Sulfated Insulin in Mild, Moderate, Severe and Insulin-resistant Diabetes Mellitus

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SUMMARY

Moloney and co-workers prepared sulfated insulin and showed it to be less antigenic and less neutralizable by antibodies than unmodified insulin. The present study compares sulfated insulin with commonly used insulins (Regular, Protamine Zinc, Lente and NPH) in twenty-four hospitalized adult diabetics. The bases of comparison were units of insulin per day, three daily blood glucose levels and urine glucose content.

In six severe, ketosis-prone diabetic patients, sulfated insulin in approximately equal doses failed to control the disease nor did the usual long acting insulins.

In four mild diabetic patients sulfated insulin had no apparent advantage over other insulins.

For nine of twelve moderate diabetic patients less or equal units of sulfated insulin provided better control.

In two insulin resistant patients sulfated insulin very greatly reduced insulin requirements (to 5 per cent and 37 per cent) and provided much better control.

Hypoglycemia was no more frequent with sulfated insulin and there were no important toxic side effects. It appears to be a promising advance in the treatment of insulin resistant diabetes and prolonging its action may further enhance its usefulness. DIABETES *15*:457-65, July, 1966.

Moloney and co-workers[1] developed sulfated insulin at the Connaught Medical Research Laboratories, Toronto, by treating pure beef insulin with sulfuric acid under controlled conditions. The resulting sulfated insulin was found to be less antigenic and less neutralizable by antibodies. It greatly reduced the insulin requirements of patients with insulin resistant diabetes. This report compares the action of this new drug with commonly used insulins in a variety of patients with diabetes mellitus.

MATERIAL AND METHODS

Twenty-four adults with diabetes mellitus were studied. The basis for selection was willingness to cooperate for a sufficiently long time in hospital and an apparent need for exogenous insulin. All varieties of the "diabetic syndrome" were accepted as they presented themselves to the hospital in- and outpatient Diabetic Service. Table 1 shows types of diabetes, age, sex, weight and total duration of study in each patient.

It was usually necessary to inject sulfated insulin twice daily because of its twelve-hour duration of action. The "other" insulin used for comparison was the one which the patient was already taking or, in the case of a new diabetic, one which we thought suitable for future continued use. Capillary blood glucose was estimated by the micro-autoanalyzer technic, fasting, 11 a.m. and 3 p.m. daily. Glucose in the urine was estimated qualitatively four times daily and quantitatively on twenty-four hour collections. Insulin antibodies were determined by Dr. P. J. Moloney using previously described methods.[2,3] Dr. Judith Cutler determined plasma insulin levels by an immunoassay method.[4]

All patients were maintained in caloric balance with a constant, weighed, diabetic diet. Half the patients received sulfated insulin followed by the other insulin. This order was reversed in the other half. The insulin dosage was varied in order to achieve a period of optimum diabetic control with each type of insulin short of producing hypoglycemia. The indices for comparison of two insulins were units of insulin per day, the blood glucose levels and urine glucose content during the periods of optimum diabetic control. These are listed for eighteen patients in the Appendices. It was impossible to achieve periods of "optimum" control with two kinds of insulin in every case of mild and severe diabetes (vide infra). However, the moderate, stable diabetics were more satisfactory subjects and figure 1 for patient No. 4 and figure 2 for patient No. 7 show the titration with two kinds of insulin and the blood glucose levels. The periods of optimum control with each kind of insulin, which were used for statistical analysis and comparison, were Day 9 to 16 and 23 to 29 in figure 1 and Day 9 to 13 and 14 to 28 in figure 2.

RESULTS

Moderate diabetes

The results in the twelve moderate diabetics are

Presented at the Twenty-fifth Annual Meeting of the American Diabetes Association in New York City on June 19, 1965.

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TABLE 1

Patient classification and duration of study

Patient No.	Age	Sex	Weight (lb.)	Total days studied on Sulfated insulin	Total days studied on "Other" insulin*
			Moderate diabetes		
1	69	F	122	19	11
2	65	F	135	17	20
3	51	M	123	14	19
4	60	M	163	12	17
5	57	F	154	27	11
6	68	F	125	28	9
7	81	F	138	15	22
8	73	M	155	19	14
9	59	M	151	30	10
10	63	F	128	23	14
11	66	F	149	14	7
12	60	F	164	14	6
			Mild diabetes		
13	60	F	122	9	8+(6)
14	60	F	218	23	(39)
15	62	M	171	17	12+(28)
16	71	M	151	35	(6)
			Severe, ketosis-prone diabetes		
17	68	F	117	12	27
18	32	M	150	12	36
19	46	M	170	7	20
20	41	M	158	4	10
21	50	F	115	6	21
22	71	F	153	28	16
			Insulin resistant diabetes		
23	67	M	172	48	24
24	60	M	142	19	22

*Numbers in brackets represent days on no insulin.

shown in Appendix I. Patient 10 had insufficient 11 a.m. and 3 p.m. blood glucose estimations for comparison of these values. Figures 1 and 2 show the insulin dosage and the blood glucose levels for patients No. 4 and No. 7, respectively. Note that on sulfated insulin the fasting blood glucose tended to be higher than on Lente while the postprandial levels were lower.

Table 2 indicates the difference between the units of insulin required and the average blood glucose during the two periods of optimum control shown in Appendix I. The minus sign means less sulfated insulin was required or that the blood glucose was lower on sulfated insulin. Less or approximately equal doses of sulfated insulin provided better or equal control than other insulin in all except patients 9, 11 and 12.

Table 3 gives further details. By coincidence the average dose of sulfated insulin was the same as for the "other" insulin, namely 38.8 U. daily. The mean fasting blood glucose was significantly higher on sulfated insulin but the 11 a.m. values were significantly less and the 3 p.m. values also tended to be lower. The average of the three mean blood glucose values was significantly lower for sulfated insulin.

There were no significant differences in the number of hypoglycemic reactions, quantities of urine glucose or days of observation on the two types of insulin. No patient lost weight during the study.

Mild diabetes

Four mild diabetics (patients Nos. 13 to 16) requiring 20 or less U. insulin were studied. No advantage of sulfated over other insulin could be demonstrated partly because insulin requirement gradually disappeared in these patients.

In two new diabetics this occurred before another insulin could be tried (table 1). Patient No. 16 develeloped a generalized urticaria with fever, swelling of the extremities and elevated sedimentation rate, which may have been an allergy to sulfated insulin.

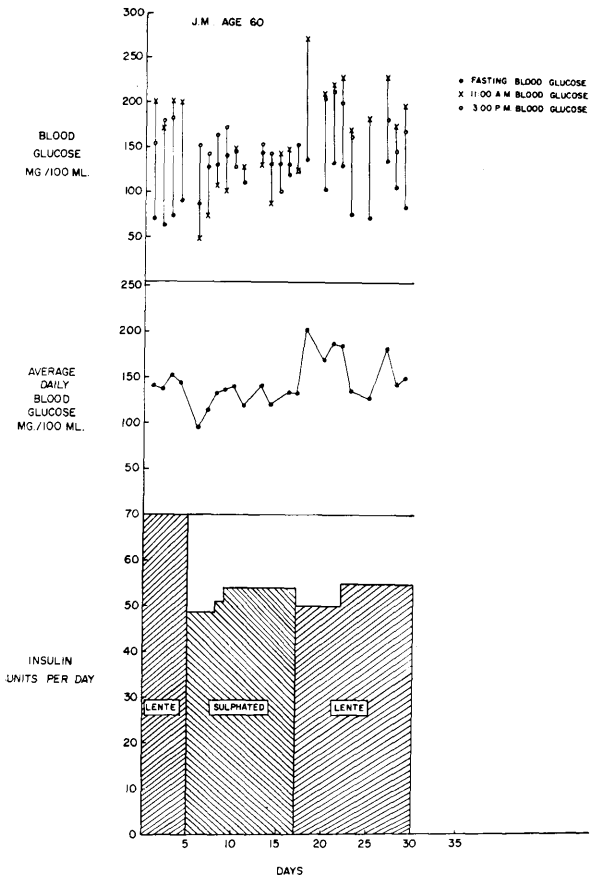

FIG. 1. Patient No. 4: Comparison of sulfated insulin and Lente Insulin in a patient with moderate diabetes.

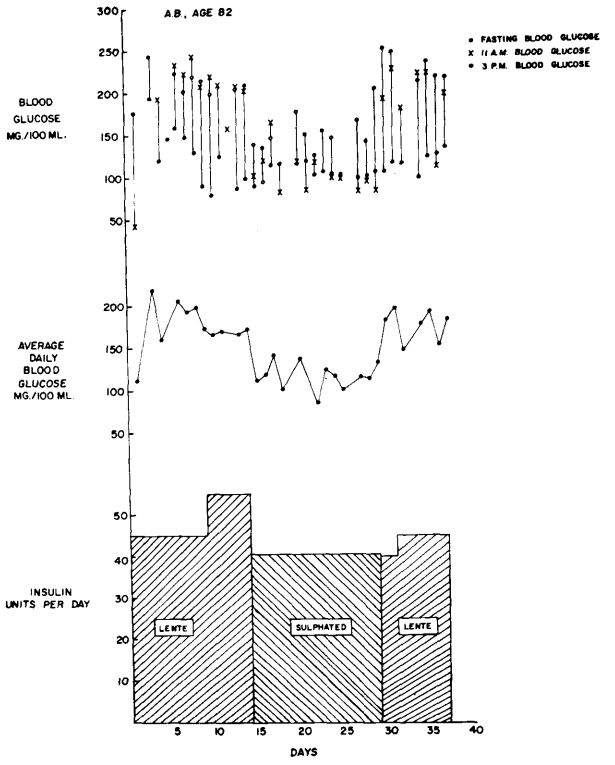

FIG. 2. Patient No. 7: Comparison of sulfated insulin and Lente Insulin in a patient with moderate diabetes.

However, these signs disappeared during continued therapy with the drug.

Severe diabetes

Six patients (Appendix II) with severe, ketosis-prone diabetes were studied. Table 4 summarizes their results. Here again, by coincidence the mean dose of the "two" insulins was the same, 73 units. On sulfated insulin the mean fasting blood glucose was markedly elevated but the postprandial levels were lower. The average mean blood glucose values were slightly higher with sulfated insulin. Five of these patients tended to become more ketotic on an equal or greater dose of sulfated insulin, necessitating a change back to one of the long acting insulins. (The sixth patient also became acidotic on sulfated insulin at home.)

The failure of twice daily sulfated insulin in these severe, ketosis-prone diabetics is probably explained by its relatively short duration of action. Possibly, if it had been tried, Regular Insulin would have been even worse for these patients under these conditions.

Insulin resistant diabetes

In two insulin resistant diabetic patients sulfated insulin greatly improved control and reduced the insulin requirements.

Patient No. 23

A sixty-seven-year-old male with diabetes for eight years had been controlled on 35 U. Protamine Zinc Insulin daily, but in the last month his requirements increased to 2,000 U. Regular Insulin daily. In the last few months he had noted fatigue, hoarseness, dry skin, stiff joints, evening swelling of ankles and feet, and numbness of his fingers.

He appeared chronically ill. A firm, two centimeter nodule was present in the right lobe of the thyroid, and over the next few weeks the entire gland became enlarged and tender. The eyebrows were coarse, the voice hoarse, and there was pretibial and ankle edema.

He had marked glycosuria, acetonuria and a serum protein bound iodine of 2.2 mcg. per 100 ml. Plasma antithyroid globulin titres rose from 1:180,000 to 1:1,600,000. A biopsy revealed Hashimoto's thyroiditis.

While on Regular Insulin alone and eleven hours after the last dose of 40 U., an oral glucose tolerance test gave the following results:

APPENDIX I

Comparison of sulfated insulin with other insulins during periods of optimum diabetic control in moderate diabetes

	Sulfated insulin†							"Other" insulin‡						
Patient number	U./day	Number of reactions	Days of optimum control	Blood glucose§ Time	No.	Mean	Mean urine glucose (gm./day)	Type	U./day	Number of reactions	Days of optimum control	Blood glucose§ Time	No.	Mean	Mean urine glucose (gm./day)
1	44.6	0	6	F	5	115		PZ	48	0	8	F	6	91	
				11 a.m.	4	120						11 a.m.	6	230	
				3 p.m.	4	136						3 p.m.	5	181	
				Average mean**		123	0					Average mean		167	1.01
2	30	1	5	F	4	115		Lente	52.5	0	8	F	5	109	
				11 a.m.	4	119						11 a.m.	7	233	
				3 p.m.	3	139						3 p.m.	3	186	
				Average mean		124	4.5					Average mean		176	8.3
3	27	0	5	F	4	98		Regular	36	0	6	F	5	101	
				11 a.m.	3	81						11 a.m.	4	140	
				3 p.m.	3	131						3 p.m.	4	122	
				Average mean		103						Average mean		121	
4	54	0	8	F	7	136		Lente	55	0	6	F	4	100	
				11 a.m.	7	129						11 a.m.	4	196	
				3 p.m.	6	128						3 p.m.	3	166	
				Average mean		131	0					Average mean		153	0
5	48.6	0	8	F	7	113		Lente	50	0	8	F	7	83	
				11 a.m.	7	101						11 a.m.	7	145	
				3 p.m.	6	132						3 p.m.	6	126	
				Average mean		115	0					Average mean		118	0
6	20.2	0	4	F	3	123		Lente	20	0	7	F	5	121	
				11 a.m.	3	90						11 a.m.	5	166	
				3 p.m.	2	164						3 p.m.	4	134	
				Average mean		126	0					Average mean		141	0
7	40.5	0	15	F	13	105		Lente	55	0	5	F	4	95	
				11 a.m.	13	102						11 a.m.	5	197	
				3 p.m.	11	147						3 p.m.	3	202	
				Average mean		118	0					Average mean		164	0
8	27	1	9	F	7	114		Lente	40	0	9	F	5	136	
				11 a.m.	8	100						11 a.m.	7	163	
				3 p.m.	7	154						3 p.m.	5	145	
				Average mean		122	0					Average mean		148	2.3
9	27	0	9	F	8	146		PZ	20	0	10	F	6	108	
				11 a.m.	6	86						11 a.m.	4	123	
				3 p.m.	7	151						3 p.m.	3	139	
				Average mean		128	0					Average mean		123	0
10	40*	0	4	F	4	122		PZ	100	0	13	F	6	100	not done
				11 a.m.	4	205	not								
				3 p.m.	3	199	done								
				Average mean		175									
11	67.5	0	7	F	6	154		PZ	25	0	7	F	3	104	
				11 a.m.	6	108						11 a.m.	1	142	
				3 p.m.	5	78						3 p.m.	1	115	
				Average mean		113	0					Average mean		120	0
12	40.5	0	7	F	6	144		PZ	25	0	6	F	3	99	
				11 a.m.	6	113						11 a.m.	1	143	
				3 p.m.	5	88						3 p.m.	1	108	
				Average mean		115	0					Average mean		117	0

*Protamine sulfate, 0.05 ml. of a 5 per cent solution, was added to 3 ml. of 100 U. per ml. sulfated insulin to produce a protamine sulfated insulin suspension in an attempt to prolong insulin action.

†Sulfated insulin given as divided doses before breakfast and supper.

‡The "other" insulin given as one dose before breakfast except to patients Nos. 10 and 3 who had divided doses of Protamine Zinc (PZ) and Regular Insulin respectively before breakfast and supper.

§Average mean is the average of the means of the fasting, 11 a.m., and 3 p.m. blood glucose values.

**Milligrams per 100 ml.

Time	Blood glucose mg./100 ml.	Immunologically active plasma insulin, μ units/ml. 1:40 dilution	1:80 dilution
Fasting	271	7,000	14,600
30 min.	386	7,600	>16,000
60 min.	478	7,400	>16,000
120 min.	542	7,300	>16,000
180 min.	546	6,350	>16,000

Also, at this time, one milliliter of fasting serum neutralized 4,000 μU. insulin with the mouse diaphragm method[2] and 7,500 μU. by the tanned sheep red cell hemagglutination method.[3] We have not detected antibodies with these tests in ordinary diabetics on insulin.

TABLE 2

Differences* between insulin requirement and average mean blood glucose values during optimum control on sulfated insulin and the "other" insulin in moderate diabetes

Patient No.	Units of insulin Sulfated—"Other"†	Average mean blood glucose (mg./100 ml.) Sulfated—"Other"†
1	— 3.4	—44
2	—22.5	—52
3	— 9	—18
4	— 1	—22
5	— 1.4	— 3
6	+ 0.2	—15
7	—14.5	—46
8	—13	—26
9	+ 7	+ 5
10	—60	not available
11	+42.5	— 7
12	+15.5	— 2

*Calculated from Values in Appendix I.
†A minus sign means that less sulfated insulin was required or that blood glucose was lower on sulfated insulin.

These results, which will be commented on later, were interpreted as indicating insulin resistance due to antibodies.

As shown in figure 3, treatment began with 75 U. Protamine Zinc Insulin and supplemental Regular Insulin. Despite increasing amounts of both, up to a total of 280 U. daily, marked hyperglycemia and glycosuria of 100 gm. persisted. Using Regular Insulin alone, 740 U. daily were required for control.

Sulfated insulin was then begun with 54 U. twice daily and subsequently increased to 94 U. at breakfast and 81 U. at supper with disappearance of glycosuria and lowering of blood glucose. After Day 28 repeated hypoglycemic reactions occurred and insulin was decreased to a total of 80 U. daily.

On a second trial of Regular Insulin, 425 U. were required (figure 3). This decline in insulin resistance was not apparently related to activity of thyroiditis, which, though being treated with thyroid hormone, remained acute throughout the study. Sulfated insulin was restarted at 95 U. daily. Because of hypoglycemia it was gradually reduced to a single morning dose of 40 U. which provided excellent control. This and smaller amounts of sulfated insulin have continued to provide good control in the succeeding year at home in spite of the fact that the plasma insulin antibody titre remains high, neutralizing 10,000 μU. of insulin per milliliter.

There can be no doubt that sulfated insulin greatly reduced exogenous insulin requirements in this patient.

Patient No. 24

A sixty-year-old male had been diabetic for one year and was controlled on 30 U. Protamine Zinc Insulin daily. Then, his insulin requirement increased to 60 U.

TABLE 3

Comparison of sulfated insulin with other insulins* in moderate diabetes

	Number of patients	Sulfated insulin	Other* insulin	p
Mean dose	11†	38.8 U.	38.8 U.	n.s.‡
Mean fasting blood glucose	12	124 mg./100 ml.	104 mg./100 ml.	<.01
Mean 11 a.m. blood glucose	11†	104 mg./100 ml.	170 mg./100 ml.	<.01
Mean 3 p.m. blood glucose	11†	132 mg./100 ml.	148 mg./100 ml.	n.s.
Average mean blood glucose	11†	120 mg./100 ml.	139 mg./100 ml.	<.05

*Protamine Zinc in five, Lente in six, and Regular Insulin in one patient.
†Patient No. 10 excluded because of insufficient 11 a.m. and 3 p.m. blood glucose estimations.
‡n.s. = not significant.

APPENDIX II

Comparison of sulfated insulin with other insulins in severe diabetes

Sulfated insulin								"Other" insulin							
Patient number	Days studied	Dose Mean total per day	Divided*	Blood glucose (mg./100 ml.) Time	No.	Mean	Mean urine glucose (gm./day)	Days studied	Type	Dose Mean total per day	Divided*	Blood glucose (mg./100 ml.) Time	No.	Mean	Mean urine glucose (gm./day)
17	9	104	b.i.d. and t.i.d.	F	4	255		10	NPH	75	b.i.d.	F	8	71	
				11 a.m.	4	139						11 a.m.	8	274	
				3 p.m.	3	147						3 p.m.	6	161	
				Average mean		180	8					Average mean		169	12
18	4	81	b.i.d.	F	3	276		6	NPH	51.6	b.i.d.	F	5	78	
				11 a.m.	3	174						11 a.m.	5	277	
				3 p.m.	2	220						3 p.m.	4	322	
				Average mean		224	140					Average mean		226	83
19	5	64	b.i.d.	F	4	224		10	Lente	81	b.i.d.	F	6	120	
				11 a.m.	4	148						11 a.m.	4	175	
				3 p.m.	3	188						3 p.m.	5	172	
				Average mean		186	33					Average mean		156	47
20	3½	25	b.i.d. and q.i.d.	F	4	295		7	Lente Regular	80 18	b.i.d. q.i.d.	F	5	158	
				11 a.m.	4	177						11 a.m.	5	276	
				3 p.m.	3	251						3 p.m.	5	241	
				Average mean		240	49					Average mean		225	31
21	4	46	b.i.d.	F	5	231		11	Lente	50	o.d. and b.i.d.	F	9	176	
				11 a.m.	5	128						11 a.m.	9	268	
				3 p.m.	4	193						3 p.m.	7	184	
				Average mean		184	3					Average mean		209	20
22	9	61	b.i.d.	F	9	181		7	Lente	80	o.d.	F	5	131	12.6
				11 a.m.	7	99									
				3 p.m.	6	69									
				Average mean		116	1.1								

*o.d. = once daily; b.i.d. = twice daily; t.i.d. = three times daily; q.i.d. = four times daily.
Patient Nos. 23 and 24 are not included in the Appendix. See text and Figures 3 and 4 for their data.

daily. Biopsy of an enlarged liver and serum iron studies revealed hemochromatosis.

Figure 4 shows that despite 75 U. Lente Insulin and 45 U. of Regular Insulin in divided doses, he had markedly elevated blood glucose levels. Sulfated insulin was then started. Initial poor control was due to lack of dietary cooperation, but when this was corrected 27 U. before breakfast and 13.5 U. before supper provided excellent control with no glycosuria. The one sharp deviation in blood glucose on Day 20 was due to the patient again eating extra food.

On a second trial of Lente Insulin, 85 U. before breakfast and 25 U. before supper provided reasonable control, but it was inferior to that on 40 U. of sulphated insulin. In the year since then, 20 U. of sulfated insulin in the morning and 15 U. at supper have provided better control at home than Lente or twice daily NPH Insulin.

When the patient was on Regular Insulin alone, and after it was withheld for twelve hours, there were no detectable circulating insulin antibodies by the mouse diaphragm[2] or tanned sheep red cell hemagglutination method.[3] However, these tests are not sensitive and the striking reduction in dosage with sulfated insulin suggests that insulin antibodies were present and causing insulin resistance.

DISCUSSION

Obviously, relatively small doses of sulfated insulin greatly improved diabetic control in the insulin resistant

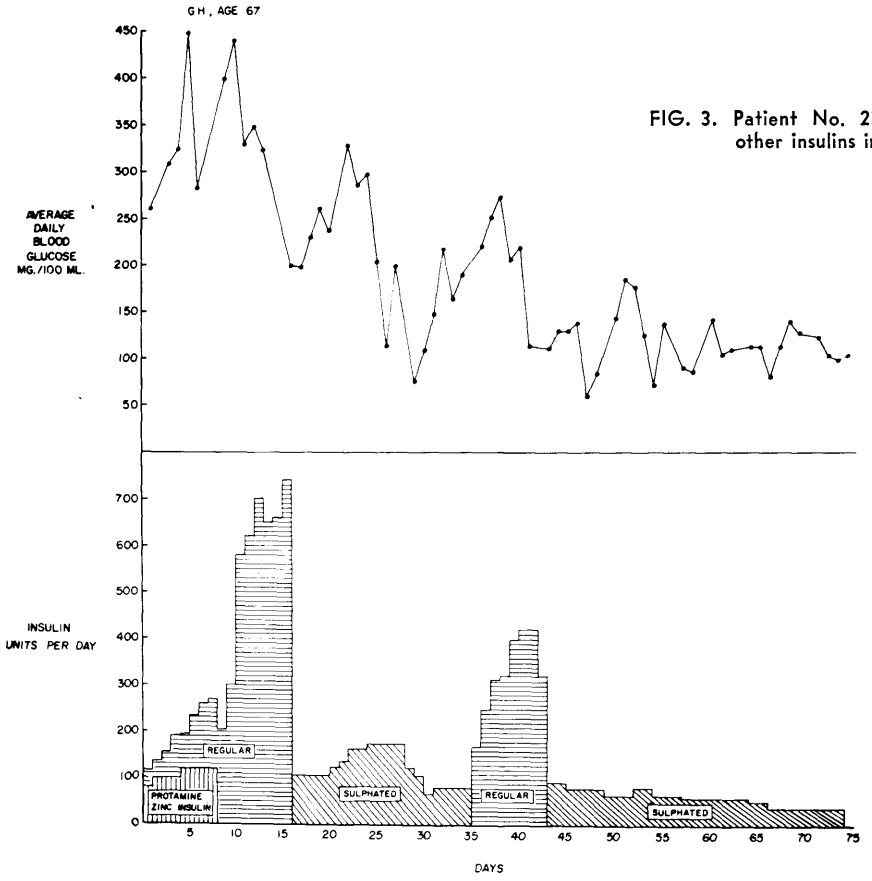

FIG. 3. Patient No. 23: Comparison of sulfated insulin with other insulins in a patient with insulin resistant diabetes.

TABLE 4

Comparison of sulfated insulin with "other" insulins in severe diabetes

	Number of patients	Sulfated insulin	"Other"* insulin	p
Mean dose	6	73 U.	73 U.	n.s.†
Mean fasting blood glucose	6	244 mg./100 ml.	126 mg./100 ml.	<.001
Mean 11 a.m. blood glucose	5	153 mg./100 ml.	254 mg./100 ml.	<.001
Mean 3 p.m. blood glucose	5	200 mg./100 ml.	216 mg./100 ml.	n.s.
Average mean blood glucose	5	203 mg./100 ml.	195 mg./100 ml.	n.s.
Mean daily urine glucose	5	39 gm.	34 gm.	n.s.
Tendency for ketosis	6	5	0	<.05

*NPH Insulin twice daily in two patients; Lente twice daily in two patients; Lente once daily in one patient; Lente plus Regular in one patient.

†n.s. = not significant.

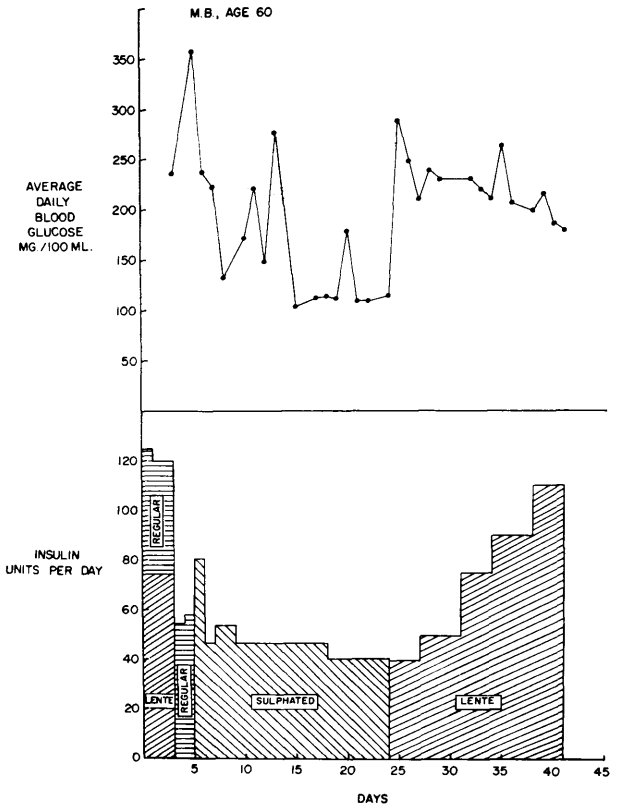

FIG. 4. Patient No. 24: Comparison of sulfated insulin with other insulins in a patient with insulin resistant diabetes.

patients. It is equally obvious that our present sulfated insulin preparation is inadequate for severe ketosis-prone, brittle diabetics. However, the majority of adult diabetics are moderate, stable and ketosis-resistant, and most patients in this group had improved control with equal or less units of sulfated insulin. In mild diabetics, requiring less than 20 U., sulfated insulin had no demonstrable advantage over other insulins.

The twelve-hour duration of action and four to six-hour peak action of this insulin means that two daily doses are usually required. This resulted in lower 11 a.m. and 3 p.m. blood glucose levels and higher fasting values than with the usual long acting insulins. However, patient No. 23, with a high titre for insulin antibodies, eventually achieved excellent control with only a single morning injection. Possibly this was due to the circulating antibodies prolonging the action of sulfated insulin. Fortunately, the relatively weak binding of sulfated insulin by antibody,[1] as compared to that with nonsulfated insulins, probably resulted in sufficient release of insulin for metabolic needs.

Plasma insulin was estimated in patient No. 23 because of the unusual insulin resistance despite the fact that interpretation of results is difficult in the presence of antibodies from exogenous insulin. Where insulin antibodies are present in plasma there will be insulin-antibody complex in equilibrium with free insulin and free antibody. Insulin antibodies were demonstrated in patient No. 23 by the mouse diaphragm and hemagglutination methods and the results were of the same order of magnitude. Because in both assay methods insulin is added to the plasma, equilibrium conditions are disturbed and it is not to be expected that the results will be a measure of the free antibody level. Similarly, the estimated plasma insulin level, namely 7,000 μU. per ml., is not a measure of free insulin. And this is also to be expected since in the immunoassay method insulin-antibody is added to the plasma, which alters the antigen-antibody equilibrium. In this insulin assay no effort was made to eliminate the antibody or correct for its presence. Dilution of the plasma may have freed insulin from its antibody accounting for the different insulin concentrations in the two dilutions. Plasma insulin levels of 7,000 or 16,000 μ units per ml. are not consistent with the high blood glucose concentrations and undoubtedly the free insulin levels were much lower.

Causes for decreased dosage other than the change to sulfated insulin were considered. It is unlikely that a Somogyi effect[5] accounted for improved control with decreased doses of sulfated insulin because each patient was also titrated with changing doses of the "other" insulin and the order of usage of the two insulins was reversed in alternate patients. Changing the species source of insulin has been shown to reduce insulin requirement in diabetes.[6,7] However, the species source (beef) of sulfated insulin did not account for decreased dosage. In many cases where reduced dosage and improved control occurred the other insulin was Lente or Regular, also made from beef. In the remaining patients the other insulin was PZ or NPH, both being mixtures of beef and pork.

In the moderate diabetic group the comparison might better have been made with only a short or intermediate acting insulin such as Regular or Globin, also given twice daily. It is possible that for sulfated insulin the twice daily injection, as compared to the single injection of other insulin, was more important in reducing the dose than was the sulfation. However, in the two insulin-resistant patients this could not have been the case since sulfated insulin reduced both the dose and the number of daily injections. Also, in the moderate diabetic group two patients who were receiving two doses

of "other" insulin daily required less units of sulfated insulin.

It is our impression that a stable, longer acting sulfated insulin (e.g. Protamine Zinc sulfated) might be more useful for the moderate diabetics and might even control the severe group. With a suitable long acting sulfated insulin, comparison with other long acting insulins would be easier and probably better since both types could be given once daily.

Our own attempts to combine Protamine with sulfated insulin were unsuccessful. The resulting material was a sticky precipitate tending to cling to the wall of the glass vial. Therefore, the amount remaining in suspension decreased with time and its action was unpredictable. Perhaps this technical problem can be overcome. It is our opinion that sulfated insulin is a promising therapeutic advance especially in the treatment of insulin resistant diabetes and further modification of the insulin molecule, with prolongation of action, should be attempted.

ACKNOWLEDGMENT

This study was supported by grants from the Medical Research Council of Canada and Denison Mines Limited.

The authors wish to thank Dr. P. J. Moloney and Dr. Judith Cutler for their help and advice in the investigation of this new insulin. We are also indebted to Dr. W. R. Campbell, Mr. M. O'Sullivan, Mr. A. Smialowski, Miss R. Yano, Mrs. A. Csima, Mrs. K. Sullivan and the dietitians and nurses of the Clinical Investigation Unit.

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Endocrine Glands and Hypothalamic Obesity

Various factors have been considered as primary or secondary causes of the obesity observed following hypothalamic ventromedial lesions. It is not clear from the literature what are the effects of many of the endocrine changes accompanying this type of obesity. Especially inconsistent have been the changes observed in pancreatic beta cells (F. X. Hausberger, Fed. Proc. *17*:67, 1958; W. Gepts, Ann. Endocrinol. *24*:140, 1963; Hausberger, G. L. Broadhead, Jr., and B. C. Hausberger, Acta Endocrinol. *45*:600, 1964).

G. Sétáló (Acta Physiol. Acad. Sci. Hung. *27*:375, 1965) has conducted an experiment designed to collect data on changes of the endocrine glands in general, and especially of the pancreatic islets of rats with hypothalamic obesity. The author was particularly interested in whether an altered function of these organs might play a role in causing hypothalamic obesity.

Electrolytic lesions in the hypothalamic ventromedial nuclei were caused in a group of 60 to 70 gm. male rats. Thirteen were defined as obese; these rats and eight control rats were studied for one year. Eleven months after the lesions were caused, blood glucose was determined in the fasted state after 400 mg. of glucose per 100 gm. body weight were injected intragastrically. One year after the lesions were caused, the animals were sacrificed; the following parameters were measured: I-131/s-ratio, body weight, nose to anus length, tibial length, and pituitary, thyroid, adrenals, and testes weights.

Histological studies were done on the pituitaries, pancreas, testes, thyroid, brains, and adrenals. Nuclear volume of the pancreatic beta cells and of cells in the fascicular zone of the adrenals was determined. Spermiogenesis was quantitatively studied.

Rats with lesions of the hypothalamus weighed more than controls at the end of the experiment: 417 gm. versus 345 gm. ($P < 0.01$). The obese rats had slightly shorter bodies and tibiae. The mean absolute weight of the adrenals of the obese rats was greater than the controls: 68.5 mg. versus 47.5 mg. The hypophyses of the obese rats weighed less than those of the controls

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