Effect of Multiple Daily Insulin Injections on the Course of Diabetic Retinopathy

Didier Job, M.D., Eveline Eschwege, M.D., Claudine Guyot-Argenton, M.D., Jean-Pierre Aubry, M.D., and Georges Tchobroutsky, M.D., Paris, France

SUMMARY

Forty-two diabetic patients on insulin once a day in the early stage of diabetic retinopathy were randomly assigned to one of two kinds of insulin regimen, i.e., single or multiple daily injections. Retinal changes were quantitatively estimated by counting the microaneurysms (MAs) observed on fluorescein angiograms at the posterior pole of the more diseased eye. Baseline characteristics of the two groups were not significantly different. These included duration of diabetes, age at diagnosis, daily dose of insulin, amount of urinary sugar excreted in 24 hours, fasting blood sugar (FBS), and number of MAs. During the follow-up (mean duration of three years) the mean yearly progression in the number of MAs was significantly less in the multiple- than in the single-injection group: 1.8 ± 0.7 versus 7.2 ± 1.9 ($p<0.01$; nonparametric test: $p<0.02$). Final values were, respectively, MAs: 15.2 ± 4.9; 33.0 ± 7.9; glycosuria (gm./24 hrs): 20.6 ± 2.5; 27.5 ± 4.3; FBS (mg./100 ml.): 154 ± 15; 195 ± 11. P values comparing the two groups were <0.02, <0.02, and <0.05.

Thus, in this clinical trial, made under routine treatment conditions, the use of divided daily insulin injections was effective in improving diabetic control and delaying retinal changes. DIABETES *25*:463-69, May, 1976.

Prospective studies on the effect of the treatment of diabetes on the course of microangiopathy are scarce but strongly suggest that insulin administration and/or the control of diabetes reduce and delay the development of retinal and glomerular changes in diabetic humans [1-4] and animals.[5-8] Indirect arguments have also been given by biochemists.[9-11] Previously retrospective studies have claimed that the quality of diabetes control influences the course of retinopathy.[12-16] Not all authors[17,18] have agreed with such a hypothesis—which, however, has been accepted by most physicians engaged in the treatment of diabetes as recently reviewed.[19-21]

In order to improve the control of diabetes in insulin-dependent patients, the use of divided daily doses of insulin was emphasized or advocated by several authors.[14,22-29] Such a technic in short-term studies has resulted in lower blood glucose values[30] and higher plasma insulin levels[31-33] than conventional insulin regimens.

The aim of this clinical trial was to compare the effect of divided daily insulin administrations versus one daily insulin injection on the course of early diabetic retinopathy.

MATERIALS AND METHODS

Patients and Treatments

The study was begun on 52 insulin-dependent

Presented in part at the Thirty-fifth Annual Meeting of the American Diabetes Association in New York, June 15-17, 1975, and published in abstract form in Diabetes *24*, suppl. 2, 1975, p. 396.

From the Unité de Recherches Statistiques, 16 bis avenue P.V. Couturier, 94, Villejuif, France, the Department of Ophthalmology and the Department of Diabetes, Hôtel-Dieu Hospital, and Pierre and Marie Curie University, place du Parvis Notre-Dame, Paris 4ème, 75004.

Accepted for publication March 4, 1976.

diabetic patients selected on the basis of (1) age below 60 years, (2) treatment by one daily insulin injection, with daily doses less than 80 units and more than 18, (3) no life-threatening disease other than diabetes, (4) known reliable regular outpatients, (5) early stage of diabetic retinopathy (DR) in the more diseased retina: at least, tortuosity of perimacular veins observed by ophthalmoscopy and independently confirmed by fluorescein angiograms to maximal lesions: microaneurysms with or without retinal hemorrhages and/or exudates of any type but without proliferative retinopathy as seen by ophthalmoscopy. Patients with bilateral keratopathy, cataract, vitreous opacification, or glaucoma were excluded.

By this screening, patients with normal fundi or proliferative retinopathy and patients with social or psychologic instability were excluded.

The patients were not asked about their willingness to accept multiple daily insulin injections in the future. They were randomized in one of the two insulin regimes: single (S) or multiple (M) doses of daily insulin.

The first group used long-acting insulin alone or with short- or intermediate-acting in the same injection. The second group used short-acting before breakfast and lunch and intermediate insulin (with or without short-acting) before dinner or, if the three-injection regime was not accepted by the patient after the random allocation, intermediate insulin before breakfast and before dinner.

Twenty-seven patients were assigned to the single-injection group and 25 to the multiple-injection group. During the first year of the study 10 patients were lost to follow-up, six of the S and four of the M group. These latter patients did not differ from the remaining subjects for any baseline parameters, including retinal lesions. Thus, this trial deals with 21 patients in each group followed for one to four years.

Baseline Characteristics

The baseline clinical features are shown in table 1. Only one of the 17 comparisons showed a significant difference between the two groups, i.e., diastolic blood pressure was higher in the M group than in the S group but still below 90 mm. Hg.

The mean daily doses of insulin and the mean values of the total glucose excreted in the urine in 24 hours and fasting blood sugar were calculated from the results given by routine examinations every three months during the year before the beginning of the study plus a complete determination at the beginning.

Retinal Study

All patients were studied by the same ophthalmologists (C.G.A. and J.P.A.), who performed annual fluoroangiograms[34] and funduscopies. Fluorograms were taken of the posterior pole, temporal from the macula to the nasal side of the nervehead, including an area of five disc diameters in diameter, vertically as well as horizontally. Microaneurysms were counted on the best film.

Since the study was designed in 1968 and started in 1969, this criterion of DR was selected and no study of retinal areas of vascular nonperfusion was done.

During the survey no other treatment except diet and insulin was given.

TABLE 1

Baseline characteristics (means ± S.E.M.)

	Single-injection group	Multiple-injection group	P value
Number of patients	21	21	
Number of men	13	10	NS
Weight (kg.)	64.6 ± 2.2	63.1 ± 2.4	NS
Height (cm.)	166 ± 2	166 ± 2	NS
Systolic blood pressure (mm. Hg)	133 ± 4.6	146 ± 5.4	NS
Diastolic blood pressure (mm. Hg)	80 ± 1.9	86 ± 2.0	<0.05
Serum cholesterol (mg./100 ml.)	221 ± 9	218 ± 10	NS
Total serum lipids (gm./L.)	6.1 ± 0.4	5.7 ± 0.3	NS
Plasma creatinine (mg./100 ml.)	9.9 ± 0.3	9.8 ± 0.3	NS
Duration of diabetes (yrs.)	12.7 ± 1.5	13.4 ± 1.2	NS
Duration of insulin treatment (yrs.)	10.7 ± 1.5	11.4 ± 1.4	NS
Age at diagnosis of diabetes (yrs.)	24.9 ± 2.1	28.9 ± 2.2	NS
Age at onset of the trial (yrs.)	36.9 ± 2.2	42.4 ± 2.5	NS
Number of microaneurysms	12.7 ± 3.5	9.0 ± 3.3	NS
Daily dose of insulin (U./24 hrs.)	42.1 ± 2.9	40.6 ± 3.1	NS
Urinary sugar (gm./24 hrs.)	22.1 ± 4.7	28.5 ± 4.2	NS
Fasting blood sugar (mg./100 ml.)	210 ± 12	200 ± 19	NS

Follow-up

The patients were followed up for one to four years, with a mean duration time of 34.9 ± 2.5 months and 38.1 ± 2.2 months for the S and M groups, respectively (N.S.). The study was started in October, 1969, and the last patient included in June, 1971; only four patients (three S and one M) were lost to follow-up after the first year, as they had left Paris.

Patients were followed by their own physicians in three diabetic clinics in Paris.*

Annual funduscopies, fluorescein angiograms, and serum lipid and creatinine determinations were performed. Every three months the patients were examined clinically and 24-hour urine and fasting blood sugar levels were recorded. The mean number (± number) of ophthalmologic examinations during the trial was 3.3 ± 0.2 in the S group and 3.7 ± 0.2 in the M group (N.S.).

Changes in Treatment During Follow-up

In the S group, five patients' regimes were later changed by their physicians to insulin twice or three times a day after a mean duration of 9.2 ± 3.4 months. This was because of poor control of diabetes and/or large daily doses of insulin needed.

In the M group, 13 patients accepted the three-injection regime after randomization, four accepted two injections, and four refused any change from their single-injection therapy. Later, nine of the 13 patients asked for insulin twice a day instead of three times after a mean duration of 12.2 ± 3.4 months, one subject changed from two injections to one after 32 months, and one changed from one to two after three months.

Finally, 16 out of 21 subjects in the S group were treated during the whole trial with one injection and 17 out of 21 subjects in the M group were on divided doses during the whole trial.

Analysis

Retinal changes were determined by the mean yearly increase in the number of microaneurysms, calculated for each individual as the slope of the regression of the number of microaneurysms versus time. Statistical methods used for comparison of the two groups were the Student *t* test for paired and nonpaired data and nonparametric tests (Mann and Whitney) for nonnormal distributions.

RESULTS

Retinal Changes

Individuals showed a great variability in the number of microaneurysms (MAs) observed every year

*This work was done under the auspices of the Association Française pour l'Etude de l'Epidémiologie du Diabète. Members are R. Assan, J. Canivet, G. Cathelineau, H. Chimènes, G. Debry, M. Dérot, R. Deuil, E. Eschwege, R. Lebouc, J. Lubetzki, G. Pequignot, P. Rambert, M. Rathery, G. Rosselin, F. Rousselie, D. Schwartz, J. Soulie, G. Tchobroutsky, F. Thervet, and A. J. Valleron.

TABLE 2a

Number of microaneurysms in the single-injection group (delay in months from baseline examinations in parentheses)

Patient no.	Baseline	1st Exam.	2nd Exam.	3rd Exam.	4th Exam.	Mean yearly increase in the number of microaneurysms
1	14	6 (14)	22 (26)	8 (37)	17 (50)	0.84
2	24	74 (12)	38 (28)	—	37 (51)	−0.84
3	0	3 (12)	—	—	10 (48)	2.40
4	0	4 (12)	—	1 (35)	—	0.12
5	4	—	0 (19)	8 (36)	20 (48)	0.72
6	0	0 (11)	—	—	0 (42)	0.00
7	12	26 (11)	36 (22)	14 (33)	18 (46)	−0.60
8	0	—	5 (28)	—	—	2.16
9	39	41 (17)	102 (26)	47 (36)	62 (48)	5.64
10	10	25 (13)	49 (23)	—	—	20.4
11	16	—	31 (18)	47 (32)	—	11.5
12	1	—	29 (25)	27 (34)	—	10.1
13	0	10 (14)	—	—	—	8.5
14	3	19 (11)	28 (22)	24 (32)	—	8.2
15	7	8 (14)	—	—	—	0.84
16	3	—	9 (18)	17 (37)	—	4.56
17	9	74 (16)	27 (24)	52 (37)	—	10.70
18	65	44 (16)	77 (24)	160 (32)	—	30.96
19	8	9 (16)	—	—	—	0.72
20	30	43 (13)	—	81 (31)	—	20.04
21	21	—	110 (21)	41 (33)	—	12.12

TABLE 2b

Number of microaneurysms in the multiple-injection group (delay in months from baseline examination in parentheses)

Patient no.	Baseline	1st Exam.	2nd Exam.	3rd Exam.	4th Exam.	Mean yearly increase in the number of microaneurysms
1	2	14 (15)	—	5 (34)	14 (45)	3.96
2	6	8 (16)	—	0 (34)	3 (47)	−1.32
3	2	5 (12)	10 (26)	—	—	3.72
4	11	4 (13)	2 (26)	—	—	−4.20
5	15	16 (14)	23 (30)	—	9 (49)	−0.96
6	8	2 (12)	18 (23)	0 (34)	—	−0.36
7	19	56 (13)	—	—	55 (52)	6.36
8	1	1 (12)	1 (25)	—	—	0.00
9	1	13 (13)	46 (24)	—	16 (47)	4.32
10	26	39 (12)	63 (26)	—	36 (44)	3.60
11	1	1 (12)	1 (25)	1 (40)	—	0.00
12	1	0 (14)	6 (27)	7 (42)	6 (50)	1.68
13	2	12 (12)	4 (28)	—	10 (46)	1.08
14	0	18 (13)	16 (24)	—	6 (45)	0.72
15	1	10 (14)	0 (22)	6 (30)	—	0.96
16	5	23 (12)	4 (24)	8 (40)	8 (45)	−0.96
17	1	—	22 (22)	—	—	11.40
18	3	0 (12)	2 (20)	—	—	−0.84
19	10	0 (11)	19 (26)	6 (38)	—	0.96
20	5	—	26 (25)	11 (38)	—	3.00
21	68	—	28 (26)	95 (34)	—	2.64

(table 2), but, expressed as a mean, a significant increase in the number of microaneurysms was observed in both groups. Patients in the multiple-injection regimen showed a yearly increase of 1.8 ± 0.7 MAs, while that in patients in the single-injection regimen was 7.2 ± 1.9 MAs. This difference was highly significant ($p<0.01$). Thus, the increase was significantly greater in the single-daily-insulin-injection group (S) than in the multiple one (M) (table 3).

At the beginning of the study no new vessels were observed by funduscopy, but on fluorescein angiograms three subjects out of 21 in the S group and two out of 21 in the M showed small areas of new vessels. At the end of follow-up, six patients in the S group and one in the M group showed new vessels. This difference is not significant.

TABLE 3

Comparison of the increase in the number of microaneurysms between the two groups (means ± S.E.M.)

		Single-injection group	Multiple-injection group	P values
Number of microaneurysms	—At baseline	12.7 ± 3.5	9.0 ± 3.3	N.S.
	—At the last examination	33.0 ± 7.9	15.2 ± 4.9	<0.05
	—Difference	20.3 ± 4.9	6.2 ± 2.5	<0.02
Mean yearly increase in the number of microaneurysms		7.2 ± 1.9	1.8 ± 0.7	<0.01 <0.02*
Mean yearly increase in the square root of the number of microaneurysms		0.85 ± 0.16	0.24 ± 0.13	<0.01

*Nonparametric test. As the variances of the mean yearly increase in the number of microaneurysms differed between the two groups, they were also compared by a nonparametric test (Mann and Whitney) and the square-root transformation, which equalized the variances.

Insulin Doses (Table 4)

In the M group there was a slight but significant increase in the daily requirement of insulin ($p<0.05$). However, no significant difference between the two groups was observed.

Diabetic Control

(a) *Fasting blood sugar* (table 5): The mean values of fasting blood sugar levels decreased during the survey in the M group but not in the S group. The difference between the two groups barely reaches the level of significance when the whole period of survey is considered but is more significant in the last year of the survey.

(b) *Glycosuria* (table 6): During the period of follow-up mean daily 24-hour urinary sugar levels remained in the same range as the baseline values in both groups. But mean values showed a tendency to

TABLE 4

Comparison of the insulin dosage (units per 24 hr.) between the two groups (means ± S.E.M.)

	Single-injection group	Multiple-injection group	P values
Baseline value	42.1 ± 2.9	40.6 ± 3.1	N.S.
Mean survey value	43.9 ± 3.5	47.4 ± 4.4	N.S.
Last-year value	44.6 ± 3.7	47.1 ± 5.0	N.S.

TABLE 5

Comparison of the fasting blood sugar (mg./100 ml.) between the two groups (means ± S.E.M.)

	Single-injection group	Multiple-injection group	P values
Baseline value	210 ± 12	200 ± 19	N.S.
Mean survey value	197 ± 9	171 ± 10	<0.06
Last-year value	195 ± 11	154 ± 15	<0.05

decrease in the multiple and to increase in the single group ($p<0.10$). When comparisons are made between baseline and final mean values, the results show that the amount of 24-hour glycosuria was higher at the end of the study than at the beginning in the S group and lower in the M group. These changes from baseline are significantly different between the two groups ($p<0.02$).

Other Parameters

No significant variations from baseline values were observed in any other parameters studied in both groups. Diastolic blood pressure, which was the only parameter showing a significant difference between the S and M groups at baseline (80.0 ± 1.9; 86.0 ± 2.0 $p<0.05$), was similar during the entire survey in both groups (80.0 ± 2.0 versus 83.0 ± 1.8, N.S.).

DISCUSSION

Thus, in this clinical trial made under routine treatment conditions, the use of divided daily insulin injections was effective in improving diabetic control and delaying retinal changes in insulin-dependent diabetics.

Restriction of the retinal study to the posterior pole of the eye was judged acceptable because of the well-documented predominance of microaneurysms in this area in diabetic retinopathy.

In a single subject, the number of microaneurysms shows some variations with time.[35,36] Nevertheless, it was thought that in a prospective study dealing with early stages of diabetic retinopathy this criterion is a valuable index. This choice was justified a posteriori by the progressive increase in the mean number of microaneurysms observed in both groups during the survey.

More sophisticated methods[37,38] to determine the areas of retinal capillary nonperfusions were not used at the beginning of this study. The angiofluorographic examination was not designed for such an assay, and this could not be performed a posteriori.

In this study the control of diabetes roughly estimated by 24-hour glycosuria and fasting blood sugar levels was improved when multiple daily insulin injections were used. Previous studies have emphasized the usefulness of such a treatment: short-acting insulin was useful in improving the control of diabetic patients, even unstable ones.[24,30,31,39-42] NPH plus regular insulin twice a day is widely used systematically or in unstable diabetic patients[24,25,29] and also during pregnancy.[29,43] Several authors think that until ideal treatments are available it is probably wise to strive for optimal control and to seek more physiologic insulin provision. They have advocated or suggested the use of multiple daily insulin injections.[22-29] In short-term studies, some of us have shown that such treatment leads to lower blood sugar levels and 24-hour urinary sugar excretion and to higher plasma insulin levels than single-injection regimes of insulin administration.[30-33] Regular insulin before meals gave similar control of blood glucose levels than did continuous intravenous infusion of insulin for days.[44]

TABLE 6

Comparison of the urinary sugar excretion (gm. per 24 hr.) between the two groups (means ± S.E.M.)

	Single-injection group	Multiple-injection group	P values
Baseline value	22.1 ± 4.7	28.5 ± 4.2	N.S.
Mean survey value	27.4 ± 4.2	24.8 ± 2.2	N.S.
Last-year value	27.5 ± 4.3	20.6 ± 2.7	N.S.
Difference between mean survey and baseline values	+5.3 ± 3.1	−3.7 ± 4.1	<0.10
Difference between last-year and baseline values	+5.4 ± 3.9	−7.9 ± 4.1	<0.02

That better diabetic control and/or the widespread use of insulin delay or prevent diabetic lesions was not firmly established. Experimental,[5-8] biochemical,[9-11] morphologic,[45,46] and clinical data[1-4,12-16,19-21] strongly suggest that vascular changes in diabetes are related to the degree and duration of hyperglycemia and/or insulin deficiency or their consequences.

The opinion that good control delays or prevents diabetic lesions is, however, not shared by all,[17,18] perhaps because clinical studies, even prospective ones, are very difficult to carry out.[47]

Prospective studies in this field are very scarce, but the work of Joplin et al.[1] and Miki et al.[2,3] in humans has shown less progression in clinical advanced forms of microangiopathy in well-controlled than in poorly controlled patients. The recent study by Takazukara et al.[4] on serial renal biopsies has shown, despite a

great heterogeneity in patients, the possibility that the type of diabetes—i.e. maturity-onset or juvenile, insulin-dependent or non-insulin-dependent—may play a prominent role and that the control of blood glucose may delay the course of glomerulosclerosis.

In animals with induced experimental diabetes the studies by Spiro and Spiro,[9] Beisswenger and Spiro,[10] Hägg,[8] Bloodworth and Engerman,[7] and Fushimi and Tarui[11] have shown that insulin administration delays or prevents the biochemical changes induced by the disease.

Lastly, Mauer et al.[5,6] have shown that pancreatic islet transplantations in streptozotocin-treated rats resulted in regression or arrest of the diabetic glomerular lesions.

In the present trial the treatment by divided daily insulin injections provided better diabetic control and resulted in lower progression in retinal changes than the conventional use of insulin once a day. Because the selected patients were randomly allocated to the two kinds of insulin regimen and because baseline characteristics were not significantly different between the two groups, the difference in the progression of the number of MAs cannot be related to a peculiar severity of diabetes and/or to instability or other conditions in the patients of the S group but can be causally ascribed to the differences in treatments.

CONCLUSION

As this study has shown that the use of divided doses of insulin in insulin-dependent patients has helped in improving diabetic control and in lowering the rate of increase of retinal microaneurysms, such an insulin regimen might be useful in the treatment of diabetic patients with a reasonable life expectancy and no social or psychologic handicaps, at least until ideal treatments are available for all patients.

ACKNOWLEDGMENTS

This study was supported by the Institut National de la Santé et de la Recherche Médicale contract 74.5.205.4 and the Caisse Nationale de l'Assurance Maladie des Travailleurs Salariés. We are greatly indebted to Dr. H.C. Zweng (Palo Alto, Calif.) for criticisms and advice and for reviewing the manuscript. We thank Mrs. Girard-Vandais for her invaluable technical assistance.

REFERENCES

[1]Joplin, G.F., Blach, R.K., Chang, H., Elkeles, R.S., Fraser, T.R., Kohner, E.M., and Oakley, N.W.: Treatability of diabetic retinopathy. *In* Diabetes, Proceedings of the Seventh Conference of the IDF. Rodriguez, R.R., and Vallance-Owen, J., Eds., International Congress no. 231. Amsterdam, Excerpta Medica, 1971, pp. 276-80.

[2]Miki, E., Fukuda, M., Kuzuya, T., Kosaka, K., and Nakao, K.: Relation of the course of retinopathy to control of diabetes, age and therapeutic agents in diabetic Japanese patients. Diabetes *18*:773-79, 1969.

[3]Miki, E., Kuzuya, T., Ide, T., and Nakao, K.: Frequency, degree and progression with time of proteinuria on diabetic patients. Lancet *1*:922-24, 1972.

[4]Takazakura, E., Nakamoto, Y., Hazakawa, H., Kawai, K., Muramoto, S., Yoshide, K., Shimizu, M., Shinoda, A., and Takeuchi, J.: Onset and progression of diabetic glomerulosclerosis. Diabetes *24*:1-9, 1975.

[5]Mauer, S.M., Sutherland, D.E.R., Steffes, M.W., Leonard, R.J., Najarian, J.S., Michael, A.F., and Brown, D.M.: Pancreatic islet transplantation. Diabetes *23*:748-53, 1974.

[6]Mauer, S.M., Steffes, M.W., Sutherland, D.E.R., Najarian, J.S., Michael, A.F., and Brown, D.M.: Studies of the rate of regression of the glomerular lesions in diabetic rats treated with pancreatic islet transplantation. Diabetes *24*:280-85, 1975.

[7]Bloodworth, J.M.B., Jr., and Engerman, R.L.: Diabetic microangiopathy in the experimentally-diabetic dog and its prevention by careful control with insulin (abstr.). Diabetes *22*, suppl. 1:290, 1973.

[8]Hägg, E.: On the pathogenesis of glomerular lesions in the alloxan diabetic rat. Acta Med. Scand. suppl. *558*:1-31, 1974.

[9]Spiro, R.G., and Spiro, M.J.: Effect of diabetes on the biosynthesis of the renal glomerular basement membrane. Diabetes *20*:641-48, 1971.

[10]Beisswenger, P.J., and Spiro, R.G.: Studies on the human glomerular basement membrane. Composition, nature of the carbohydrate units and chemical changes in diabetes mellitus. Diabetes *22*:180-93, 1973.

[11]Fushimi, H., and Tarui, S.: Kidney and serum beta-N-acetylglucosaminidase activities in streptozotocin diabetic rats and their responses to insulin and glucagon. J. Biochem. *76*:225-27, 1974.

[12]Keiding, N.R., and Marble, A.: Importance of control of diabetes on prevention of vascular complications. J.A.M.A. *8*:964-69, 1952.

[13]Hardin, R.C., Jackson, R.L., Johnston, T.L., and Kelly, H.G.: The development of diabetic retinopathy. Effect of duration and control of diabetes. Diabetes *5*:397-405, 1956.

[14]Lawrence, R.D.: Treatment of 90 severe diabetics with soluble insulin for 20-40 years: effect of diabetic control on complications. Br. Med. J. *5373*:1624-32, 1963.

[15]Colwell, J.A.: Effect of diabetic control on retinopathy. Diabetes *15*:497-99, 1966.

[16]Pirart, J., Lauvaux, J.P., and Vassart, A.: Evolution de la rétinopathie diabétique en fonction de la durée et du degré de contrôle du diabète. Diabète *20*:209-13, 1972.

[17]Ricketts, H.T.: The influence of diabetic control on angiopathy. *In* On the Nature and Treatment of Diabetes. Proc. of the Fifth Conference of the IDF. Leibel, B.S., and Wrenshall, G.A., Eds. no. 84 International Congress Series. Amsterdam, Excerpta Medica, 1965, pp. 588-600.

[18]Knowles, H.C.: Long term juvenile diabetes mellitus and unmeasured diet. *In* Diabetes, Proc. of the Seventh Conference of

the IDF. Rodriguez, R.R., and Vallance-Owen, J., Eds. Intern. Congress series no. 231, Amsterdam, Excerpta Medica, 1971, pp. 209-15.

[19]Marble, A.: Long term diabetes and the effect of treatment. *In* Diabetes, Proc. of the Seventh Conference of the IDF. Rodriguez, R.R., and Vallance-Owen, J., Eds. Intern. Congress series no. 231, Amsterdam, Excerpta Medica, 1971, pp. 25-35.

[20]Constam, G.R.: Contrôle du diabète et prévention des complications. *In* Journées de Diabétologie de l'Hôtel-Dieu. Paris, Flammarion edt., 1972, pp. 314-20.

[21]Fajans, S.S.: Current unsolved problems in diabetic management. Diabetes *21*, suppl. 2:678-84, 1972.

[22]Deuil, R.: La redécouverte de l'insuline ordinaire. Presse Méd. 75:27, 1967.

[23]Lukens, F.D.W.: The rediscovery of regular insulin. N. Engl. J. Med. *272*:130-37, 1965.

[24]Oakley, W., Hill, D., and Oakley, N.: Combined use of regular and crystalline protamine (NPH) insulin in the treatment of severe diabetes. Diabetes *15*:219-22, 1966.

[25]Forsham, P.H.: Insulin twice a day suggested for control. J.A.M.A. *202*:26, 1967.

[26]Earll, Maj. J.M.: Time for more physiologic management of diabetes. Milit. Med. *133*:39-42, 1968.

[27]Tutin, M., Sanmori, M., Tchobroutsky, G., Bour, H., and Dérot, M.: Regular versus depot insulin and the course of diabetic retinopathy. Diabetologia *5*:57 (abstr.), 1969.

[28]Rull, J.A., Conn, J.W., Floyd, J.C., and Fajans, S.S.: Levels of plasma insulin during cortisone glucose tolerance tests in "non-diabetic" relatives of diabetic patients. Diabetes *19*:1-10, 1970.

[29]Oakley, W.G., Pyke, D.A., and Taylor, K.W.: Diabetes and its management. Oxford, Blackwell Scientific Publications, 1973.

[30]Kopf, A., Tchobroutsky, G., and Eschwege, E.: Serial postprandial blood glucose levels in 309 subjects with and without diabetes. Diabetes *22*:834-46, 1973.

[31]Tchobroutsky, G., Kopf, A., Eschwege, E., and Assan, R.: Serial postprandial plasma insulin levels in 117 subjects with and without diabetes including insulin-treated patients. Diabetes *22*:825-33, 1973.

[32]Tchobroutsky, G.: How to achieve better diabetic control? Studies with insulin three times a day. *In* Diabetes, Proc. of the Eighth Congress of the Intern. Diabetes Fed., Brussels, 1973. Malaisse, W.J., Pirart, J., and Vallance-Owen, J., Eds., Amsterdam, Excerpta Medica, 1974, pp. 667-79.

[33]Tchobroutsky, G., Lenormand, M.E., Michel, G., and Assan, R.: Lack of postprandial exercise-induced growth hormone secretion in normoglycemic insulin-treated diabetic men. Horm. Metab. Res. 6:184-87, 1974.

[34]Almaric, P., and Coscas, G.: Rétinopathie diabétique. Angiogluorographie fluorosceinique dans la rétinopathie diabétique. Arch. Ophtalm. *27*:553-58, 1967.

[35]Caird, F.I., and Garret, C.J.: Progression and regression of diabetic retinopathy. Proc. R. Soc. Med. *35*:477-81, 1962.

[36]Kohner, E.M., and Dollery, C.T.: The rate of formation and disappearance of microaneurysms in diabetic retinopathy. Eur. J. Clin. Invest. *1*:107-79, 1970.

[37]Kohner, E.M., Dollery, C.T., Fraser, T.R., and Bulpitt, C.J.: Effect of pituitary ablation on diabetic retinopathy studied by fluorescein angiography. Diabetes *19*:703-14, 1970.

[38]Davis, H.D., Morton, W.D., and Myers, F.L.: *In* The Airlie Classification of Diabetic Retinopathy. Goldberg, M., et al., Eds. U.S. Department of Public Health, 1969, pp. 7-22.

[39]Metz, R., Nice, M., and Laplaca, G.: Evaluation of an eight-hour therapeutic regimen in uncontrolled diabetes. Diabetes *16*:341-45, 1967.

[40]Service, F.J., Molnar, G.D., Rosevear, J.W., Ackerman, E., Gatewood, L.C., and Taylor, W.F.: Mean amplitude of glycemic excursion, a measure of diabetic instability. Diabetes *19*:644-55, 1970.

[41]Spathis, G.S.: Continuous monitoring of blood sugar in brittle diabetics. Diabetologia 6:586-92, 1970.

[42]Vining, K.K., Jr., and Strong, L.E.: The effectiveness of a highly structured diabetic control program on progress of proliferative diabetic retinopathy. (abstr.). Diabetes *21*, suppl. 1:382, 1972.

[43]Pedersen, J.: The Pregnant Diabetic and Her Newborn. Problems and Management. Copenhagen, Munksgaard, 1967.

[44]Slama, G., Hautecouverture, M., Assan, R., and Tchobroutsky, G.: One to five days of continuous intravenous insulin infusion on seven diabetic patients. Diabetes *23*:732-38, 1974.

[45]Osterby, R.: Course of diabetic glomerulopathy. *In* Blood Vessel Disease in Diabetes Mellitus. Lundbaek, K., and Keen, H., Eds. Milan, Il Ponte, 1971, pp. 179-91.

[46]Kilo, C., Vogler, N., and Williamson, J.R.: Muscle capillary basement membrane changes related to aging and diabetes mellitus. Diabetes *21*:881-96, 1972.

[47]Kaplan, M.H., and Feinstein, A.R.: A critique of methods in reported studies of long-term vascular complications in patients with diabetes mellitus. Diabetes *22*:160-74, 1973.