Retinopathy and Plasma Growth Hormone Levels in Idiopathic Hemochromatosis with Diabetes

Philippe Passa, M.D., Françoise Rousselie, M.D., Christiane Gauville, and Jean Canivet, M.D., Paris, France

SUMMARY

In patients with idiopathic hemochromatosis, retinopathy was investigated by ophthalmoscopy and fluorescein angiography and was present in eight out of 23; this prevalence is similar to that reported in patients with diabetes aged between 30 and 60 years at onset of diabetes and with the same duration of the disease; in these eight patients retinopathy was of mild degree, with no impairment of visual acuity, fewer than 10 microaneurysms in each fundus, and no other retinal abnormalities. Plasma growth hormone response to arginine stimulation was studied in 10 patients with hemochromatosis and diabetes and was significantly lowered compared with uncomplicated diabetes and with nondiabetic subjects. Plasma growth hormone response to insulin-induced hypoglycemia was studied in six patients with hemochromatosis and diabetes and was significantly lowered as compared with nondiabetics. It is suggested that the blunted growth hormone secretion in idiopathic hemochromatosis acts as a protective factor and could explain the mild degree of the retinopathy; this would support the hypothesis on a possible role of growth hormone in the development of diabetic microangiopathy.

DIABETES *26*:113-20, February, 1977.

Diabetes mellitus is regarded as one of the classic features of hemochromatosis.[1-3] It has been commonly stated that these diabetic patients are not prone to long-term vascular complications.[1,4-9] However, regarding microangiopathy, namely glomerulosclerosis and retinopathy, this assumption has been challenged.[10-14] The first aim of this study was a reëvaluation of the prevalence and severity of the retinopathy in a series of 23 patients with idiopathic hemochromatosis (IH) and diabetes.

The assumed infrequency of the microangiopathy in hemochromatosis has yet received no satisfactory explanation. A hypothesis has been put forward that overproduction of growth hormone (GH) is a causal factor in the development of diabetic vasculopathy;[15] thus it could be possible that a low GH secretion would protect these patients from microangiopathy. Plasma GH levels previously reported in hemochromatosis with diabetes result in conflicting data.[16-18] The second purpose of this study was to determine GH response to arginine and to insulin-induced hypoglycemia.

MATERIALS AND METHODS

Patients

Twenty-three patients with IH and diabetes were

A preliminary report of this work was given at the Eleventh Annual Meeting of the European Association for the Study of Diabetes at Munich, Germany, on September 4-6, 1975.

From the Department of Metabolism and Endocrinology, University of Paris VII, Hôpital Saint Louis.

Address reprint requests to Jean Canivet, Hôpital Saint Louis; 2, place du Dr A. Fournier, 75475 Paris Cedex 10, France.

Accepted for publication August 25, 1976.

selected. The diagnosis of IH was based on (1) clinical manifestations including hyperpigmentation, liver enlargement, and diabetes, (2) raised serum iron level over 190 μg./100 ml. with increased saturation of serum total iron-binding capacity over 80 per cent, (3) evidence of iron overload estimated in liver biopsy specimens,[19] (4) positive data for hemochromatosis among family members or absence of any possible nonfamilial origin of iron overload—that is, severe alcoholism, hematologic abnormalities, or anemia even after long-term phlebotomy.[20]

Eye examination was performed in the 23 patients; these were 19 men and four women of average age 53.3 years (range 26 to 77 years) and of mean total body weight of 103 per cent (range 77 to 134 per cent) according to Metropolitan Life Insurance tables, 1959. Sixteen had positive family data for hemochromatosis. Fifteen were insulin-requiring diabetics, six were taking oral hypoglycemic drugs, and two were managed by diet alone; none of them were ketosis-prone. Clinical features are recorded in table 1.

An arginine-infusion test was carried out in 10 patients with IH and diabetes: eight men and two women (cases 1, 4, 5, 10, 12, 13, 17, 20, 21, and 23); none of them had evidence of hepatic dysfunction as assessed by sulfobromphthalein test; none of them had evidence of renal failure since their plasma creatinine level was below 10 mg. per liter. Ten insulin-requiring diabetics and 10 nondiabetic subjects matched for sex, age, weight, and diabetes duration were also selected:* in nondiabetic subjects fasting blood glucose was below 100 mg./100 ml. and there was no family history of diabetes; women with active menstruation were tested out of the ovulation period.

An insulin-induced hypoglycemia test was made in six patients (cases 5, 6, 10, 12, 20, and 23) with IH and diabetes out of the 23 and six nondiabetic subjects matched for sex and weight.

Eye Examination

Eye examination was performed by one of us (F.R.) and involved visual acuity evaluation, ophthalmoscopy, color fundus photography, and fluorescein angiography of the retina; fluorescein angiography was performed after intravenous antecubital injection of the dye, by means of a Topcon II retinograph; the fluorescein transit through the macula and the adjacent area was photographed in both eyes, and late photographs were also taken. A diagnosis of diabetic retinopathy was made in those patients showing more than one microaneurysm in the area of the macula and optic disc under ophthalmoscopy or after fluorescein angiography; microaneurysms were counted on photographs taken under fluorescein angiography.

TABLE 1

Clinical data for patients with IH and diabetes

Case	Sex	Age (yrs.)	Ideal body weight %	Clinical diabetes duration (yrs.)	Treatment of diabetes*
1	M	62	96	24	I
2	M	67	98	15	OHD
3	M	55	101	15	OHD
4	M	68	130	14	I
5	M	60	106	14	OHD
6	M	48	85	14	I
7	M	53	100	13	I
8	M	44	110	10	I
9	F	44	97	10	I
10	M	44	104	9	I
11	F	67	134	8	I
12	M	49	103	8	I
13	F	66	130	7	I
14	M	66	118	6	I
15	M	48	77	6	I
16	M	53	115	4	OHD
17	M	44	105	4	I
18	M	51	92	3	D
19	M	77	95	2	OHD
20	M	36	103	2	I
21	F	26	95	2	I
22	M	46	95	1	OHD
23	M	52	91	1	D

*I = insulin; OHD = oral hypoglycemic drugs; D = diet alone.

Arginine Infusion Test

Patients and nondiabetic subjects were admitted in the hospital and the test was carried out the following day at 8 a.m., after an overnight fast. On the morning of the test day, insulin therapy was withdrawn and arginine monochloride was infused at a steady rate (11.7 mg./kg./min.) for 40 minutes with an Infusomatpump (Braun-Melsungen). Blood samples were taken through a Teflon catheter at intervals of −20, −10, 0, 5, 10, 20, 30, 40, 55, 70, and 90 minutes, time 0 corresponding to beginning of the infusion.

Insulin-induced Hypoglycemia Test

Patients were admitted in the department and the test was carried out after an overnight fast. On the morning of the test day, insulin therapy was withdrawn and the patient was given 0.1 unit insulin (Actrapid Novo) per kg. body weight by rapid intravenous injection. Blood samples were collected

*After informed consent.

through a Teflon catheter at times −5, 30, 60, and 120 minutes, time 0 corresponding to insulin injection. When lowering of blood glucose did not reach at least the level of 50 mg./100 ml. because of a high fasting level, GH determination was not made; a better control was achieved and the test was repeated.

Determinations

Liver function, serum iron, and degree of saturation of iron-binding protein were measured by conventional methods. Blood glucose was determined by the orthotoluidine method[21] adapted to the Technicon AutoAnalyzer. Plasma GH was assayed radioimmunologically.[22] All measurements were made in duplicate. Statistical calculations were made by Fischer-Student *t* test, after determination of analysis of variance.

RESULTS

As shown in table 2, retinopathy was present in eight cases and was detected in six cases by ophthalmoscopy and in two more cases (cases 2 and 11) by fluorescein angiography, which revealed microaneurysms not visible ophthalmoscopically. Among these eight cases, retinopathy was observed in seven patients out of 12 with clinical diabetes duration of eight years or more, and in one out of 11 with duration of seven years or less. No impairment of vision could be attributed to diabetic retinopathy; cataract or lens opacities were noted in four patients and refractive changes in two. In all cases, microaneurysms were scanty, even after detection by fluorescein angiography. Mild leakage of the dye surrounding a microaneurysm was noted by fluorescein study in two patients (cases 1 and 3) with retinopathy. Hard exudates were noticeable in another patient (case 22) but could not be attributed to diabetic retinopathy in the absence of microaneurysms. No other retinal signs were observed.

The results of the arginine stimulation test are shown in figure 1. In the control group, the basal blood glucose level was 90 ± 2 mg./100 ml. (mean of the three preinfusion determinations); it increased during arginine infusion to a maximum level of 109 ± 5 mg./100 ml. after 20 minutes and then declined progressively; the plasma fasting GH level was 4.0 ± 2.2 ng./ml. (mean of the three preinfusion determinations) and rose to its maximum level of 22.1 ± 4.4 ng./ml. 15 minutes after the end of the infusion. In the uncomplicated diabetic group, the basal blood glucose level was 125 ± 2 mg./100 ml. and rose to a maximum level of 188 ± 2 mg./100 ml. 30 minutes after the end of the infusion; the plasma basal GH level was 5.7 ± 1.6 ng./ml. and reached its max-

TABLE 2

Ocular findings in patients with IH and diabetes

Case	Retinopathy: Ophthalmoscopy	Retinopathy: Fluorescein angiography	Visual Acuity* R	Visual Acuity* L	Number of microaneurysms R	Number of microaneurysms L	Other signs
1	+	+	6/9	6/6	<10	<5	leakage of the dye
2	0	+	6/6	6/6	0	<5	
3	+	+	6/15	6/6	<10	<5	leakage of the dye
4	0	0	6/30	6/60	0	0	cataract
5	0	0	6/6	6/6	0	0	
6	+	+	6/6	6/6	< 5	<5	
7	0	0	6/6	6/6	0	0	
8	+	+	6/6	6/6	< 5	0	
9	0	0	6/9	6/12	0	0	refractive changes
10	+	+	6/6	6/6	<10	<5	
11	0	+	6/9	6/12	< 5	<5	lens opacities
12	0	0	6/6	6/9	0	0	
13	0	0	6/6	6/6	0	0	
14	0	0	6/6	6/12	0	0	lens opacities
15	0	0	6/6	6/6	0	0	
16	0	0	6/6	6/12	0	0	refractive changes
17	0	0	6/6	6/6	0	0	
18	0	0	6/6	6/6	0	0	
19	0	0	6/30	6/60	0	0	cataract
20	+	+	6/6	6/6	<10	<5	
21	0	0	6/6	6/6	0	0	
22	0	0	6/9	6/6	0	0	hard exudates
23	0	0	6/6	6/6	0	0	

*Values listed in meters.

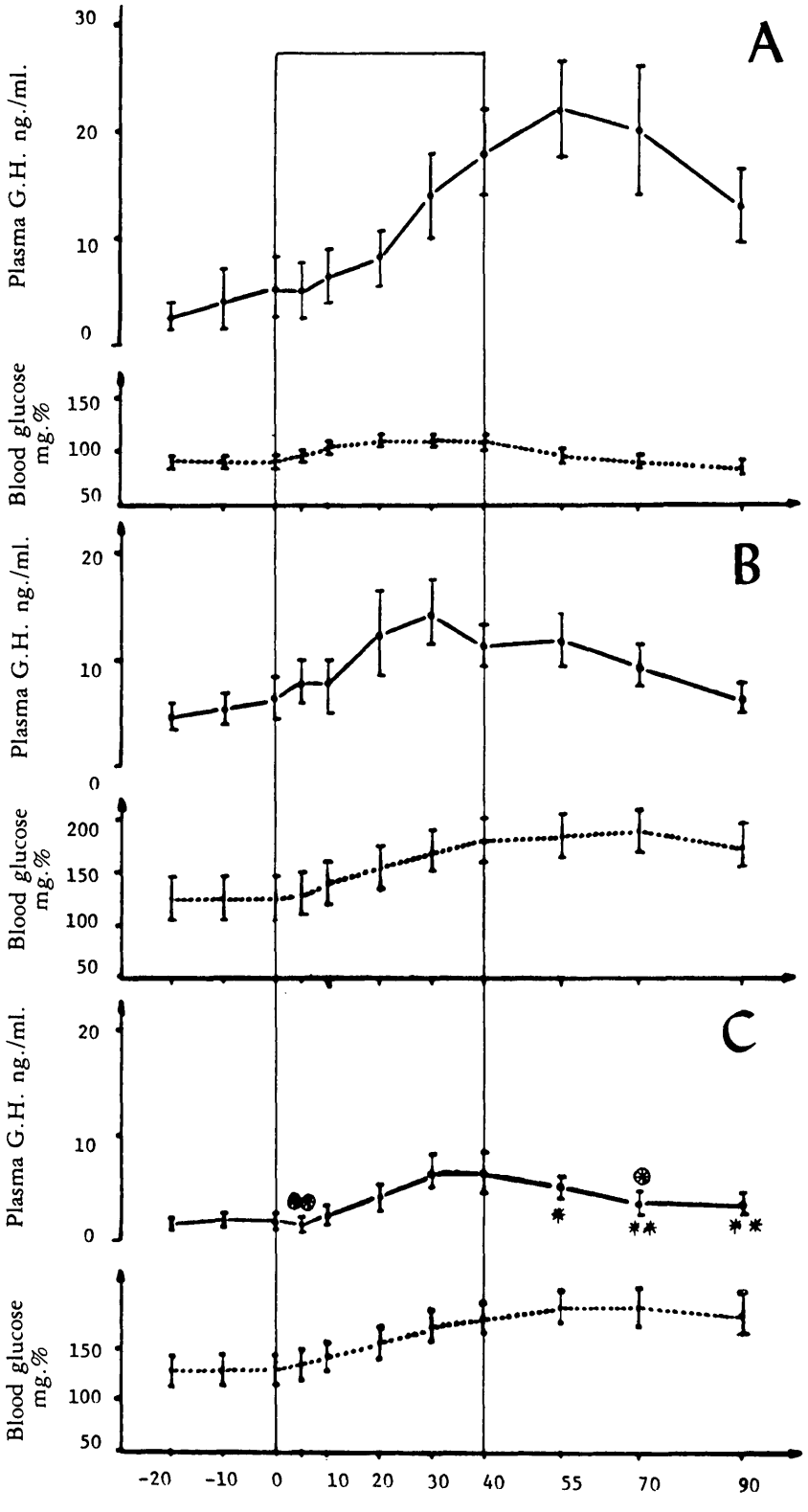

FIGURE 1

Blood glucose and plasma GH levels during arginine stimulation test (mean ± S.E.M.) in (A) nondiabetics, (B) diabetic subjects, (C) patients with IH and diabetes. In this last group: * $p < 0.02$, ** $p < 0.01$, as compared with nondiabetics; ⊛ $p < 0.02$, ⊛⊛ $p < 0.01$, as compared with common diabetics.

imum level of 14.3 ± 2.9 ng./ml. 30 minutes after beginning of the infusion. In patients with IH and diabetes, the fasting blood glucose level was 131 ± 1 mg./100 ml. and increased to a maximum level of 189

± 2 mg./100 ml. 15 minutes after the end of infusion; basal plasma GH level was 1.8 ± 0.4 ng./ml. and rose to a maximum level of 6.4 ± 1.8 ng./ml. at the end of the infusion period.

No significant difference in plasma GH levels at fast or after arginine stimulation was observed in uncomplicated diabetics as compared with nondiabetic subjects in spite of a rather high basal value in the diabetic group. In patients with IH and diabetes, plasma GH response to arginine infusion was significantly blunted as compared with normals ($p < 0.02$ 15 minutes after the end of the infusion, $p < 0.01$ 30 and 50 minutes after the end of the infusion) and with uncomplicated diabetics ($p < 0.01$ five minutes after beginning of the infusion, $p < 0.02$ 30 minutes after the end of the infusion).

The results of the insulin-induced hypoglycemia test are shown in figure 2. In the control group, fasting blood glucose level was 90 ± 6 mg./100 ml. and was lowered to 26 ± 2 mg./100 mg. 30 minutes after insulin injection; basal plasma GH level was 2.5 ± 1.4 ng./ml. and increased to a maximum level of 30.4 ± 6.7 ng./ml. 60 minutes after injection. In patients with IH and diabetes, the basal blood glucose level was 98 ± 12 mg./100 ml. and was lowered to 38 ± 8 mg./100 ml. 60 minutes after beginning of the test; the basal plasma GH level was 2.9 ± 1.3 ng./ml. and increased to a maximum level of 8.4 ± 1.4 ng./ml. 60 minutes after insulin injection. Difference in the plasma GH response to hypoglycemia was significant at 60 minutes ($p < 0.001$).

DISCUSSION

Recognition of diabetic retinopathy was based on the presence of microaneurysms in eight out of 23 patients with IH and diabetes; this figure is in agreement with the findings of Griffiths et al.[12] and Dymock et al.,[13] who reported that retinopathy is not as rare as previously found, although their study is concerned with all types of hemochromatosis. Among the eight cases of retinopathy in this series, seven were observed in the 12 patients with a clinical diabetes duration of eight years or more; this prevalence is similar to that reported in genetic-diabetic patients aged between 30 and 60 years at diagnosis of diabetes.[23] It is quite possible that the higher incidence of retinopathy observed in the latest reports may be related to the longer survival of patients with hemochromatosis as a result of venesection therapy,[12] as was suggested 10 years ago.[11]

In all the cases of this series, retinopathy, when present, was not severe; the same findings concerning the mild degree of retinal abnormalities have been reported in the most recent studies.[11-13] Thus, retinopathy in IH with diabetes may be considered of similar prevalence but of mild degree as compared with that in uncomplicated diabetes.

In hemochromatosis, obesity, hyperlipoproteinemia, and hypertension are uncommon features, and it has been suggested that this could account for the infrequency of microangiopathy.[24] But to date no statistical interrelations have been recorded to support the view that these troubles are causal factors in the development of microangiopathy. Therefore some other protective factors accounting for the mild degree of the retinopathy are to be investigated.

Arginine-induced plasma GH response was significantly lowered in patients with IH and diabetes as compared with normals and with uncomplicated diabetics. However, it must be pointed out that there was no individual relation between plasma GH response and the presence of the retinopathy. Quite wide individual variations in intensity of the response of plasma GH were observed; these individual differences are well known and explain the extent of the standard deviation from the average in these groups; however, there were no sudden transient increase in plasma GH as has sometimes been seen without apparent cause in nondiabetics and, more often, in diabetics.[25,26] The low plasma GH response in patients with IH and diabetes cannot be related to high blood glucose levels, since they were nearly the same in uncomplicated diabetics. It has been reported that a carbohydrate-rich diet may decrease the plasma GH response to arginine;[27] but the patients of these series were on a diabetic diet.

Plasma GH response to insulin-induced hypoglycemia was significantly blunted at 60 minutes in patients with IH and diabetes as compared with nondiabetic subjects. Nondiabetics were slightly younger, but there is no difference in relation to age in the GH response to this test. No determination was performed between 60 minutes and two hours, and an increase in plasma GH could have been missed during this period in patients with IH and diabetes; however, this is pure speculation.

The decreases in blood glucose levels in patients with IH were less than the decreases observed in control subjects; this could explain the reduced growth hormone response to hypoglycemia in the former group. But it has been shown that the threshold for

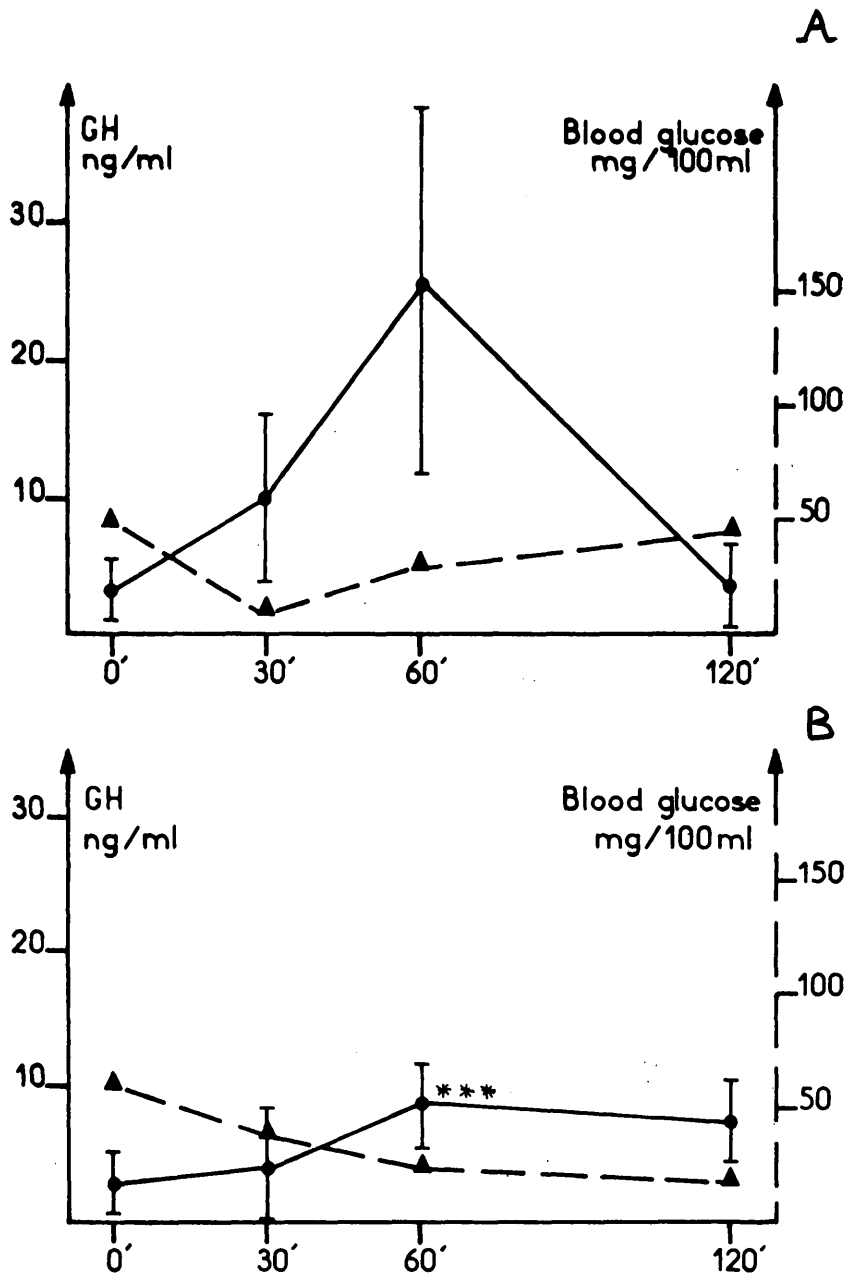

FIGURE 2

Blood glucose and plasma GH levels during insulin-induced hypoglycemia (mean ± S.E.M.) in (A) nondiabetics, (B) patients with IH and diabetes. *** $p < 0.001$.

growth hormone release was a fall of blood glucose of between 20 and 30 mg./100 ml.[28] In this study the blood glucose decrease was more dramatic, over 50 per cent from the basal value.

Variations in plasma GH levels might be influenced by factors occurring during transport, degradation, or elimination; however, cirrhosis of the liver, which might influence hormone metabolism, would result in increase and not lowering of GH response; rapid changes in plasma concentration are mainly a reflection of pituitary function, which seems disturbed in patients with IH. This opinion is supported by histopathologic studies that have demonstrated iron deposits in the hypophysis[29] and studies on gonadotropic function, disturbed in most patients.[30]

Growth hormone secretion in primary or secondary hemochromatosis has been studied.[16-18] In eight patients with IH (overt diabetes in seven and glucose intolerance in one) arginine-induced plasma GH response was said to be high in six patients and low in

two; unfortunately detailed results were not given and control subjects were not tested; after insulin-induced hypoglycemia, plasma GH response was determined in only three cases.[16] In 15 patients with hemochromatosis (diabetes in 11) the plasma GH response to insulin-induced hypoglycemia was decreased in nine and normal in the others.[17] In 31 patients with IH (diabetes in 26) plasma GH response to insulin-induced hypoglycemia was poor as compared with normals.[18] Differences reported in these studies may possibly be due to hepatic or renal dysfunction in some of these patients.

Although the role of GH in the development of diabetic microangiopathy is still uncertain,[31] several data have been reported in support of a possible role.[32-35] It has also been reported that capillary fragility[36] and platelet hyperaggregation,[37] which may play a role in the mechanism of microangiopathy, return to normal after hypophysectomy. It is suggested from this study that the reduced GH secretion may be a protective factor, accounting for the mild degree of diabetic retinopathy in IH.

ACKNOWLEDGMENTS

We thank Dr. Aa. P. Hansen for helpful discussion and for reviewing the manuscript. We are indebted to Miss Florence Niedergang for typing the manuscript.

This work was supported in part by a grant from the University of Paris VII.

REFERENCES

[1]Sheldon, J. H.: Haemochromatosis. London, Oxford University Press, 1935, p. 34.

[2]Finch, S. C., and Finch, C. A.: Idiopathic hemochromatosis, an iron storage disease. A. Iron metabolism in hemochromatosis. Medicine (Baltimore) *34*:381-430, 1955.

[3]Bothwell, T. H., and Finch, C. A.: Iron metabolism. Boston, Little, Brown, 1962, p. 366.

[4]Boulin, R.: Etude statistique de 70 cas de diabète bronzé. Presse Méd. *53*:326-27, 1945.

[5]Lawrence, R. D.: Insulin therapy: success and problems. Lancet *2*:401-05, 1949.

[6]Oakley, W., Pyke, D. A., and Taylor, K. W.: Clinical diabetes and its biochemical basis. Oxford, Blackwell Scient. Publ., 1968, p. 383.

[7]Lonergan, P., and Robbins, S. L.: Absence of intercapillary glomerulosclerosis in the diabetic patient with hemochromatosis. N. Engl. J. Med. *260*:367-70, 1959.

[8]Kreines, K., Kim, O., and Knowles, H. C., Jr.: Glomerulosclerosis, hemochromatosis and diabetes mellitus. Am. J. Clin. Pathol. *54*:47-52, 1970.

[9]Pirart, J., and Barbier, P.: Effet protecteur de l'hémochromatose vis à vis des lésions vasculaires séniles ou diabétiques. Diabetologia 7:227-36, 1971.

[10]Becker, D., and Miller, M.: Presence of diabetic glomerulosclerosis in patients with hemochromatosis. N. Engl. J. Med. *263*:367-73, 1960.

[11]Galton, D. J.: Diabetic retinopathy and hemochromatosis. Br. Med. J. *1*:1169, 1965.

[12]Tutin, M., Rousselie, F., Rathery, M., Bour H., and Derot, M.: Specific angiopathies in diabetes secondary to pancreatitis and hemochromatosis. Proc. 2nd Meeting, European Assoc. for the Study of Diabetes, Aarhus, 1966, p. 122.

[13]Griffiths, J. D., Dymock, I. W., Davies, E. W. G., Hill, D. W., and Williams, R.: Occurrence and prevalence of diabetic retinopathy in hemochromatosis. Diabetes *20*:766-70, 1971.

[14]Dymock, I. W., Cassar, J., Pyke, D. A., Oakley, W. G., and Williams, R.: Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of hemochromatosis. Am. J. Med. *52*:203-10, 1972.

[15]Lundbaek, K., Christensen, N. J., Jensen, V. A., Johansen, K., Olsen, T. S., Hansen, Aa. P., Orskov, H., and Osterby, R.: Diabetes, diabetic angiopathy and growth hormone. Lancet *2*:131-33, 1970.

[16]Gay, J., Tchobroutsky, G., Rosselin, G., Assan, R., Dolais, J., Freychet, P., and Derot, M.: Etude de 8 cas d'hémochromatose primitive comportant en particulier le dosage radioimmunologique plasmatique des hormones somatotrope, folliculo-stimulante et du glucagon. Pathol. Biol. *16*:53-60, 1968.

[17]Stocks, A. E., and Martin, F. I. R.: Pituitary function in hemochromatosis. Am. J. Med. *45*:839-45, 1968.

[18]Simon, M., Franchimont, P., Murie, N., Ferrand, B., Van Cauwenberge, H., and Bourel, M.: Study of somatotropic and gonadotropic pituitary function in idiopathic haemochromatosis (31 cases). Eur. J. Clin. Invest. *2*:384-89, 1972.

[19]Kent, G., and Popper, H.: Liver biopsy in diagnosis of hemochromatosis. Am. J. Med. *44*:837-41, 1968.

[20]Balcerzak, S. P., Westerman, M. P., Lee, R. E., and Doyle, A. P.: Idiopathic hemochromatosis. A study of three families. Am. J. Med. *40*:857-73, 1966.

[21]Hultman, E.: Rapid specific method for determination of aldosaccharides in body fluids. Nature (London) *183*:108-09, 1959.

[22]Glick, S. M., Roth, J., Yalow, R. S., and Berson, S. A.: Immunoassay of human growth hormone in plasma. Nature (London) *199*:784-87, 1963.

[23]Caird, F. I., Pirie, A., and Ramsell, T. G.: Diabetes and the Eye. Oxford, Blackwell Scient. Publ., 1969, pp. 58, 76.

[24]Pirart, J., and Bastenie, P. A.: Complications vasculaires dans 34 cas de diabète bronzé. *In* Les Hémochromatoses. Paris, Masson, 1963, p. 431.

[25]Glick, S. M.: Normal and abnormal secretion of growth hormone. Ann. N.Y. Acad. Sci. *148*:471-87, 1968.

[26]Hansen, A., and Johansen, K.: Diurnal patterns of blood glucose, serum free fatty acids, insulin, glucagon and growth hormone in normals and juvenile diabetics. Diabetologia 6:27-33, 1970.

[27]Merimee, T. J., and Fineberg, S. E.: Dietary regulation of human growth hormone secretion. Metabolism *22*:1491-97, 1973.

[28]Glick, S. M.: Hypoglycemic threshold for human growth hormone release. J. Clin. Endocrinol. *30*:619-23, 1970.

[29]Peillon, F., and Racadot, J.: Modifications histopathologiques de l'hypophyse dans six cas d'hémochromatose. Ann. Endo-

crinol. 6:800-07, 1969.

[30]Tourniaire, J., Fèvre, M., Mazenod, B., and Ponsin, G.: Effects of clomiphene citrate and synthetic LH-RH on serum luteinizing hormone (L.H.) in men with idiopathic hemochromatosis. J. Clin. Endocrinol. Metab. *38*:1122-24, 1974.

[31]Luft, R., and Guillemin, R.: Growth hormone and diabetes in man. Old concepts. New implications. Diabetes *23*:783-87, 1974.

[32]Goldberg, M. F., and Fine, S. L., Eds.: Symposium on the treatment of diabetic retinopathy. Public Health Service Publ. 1890. Washington, D. C., U. S. Government Printing Office, 1969.

[33]Ray, B. S., Pazianos, A. G., Greenberg, E., Peretz, W. L., and McLean, J. M.: Pituitary ablation for diabetic retinopathy. I. Results of hypophysectomy (a ten-year evaluation). J.A.M.A. *203*:79-84, 1968.

[34]Passa, P., Gauville, C., and Canivet, J.: Influence of muscular exercise on plasma level of growth hormone in diabetics with and without retinopathy. Lancet *2*:72-74, 1974.

[35]Merimee, T. J., Siperstein, M. D., Hall, J. D., and Fineberg, S. E.: Capillary basement membrane structure: a comparative study of diabetics and sexual ateliotic dwarfs. J. Clin. Invest. *49*:2161-64, 1970.

[36]Christensen, M. J., and Terkildsen, A. B.: Quantitative measurements of skin capillary resistance in hypophysectomized long-term diabetics. Diabetes *20*:297-301, 1971.

[37]Passa, P., Bensoussan, D., Lévy-Tolédano, S., Caen, J., and Canivet, J.: Etude de l'agrégation plaquettaire au cours de la rétinopathie diabétique. Influence de l'hypophysectomie. Atherosclerosis *19*:277-87, 1974.