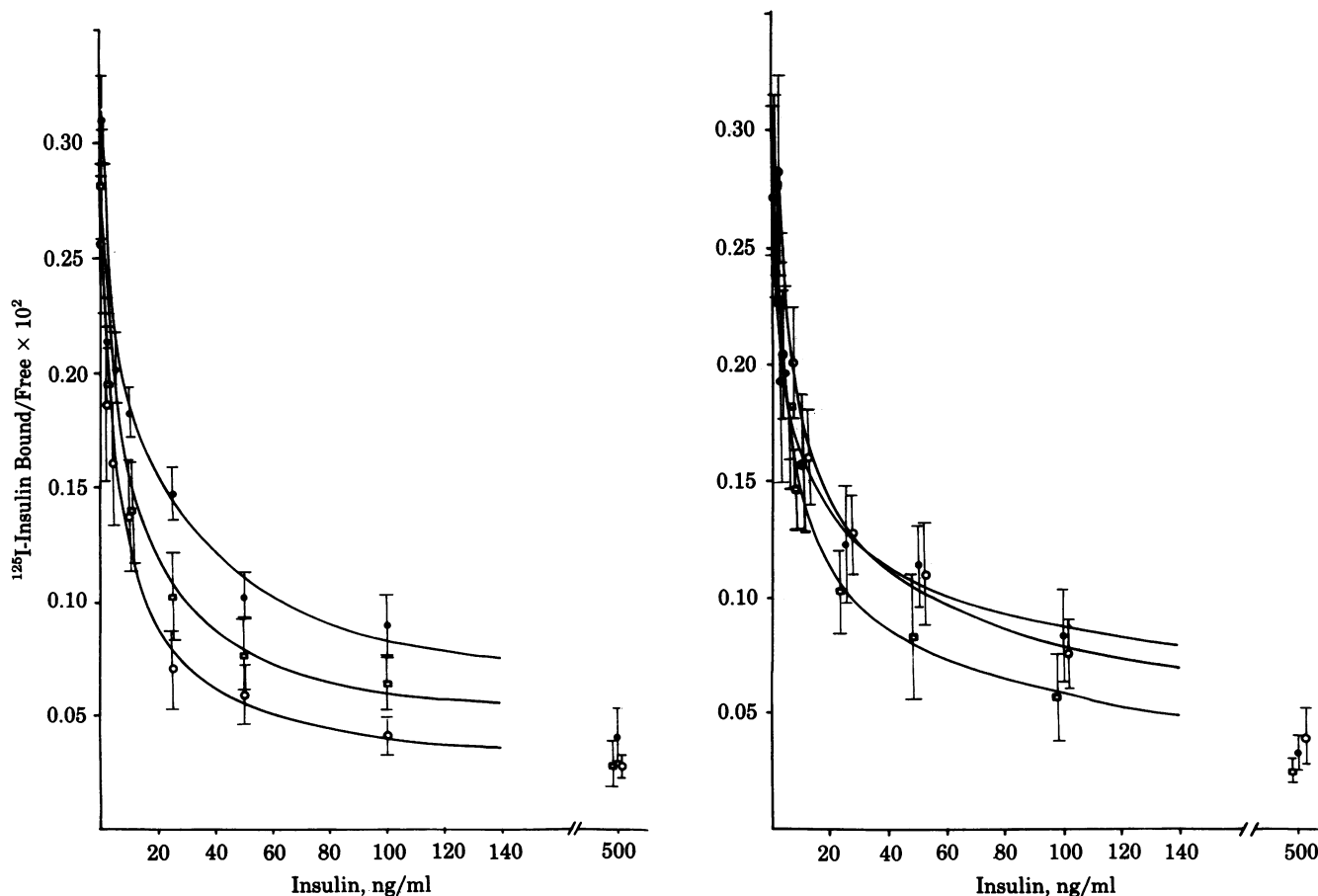

FIG. 1. Competition curves for specific ^{125}I-insulin binding to monocytes before and 2 and 5 hr after oral glucose tolerance testing in 9 myotonic dystrophy patients (*Right*) and 10 normal controls (*Left*). Mononuclear cell suspensions containing 10^6 monocytes were incubated in the absence (initial point on the curve) and presence of increasing concentrations of unlabeled insulin. Data are corrected for nonspecific binding. Each point on the curve represents the mean ± SEM. ●, Basal; □, 5 hr; ○, 2 hr.

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The significant difference in the insulin displacement curve shapes between myotonic dystrophy patients and normals after oral glucose is emphasized by the data from individual patients presented in Fig. 2. Paired t analysis comparing 0 to 2 hr and 0 to 5 hr after glucose showed no significant difference in these values (0-hr B_{50}, 14.2 ± 2.8 ng/ml; 2-hr B_{50}, 16.7 ± 1.7 ng/ml; 5-hr B_{50}, 10.8 ± 2.1 ng/ml). Six of eight B_{50} values either increased or remained unchanged at 2 hr and four of six did so at 5 hr in the myotonic dystrophy patients; none of eight normals at 2 hr and three of eight at 5 hr had B_{50} values that showed such behavior. There was no correlation between B_{50} changes and age, weight, basal insulin concentration, or degree of hyper-insulinemia after glucose digestion.

In marked contrast to the myotonic dystrophy group, normal subjects showed a 2-fold increase in cellular insulin binding affinity after the oral carbohydrate challenge. Paired t analysis of the data indicated that this change is significant at both 2 and 5 hr (0-hr B_{50}, 23.2 ± 2.2 ng/ml; 2 hr B_{50}, 11.7 ± 2.7 ng/ml; 5-hr B_{50}, 13.5 ± 2.7 ng/ml; for 0 vs. 2 hr, $P < 0.005$; for 0 vs. 5 hr, P (< 0.05).

Fig. 3 presents Scatchard plots of the mean monocyte insulin binding data for the myotonic dystrophy and normal groups at 0, 2, and 5 hr after oral glucose loading. The differences in the pattern of serial changes in the insulin displacement curves between the normals and myotonic dystrophy patients is re-emphasized, and a suggestion of a difference between these groups in the number of specific insulin binding sites per monocyte is evident. All the plots are curvilinear, and this shape contributes to uncertainty in exact determination of the intercept of the curves with the abscissa. This intersection point is used to estimate the total insulin-binding capacity per monocyte (R_o) and allows an approximate calculation of the number of specific insulin binding sites per cell. There appears to be a greater number of insulin binding sites in the monocytes from overnight fasted normal subjects than in those from the patients, normals having 18,100 per cell and myotonic dystrophy patients having 11,100 per cell. However, this difference is not statistically significant because of the large standard errors in the final point for each curve.
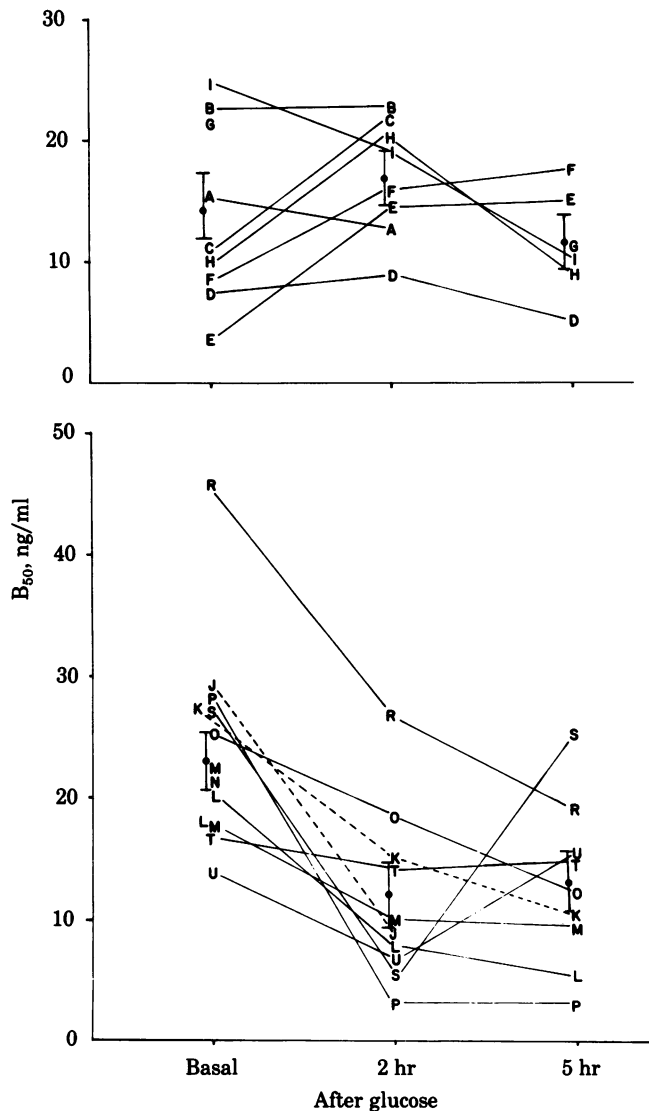
DISCUSSION

This investigation used the physiologic stimulus of an oral glucose challenge to investigate the acute regulation of monocyte insulin binding in patients with myotonic muscular dystrophy, an autosomal dominant disease frequently manifesting some degree of insulin insensitivity (1–5). Our findings show that monocytes isolated from myotonic dystrophy patients 2 and 5 hr after glucose ingestion fail to display a normal qualitative increase in insulin-binding affinity. No clear explanation for this is available. Monocytes from our fasting myotonic dystrophy patients had a higher insulin-binding affinity (B_{50} 70% lower) than did cells from fasting normals (Fig. 2). This difference in basal binding may indicate that the monocytes from myotonic dystrophy patients are already in the high-affinity form normally seen postprandially. This interpretation is supported by the data from two of the nine myotonic dystrophy patients. These patients eventually developed an increase in monocyte binding affinity 5 hr after glucose loading. They had basal B_{50} values (Fig. 2) that were higher than those of the other patients and their absolute values fell within the normal range. Whether the failure of monocytes from the seven other patients to develop a normal qualitative increase in their binding affinity after glucose ingestion was causally related to their increased basal receptor affinity is a possibility requiring further study. Future investigations of monocyte insulin binding before and after carbohydrate challenge in conditions reported to have increased basal monocyte insulin binding affinity, such as acromegaly (24), obesity, after a 72-hr fast (25), and insulinoma (26), may also clarify this hypothesis.

An alteration in the insulin receptor or a derangement subsequent to binding or a combination of these two may underlie the insulin-binding abnormalities that this study has shown. There is evidence suggesting that an altered plasma membrane environment may surround the insulin receptor in myotonic dystrophy cells. Plasma membrane-associated abnormalities have been described in different tissues [skeletal muscle (6–8), erythrocyte (9–11), platelet (12), neutrophil (13), and lens (14)]. These observations have prompted the hypothesis that myotonic dystrophy is a hereditary membrane disease and are consistent with a post-binding defect as an explanation for the failure of monocytes in these patients to display a normal postprandial increase in insulin-binding affinity. Other recent reports have described the presence of a non-insulin-binding
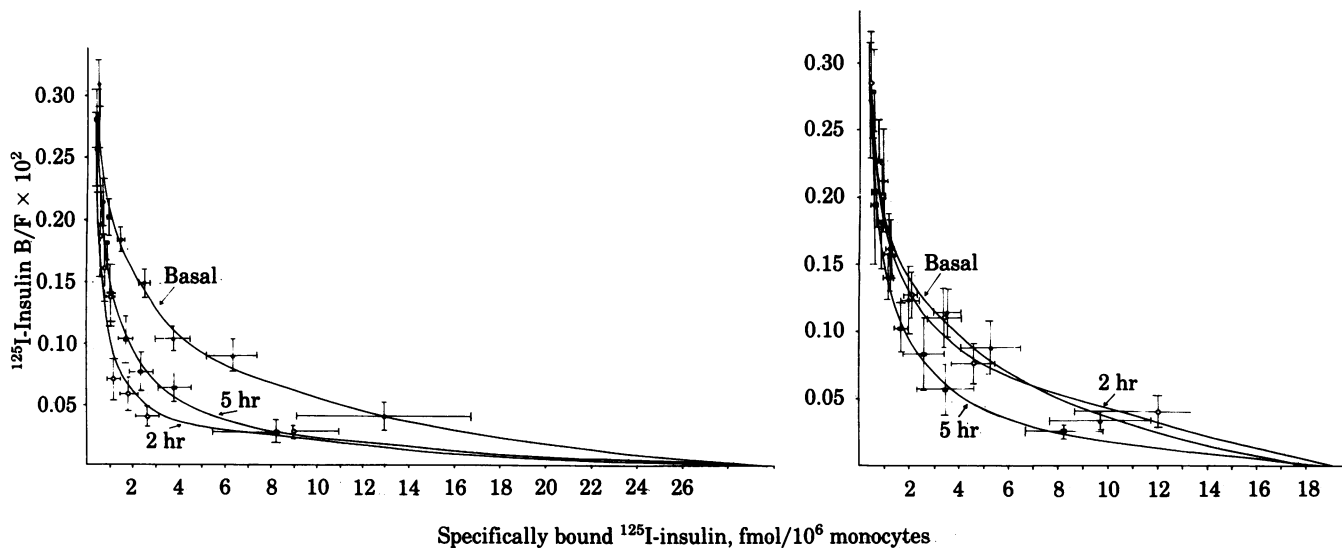

FIG. 2.   Unlabeled insulin concentrations required to reduce by 50% the specific binding of ^{125}I-insulin binding (B_{50}) to 10^6 monocytes before and 2 and 5 hr after oral glucose tolerance testing in 9 myotonic dystrophy patients (A–I) (Upper), and in 2 myotonia congenita controls (J and K) and 10 normal controls (L–U) (Lower). Normal controls L and M underwent repeat basal binding studies 4 months apart. No 5-hr samples were collected from myotonic dystrophy patients A, B, and C.

FIG. 3. Scatchard analysis of insulin binding data for the 9 myotonic dystrophy patients (*Right*) and 10 normal controls (*Left*). Each point represents the mean ± SEM. Total insulin binding capacity is indicated by the intercept of each curve at the abscissa.

component of the plasma membrane that affects the binding affinity for insulin (27–29). A hereditary lesion that deleteriously influences the function of such a membrane component may exist in myotonic dystrophy and in this way may prevent the development of the normal increase in insulin receptor affinity after oral glucose loading.

The physiologic significance of the binding abnormalities we have observed in our patients is unknown. Nevertheless, an attempt to relate these alterations to the insulin insensitivity that frequently occurs in this disease (1–5) seems appropriate. Ideally, skeletal muscle as well as monocyte insulin-binding studies should have been performed to establish a more direct correlation with the severe forearm muscle insulin insensitivity that has been reported from our laboratory (2, 3). But these investigations are not technically feasible because of the small yield and impurity of sarcolemma that is isolated from a muscle biopsy specimen. Monocytes with specific insulin receptors are easily accessible from our patients, and binding to these cells may reflect the behavior of the receptor in less-available target organs. Previous studies of other diseases that manifest decreased insulin responsiveness have demonstrated a correlation between insulin binding to circulating monocytes and diminished insulin action in other tissues (15–17). The primary abnormality that these prior investigations report is a decrease in the number of specific insulin binding sites in cells collected from subjects who fasted overnight. In this respect there is a similarity between these studies and data from two (30, 31) of three (30–32) past investigations of basal monocyte insulin binding in myotonic dystrophy, which have described a decrease in monocyte insulin receptor number. The present study has also detected a small decrease in the number of specific insulin binding sites but it is not statistically significant.

The insulin binding alterations that this report describes possibly result from complex mechanisms extending beyond the receptor itself. In this regard, previous investigations in our laboratory suggest that a decrease in insulin receptor number alone is not sufficient to explain the persistently low insulin-stimulated muscle glucose uptake seen in our patients after *in vivo* exposure to supraphysiologic levels of insulin (3). This suggests that a post-binding derangement underlies or contributes to the forearm muscle insulin insensitivity in addition to the alterations found in the modulation of receptor affinity.

1. Harper, P. S. (1979) *Myotonic Dystrophy*, Major Problems in Neurology, Vol. 9 (Saunders, Philadelphia, pp. 1–324.
2. Moxley, R. T., Griggs, R. C. & Goldblatt, D. (1978) *J. Clin. Invest.* 62, 857–867.
3. Moxley, R. T., Griggs, R. C. & Goldblatt, D. (1980) *Neurology* 30, 1077–1083.
4. Barbosa, J., Nuttall, F. Q., Kennedy, W. & Goetz, F. (1974) *Medicine* 53, 307–323.
5. Tevaarwerk, G. J. M. & Hudson, A. J. (1977) *J. Clin. Endocrinol. Metab.* 44, 491–498.
6. Roses, A. D. & Appel, S. H. (1974) *Nature (London)* 250, 245–247.
7. Schotland, D. L. & Bonilla, E. (1977) *Neurology* 27, 379 (abs.).
8. Reddy, N. M., Oliver, K. L. & Engel, W. K. (1977) *Neurology* 8, 378–379.
9. Roses, A. D. & Appel, S. H. (1973) *Proc. Natl. Acad. Sci. USA* 70, 1855–1859.
10. Butterfield, D. A., Chesnut, D. B., Roses, A. D. & Appel, S. H. (1974) *Proc. Natl. Acad. Sci. USA* 71, 909–913.
11. Plishker, G. A., Gitelman, H. J. & Appel, S. H. (1978) *Science* 200, 323–325.
12. Bousser, M. G., Conrad, J., Lecrubier, C. & Samama, M. (1975) *Lancet* ii, 307–309.
13. Seay, A., Ziter, F. & Hill, H. (1978) *J. Neurol. Sci.* 35, 25–30.
14. Eshaghian, J., March, W., Grossens, W. & Rafferty, N. S. (1978) *Invest. Ophthalmol. Vis. Sci.* 17, 289–293.
15. DeFronzo, R. A., Soman, V., Sherwin, R. S., Hendler, R. & Felig, P. (1978) *J. Clin. Invest.* 62, 204–213.
16. Olefsky, J. M. (1976) *J. Clin. Invest.* 57, 1165–1172.
17. DeFronzo, R. A., Deibert, D., Hendler, R., Felig, P. & Soman, V. (1979) *J. Clin. Invest.* 63, 939–946.
18. Muggeo, M., Bar, R. S. & Roth, J. (1977) *J. Clin. Endocrinol. Metab.* 44, 1206–1209.
19. DePirro, R., Bertoli, A.; Creco, A. V., Gelli, A. S. & Lauro, R. (1979) *Acta Endocrinol. (Copenhagen)* 90, 473–480.
20. National Diabetes Data Group (1979) *Diabetes* 28, 1039–1057.
21. Boyum, A. (1968) *Scand. J. Clin. Invest.* Suppl. 97 21, 77–89.
22. Loos, H., Blok-Schut, B., Kipp, B., VanDoorn, R. & Meerhof, L. (1976) *Blood* 48, 743–753.
23. Li, C. Y., Lam, K. W. & Yam, L. J. (1973) *J. Histochem. Cytochem.* 21, 1–12.

24. Muggeo, M., Bar, R. S., Roth, J., Kahn, C. R. & Gorden, P. (1979) *J. Clin. Endocrinol. Metab.* **48,** 17–25.
25. Bar, R. S., Gordon, P., Roth, J., Kahn, C. R. & DeMeyts, P. (1976) *J. Clin. Invest.* **58,** 1123–1135.
26. Bar, R. S., Gorden, P., Roth, J. & Siebert, C. W. (1977) *J. Clin. Endocrinol. Metab.* **44,** 1210–1213.
27. Harmon, J. T., Kahn, C. R., Kampner, E. & Schlegel, W. (1979) *Endocrinology* Suppl. 1, **104,** 118.
28. Maturo, J. M. & Hollenberg, M. D. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3070–3074.
29. Maturo, J. M., Schackelford, W. H. & Hollenberg, M. D. (1978) *Life Sci.* **23,** 2063–2072.
30. Festoff, B. W. & Moore, W. V. (1979) *Ann. Neurol.* **6,** 60–65.
31. Tevaarwerk, G. J. M., Strickland, K. P., Lin, G. H. & Hudson, A. J. (1979) *J. Clin. Endocrinol. Metab.* **49,** 216–222.
32. Kobayashi, M., Meek, J. C. & Streib, E. (1977) *J. Clin. Endocrinol. Metab.* **45,** 821–824.