Presence of Synalbumin Antagonist in Siblings of Diabetic Children

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SUMMARY

Synalbumin antagonist was investigated in a group of forty-eight siblings of diabetic children. Nine parents of these patients, ten normal controls and eleven diabetic children were included in this study. In twenty-six of the forty-eight siblings synalbumin was detected. Glucose uptake by the rat hemidiaphragm was depressed from a mean of 13.36 to 0.99 mg. per cent of glucose per 10 mg. of tissue in ninety minutes incubation in the presence of 1,000 μU. of insulin. In five of nine parents tested, synalbumin was present. In only three of fourteen families in which two or more siblings were studied was synalbumin absent. These findings suggest a familial distribution of the antagonist. Fasting blood sugar, glucose tolerance test and plasma insulin were normal in synalbumin positive siblings. Negligible amounts of immunoreactive insulin were detected in the albumin extracted from nondiabetic relatives. The synalbumin did not interfere with the immunoassay of insulin. In extracts of insulin-treated diabetics, various amounts of insulin were found. Five of eleven diabetics showed antagonism, three were border line, and three did not show antagonism. The results obtained suggest that synalbumin may be used as a genetic marker and encourage further investigation to assess the role of this inhibitor in the development of diabetes and as an indicator of prediabetes. DIABETES *15*:400-05, June, 1966.

The mode of inheritance of diabetes mellitus has been the subject of much controversy mainly because a suitable genetic marker has not been found. Recently Vallance-Owen has suggested that synalbumin (the insulin antagonist associated with plasma albumin) might be such a marker. From his investigations of several families with diabetes, he postulated a "dominant" mode of inheritance.[1-4]

We have investigated the synalbumin content of plasma in a group of siblings of diabetic children attending our clinic. The parents of some of these children with synalbumin were also studied. The data reported in this paper represent the initial results of a long term study of the role of synalbumin in the development of diabetes and its potential as an indicator of prediabetes.

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MATERIALS AND METHODS

Fasting heparinized blood was obtained in all instances except when glucose tolerance tests were performed on capillary samples. The plasma was separated by centrifugation. Insulin was determined by the immunoassay procedure of Morgan and Lazarow[5] and glucose by the glucose oxidase method of Huggett and Nixon.[6] Plasma was submitted to the trichloracetic-alcohol extraction of Debro[7] as recommended by Vallance-Owen.[8] Glucose tolerance tests (1 gm. per kilo up to 50 gm. orally) were performed on some siblings taken at random from the group in which insulin antagonism was found.

For the detection of insulin antagonist in the albumin fraction, the rat hemidiaphragm preparation of Vallance-Owen and Hurlock[9] was used. Hemidiaphragms were incubated in buffer (Gey & Gey) with 300 mg. of glucose per 100 ml. (base line); buffer plus 1,000 μU. of crystalline beef insulin per milliliter and in buffer with 1,000 μU. insulin plus 1.25 gm. per 100 ml. of the freeze-dried albumin extract. The *p*H of the incubation media was controlled after the additions and adjusted to a constant value of 7.2-7.4. All samples were determined in triplicate. Glucose uptake was determined on the deproteinized media (Somogyi Barium-Zinc filtrate) at the end of the ninety-minute incubation period. After substraction of base line uptake the results were compared with the effect of 1,000 μU. per milliliter insulin. Base line and standard were determined in each experiment.

In twelve samples taken at random the extracted albumin was assayed for the presence of insulin by the immunoassay method. In order to assess any effect of the synalbumin on the immunoreaction of insulin, recovery experiments were carried out in which 100 μU. of insulin per milliliter were added to the borate buffer with 1.25 per cent of albumin extract instead of the regular 1 per cent bovine serum albumin.

RESULTS

A total of eighty-five plasma samples was studied. In order to evaluate the results, the material was divided into four groups: (a) diabetics; (b) siblings of diabetic children in which no antagonist was found; (c) siblings in which an insulin antagonist was present; and (d) normal controls.

Before assessing the existence of antagonistic effect, the coefficient of variation of the method was calculated by measuring the glucose uptake over base line under the influence of 1,000 μU. insulin per milliliter. A mean value of 13.9 (± 6.1 S.D.) mg. per cent per 10 mg. of tissue in ninety minutes was recorded in seventy-two different experiments with a coefficient of variation of 43 per cent. Therefore, only variations larger than 50 per cent below the 1,000 μU. standard were taken as indicative of antagonistic effect.

Synalbumin insulin antagonist determination (table 1).

(a) *Diabetics.* Eleven diabetic patients were included in this group. Three showed no antagonism while in two others the effect was border line (> 40 per cent < 50 per cent).

(b) *Siblings with no insulin antagonism.* Twenty-two of forty-eight children examined did not exhibit any antagonistic effect. The values observed were not significantly different from those observed in normal subjects or the 1,000 μU. insulin standard.

(c) *Siblings with insulin antagonism.* An average depression of 92.5 per cent of glucose uptake was found in this group of twenty-six children. The age ranged from seven months to seventeen years. The familial distribution is summarized in table 2 and figure 1.

Both parents of four families and the mother of a fifth one were included in this study. In all four couples the presence of an antagonistic effect was detected in one of the members, while it was absent in the other one. One of the parents with the synalbumin antagonist present was found to be a diabetic with a fasting blood glucose of 225 mg. per 100 ml. Two other parents had values slightly over 120 mg. per 100 ml. and will be repeated (table 3).

(d) *Normals.* This group includes children with no obvious endocrine disorder selected at random from the wards of The Hospital for Sick Children. The fasting blood glucose and plasma insulin values were within normal limits. Two children were found to have antagonist. In one there was a family history of diabetes mellitus; in the other no family history was elicited. In the remaining, the mean values for antagonists were not significantly different from those of the normal siblings. Two cases in this group were set apart and not included in the normal group. They were two obese children in which the glucose tolerance test was found to be in the upper limit of normal. In one of them the fasting plasma insulin was relatively high. The test for antagonist showed an inhibition of 84.7 per cent (table 4).

Blood glucose and glucose tolerance test.

Table 5 summarizes the results. Fasting blood glucose levels in normals and siblings either with or without antagonist were within the normal range. The large spread observed in the diabetic group reflects the different degrees of control at the time the samples were taken. The higher mean value in the group of parents with antagonist present was because of the one diabetic parent mentioned above.

TABLE 1

Synalbumin antagonist

	Number	1,000 μU. Ins per ml.*	1,000 μU. Ins/ml. + 1.25 albumin/ 100 ml.*	Per cent
Normals	10	10.98± .21	9.29±1.06	—15.0
Diabetics	11	17.69±2.9	7.18±1.96	—59.4
Siblings (with antagonist)	26	13.36±1.96	0.99±0.62	—92.5
Siblings (no antagonist)	22	13.35±1.37	10.84±1.15	—18.8

*Mean glucose uptake above base line in mg. glucose per 100 ml. per 10 mg. rat diaphragm in ninety-minute incubation ± S.E.M. Base line = amount of glucose taken up by diaphragm when no insulin is present in the incubation medium.

TABLE 2

Familial distribution

Family		Siblings Total tested	Siblings Per cent synalbumin positive	Parents per cent synalbumin positive
1	Br	2	50	50
2	Bu	2	50	
3	Ka	2	100	50
4	Ro	2	0	
5	Me	3	67	*
6	Kl	3	100	50
7	Du	2	50	
8	Gl	2	0	
9	O'H	2	0	
10	Po	2	50	
11	Sa	2	100	50
12	Ta	3	100	
13	Wo	2	50	
14	Za	2	100	

*Only one parent tested. Antagonist present.

In seventeen families (not included in this table) only one sibling tested. Seven of those children had antagonist present.

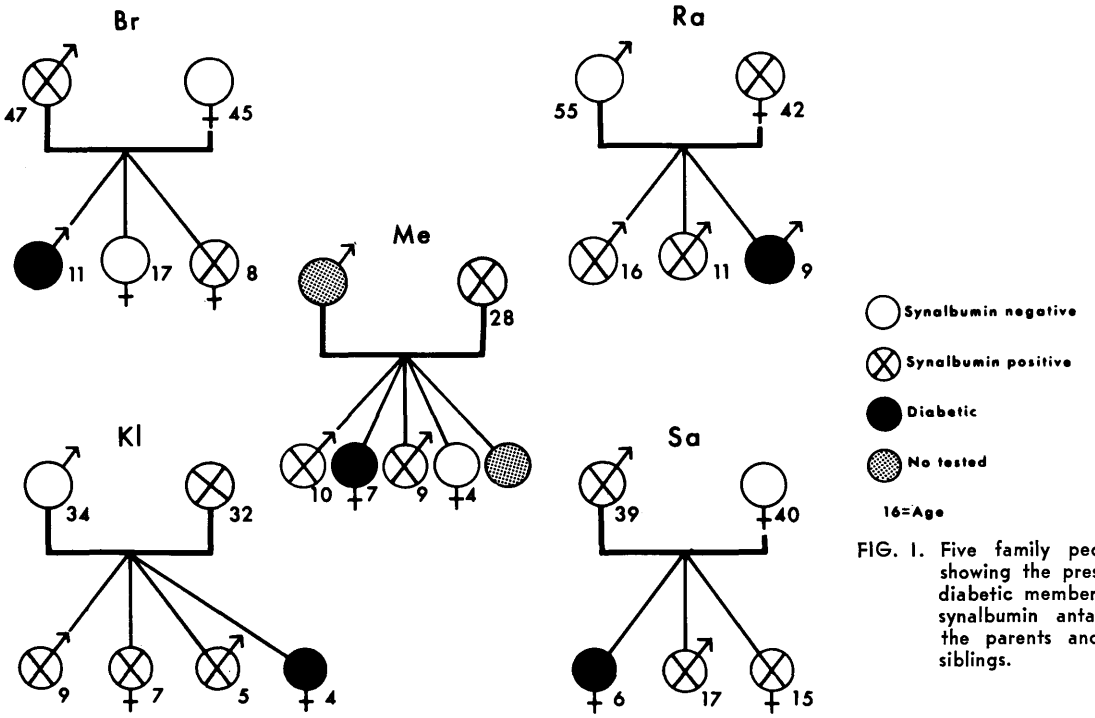

FIG. 1. Five family pedigrees, each one showing the presence of one overt diabetic member and an excessive synalbumin antagonist in one of the parents and in some of the siblings.

TABLE 3

Synalbumin antagonist—Parents

	Number	Per cent inhibition	Fasting blood glucose*	Fasting serum insulin*
Synalbumin positive	5†	—64	144±23.9	5.4±1.5
Synalbumin negative	4	—36	102.8± 6.3	8.5±0.5

*Mean value ± S.E.M.
†One of the parents in this group had a fasting blood glucose of 225 mg./100 ml.

TABLE 4

Obese children

Number	Per cent inhibition	Fasting blood glucose*	Serum insulin*
2	84.7	99.5±7.4	17.0±5

Glucose tolerance tests and plasma insulin response

	F	½ hr.	1 hr.	2 hrs.	3 hrs.
S.H. (glucose mg./100 ml.)	89	169	188	120	112
S.H. (insulin μU./ml.)	24	92	139	156	78
G.G. (glucose mg./100 ml.)	89	144	158	110	
G.G. (insulin μU./ml.)	10	10	19	15	

*Mean value ± S.E.M.

The curve depicting the GTT in siblings with an excess of synalbumin antagonist is normal (figure 2).

TABLE 5

Blood glucose and plasma insulin*

	Number	Blood glucose†	Plasma insulin†
Normals	15	71.2± 4.5	5.1 ± 1.1
Diabetics	11	192.0±42.5	81.2 ±34.3
Siblings with antagonist	25	84.4± 1.7	10.24± 1.6
Siblings without antagonist	23	82.1± 2.6	13.50± 3.0

*All values are fasting.
† Mean value ± S.E.M.

Plasma insulin. (table 5)

Normal values of plasma insulin levels were found in all groups except the diabetics. The high value in some of these cases is the result of administered insulin which can be detected in the circulation even forty-eight hours after the last injection of the crystalline product. This phenomenon occurs in children who have been treated with insulin for some time (unpublished observations). One of these obese children showed an excessive insulin response to the glucose load (S.H., table 4).

Albumin extracts and immunoassay of insulin (table 6).

Albumin fractions extracted from plasma of siblings and parents with and without antagonist had negligible amounts of insulin by the immunochemical procedure. The same extracts did not interfere with the immunoreaction of insulin as shown by the good recovery when

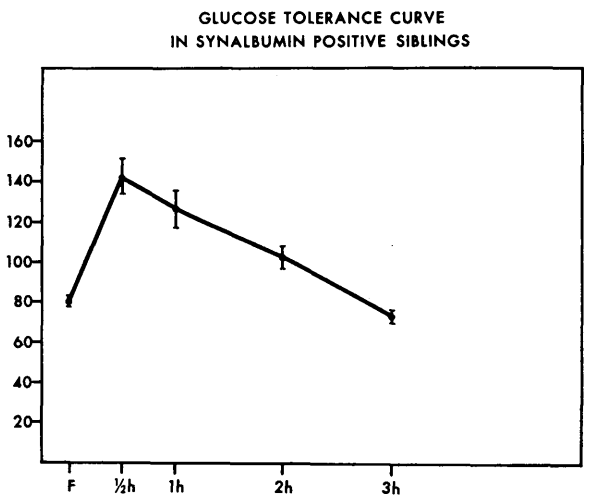

FIG. 2. Each point represents the mean value of eleven determinations and the vertical bars the S.E.M.

100 μU. per milliliter of Crystalline Insulin was added to the extract.

When the albumin was extracted from the plasma of treated diabetics various amounts of insulin were found, except in the first child who had been in treatment for only two days at the time of sampling. The recovery experiments in this group demonstrate the interference of both exogenous insulin and antibodies in the four patients on treatment for longer periods.

TABLE 6

Immunoassay of insulin on synalbumin extracts

	Insulin content of the extract μU./ml.	Insulin recovery* μU./ml.	Per cent inhibition†
Non-diabetic			
B.K.	0	113	9.3 (Normal)
C.M.	2	102	0 (Normal)
M.M.	—	118	40 (Normal)
C.V.	—	118	1.6 (Normal)
Mr. B.	1	114	46.4
Mrs. B.	1	95.5	77.4
Mrs. R.	9	118	46.6‡
Mr. R.	6	109	16.6
Mrs. K.	0	117	71.5
Mr. K.	2	121	44.0
M.V.	6	136	47.3
Diabetic			
D.M.	1	106	41.5§
M.V.	10	132	—11.0‡
V.S.	>100	>100	0
R.D.	60	64	80.0
R.F.	130	>100	55.0
B.H.	61	>100	45.4

*100 μU./ml. of insulin added.
†The presence of antagonist as determined by the rat diaphragm is expressed in per cent inhibition found in each individual plasma.
‡Untreated diabetic.
§Treated with insulin for two days.

DISCUSSION

The results of this preliminary study confirm the work of Vallance-Owen[1-4] that synalbumin is present in the close relatives of diabetic children. The present studies are insufficient to draw any genetic conclusions, but it is of interest that our findings are similar to those of Vallance-Owen.[2,3] Half the parents tested (one in each family) contained the antagonist. Of this group one was definitely diabetic and two others had fasting blood glucose greater than 120 mg. per 100 ml. Only one parent without antagonist had a fasting blood glucose greater than 120 mg. per 100 ml.

Fourteen families were studied in which more than one nondiabetic sibling was tested (table 2). Of these, eleven had 50 per cent or more of the children showing synalbumin. In seventeen families only one nondiabetic sibling was studied and, therefore, no definitive conclusion can be drawn. These findings suggest that perhaps there is a familial distribution of the synalbumin antagonist.

Oral glucose tolerance tests performed on eleven of the siblings who were synalbumin positive were normal, indicating that the presence of the antagonist was not as yet producing any apparent effect on glucose tolerance. Vallance-Owen and Ashton have reported[11] the presence of synalbumin in the sera of nineteen patients who had suffered myocardial infarction; none of these patients had any overt abnormality of carbohydrate metabolism. We plan to test the remaining children and their parents who showed antagonist and follow both groups (with and without antagonist) for a number of years. It would be of interest to study plasma lipids and biopsy material from these children as well.

This investigation of the synalbumin antagonist indicates that when the technic of Vallance-Owen is followed as he described it and factors such as *p*H are controlled, results identical with those previously published are observed. The failure of some groups[12,13] to find an antagonistic effect of albumin may be due to variations in technic (incubation procedure, or method of extraction) from that originally described. It has been shown by Kipnis[14] and Recant[10] that fatty acids do not account for the antagonistic effect of synalbumin. It seems to us that, whatever the nature of synalbumin (B chain of insulin[15] or some other factor), that it is a real phenomenon and not an artefact of technic. However, it should be stressed that, in spite of the presence of antagonist in the plasma of some of the examined subjects, their plasma insulin, blood glucose, and glucose

tolerance test were normal. If synalbumin operates in vivo as it does in vitro, it would be anticipated that elevated levels of insulin should be detected when blood glucose and glucose tolerance test were within normal limits. Therefore, the translation of the in vitro data to the in vivo situation should be done with caution.[14]

It is of interest to note that in keeping with others,[11,14] no insulin was found in the albumin extracts of nondiabetics in the concentration tested (1.25 per cent). Recovery experiments indicate no interference by the antagonist with the immunoassay procedure. Recent observations from this laboratory[16] indicate that all the insulin present in plasma of normal subjects is measured by the immunoassay technic and that no additional insulin is liberated by extraction. Although we did not measure the extracts for insulin-like activity, it seems unlikely that significant amounts would be present from the above observations.

In the extracted plasma albumin of treated diabetics, insulin was present. Five of eleven diabetics showed antagonism; three were border line (between 40 and 50 per cent inhibition) and three definitely did not show antagonism (table 7). The presence of insulin in the albumin extract of insulin-treated patients could explain the lower antagonistic effect found in the diabetic children as a group when compared to the synalbumin-positive siblings. This insulin may be able to mask some of the antagonistic action of the synalbumin.

It is of interest to note that in two of the three cases of diabetes of most recent onset (table 7), antagonism was border line in one and absent in one other. The significance of this observation is not known, although Alp and Recant[10] have recently reported diabetic patients without synalbumin antagonism.

Of great interest were the results in two obese children. Synalbumin was present in both. Glucose tolerance was normal in both, but one child showed an excessive rise in insulin similar to the finding of Karam et al.[17] The family history was negative for diabetes in both children. Vallance-Owen has stated[3] that obese individuals without a family history of diabetes mellitus do not have synalbumin. We intend to investigate more obese children for antagonist and plasma insulin response.

Few firm conclusions can be drawn from the data accumulated thus far. It seems that synalbumin is present in a large percentage of close relatives of diabetics and it is present in each generation. Carbohydrate tolerance and plasma insulin levels are normal in the synalbumin positive siblings of diabetic children. Some families do not seem to have the antagonist, although diabetes is present. Not all diabetics in this investigation demonstrated antagonist. The albumin extract of nondiabetics does not contain insulin (at 1.25 per cent concentration) and does not interfere with the immunoassay for insulin. Obese children may have antagonist even without a family history of diabetes mellitus.

TABLE 7

Synalbumin antagonist—diabetic children

Patient	Fasting blood glucose mg./100 ml.	Fasting plasma insulin μU./ml.	Per cent inhibition	Remarks
DMcC	261.6	137.5	92.2	Insulin treated
WF	262.0	308.0	68.3	Insulin treated
KC	63.0	19.0	32.3	Insulin treated
DM	82.2	2.0	41.5	Two-day treatment
RD	60.0	40.0	80.1	Insulin treated
RF	411.0	38.0	54.8	Insulin treated
BH	132.0	100.0	45.4	Insulin treated
MD	268.0	6.0	100.0	Untreated
MV	420.0	5.0	11.2	Untreated
TV	76.8	64.0	45.8	Insulin treated
US	61.8	118.0	+1.1	Insulin treated

ACKNOWLEDGMENT

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Comparison of an 800 to 1000 Calorie Diet with Fasting in Weight Reduction

(*Continued from page* 399)

markably, in the four patients who were followed for sufficient periods of time, the weight curves either remained constant or rose *only* until they had reached the extrapolated regression lines calculated from body weights measured while the patients were on the 800 or 1000 calorie diets before the period of fasting.

With continuation of the second period of 800 or 1000 calorie diets, the patients again began to lose weight at approximately the same rate as they had during the first period on the same diets. The "extra" weight loss noted during the period of near or complete fasting was thus completely regained, and each patient ended the study with a weight which did not differ significantly from that predicted by extrapolation of the rate of weight loss from the first period of 800 or 1000 calorie diet.

Two patients were lost from this study before the second period of low calorie, low carbohydrate diet could be completed. They left the hospital in dismay at their rapid weight gain on resuming the 800 to 1000 calorie diets following the period of fasting.

It would appear from the data presented in this article that, in terms of eventual weight loss, little benefit is to be derived from intermittent periods of fasting or near-fasting that cannot be had from a more liberal 800 to 1000 calorie, low carbohydrate diet which is continued over the same period of time. Although the number of patients involved in this study is small, there have been other reports which lend support to this conclusion. W. L. Bloom and G. J. Azar (Arch. Int. Med. 112:333, 1963) reported weight loss on a carbohydrate free diet which was quantitatively comparable on a very short term basis to that observed in fasting subjects. The authors of the article under review also quote a personal communication from A. Kekwick which states that the rate of weight loss does not increase with dietary restriction below 1000 calories per day.

Although the results of this study are surprising, there are possible explanations for them. The authors suggest that it is possible that starvation or fasting may result in a reduction of the basal metabolic rate (A. Keys et al., The Biology of Human Starvation. The University of Minnesota Press, Minneapolis, 1950), thereby reducing caloric needs at least temporarily. It is also possible that loss of the specific dynamic action of food and reduction of the amount of energy used for digestion and absorption of food may significantly reduce the body's caloric needs. The moderate malaise associated with fasting may result in reduced physical activity. The total of such reductions in energy expenditure taken together could thus possibly negate the advantage of complete fasting over a diet of less than 1000 calories.

This study in no way deals with the suggested psychological benefit to the obese patient of intermittent short periods of fasting. It does raise a question as to whether sufficient advantage is to be had from more prolonged periods of fasting to warrant the hospitalization which is required for such a regimen. Before a definite answer can be given, the authors themselves point out that further experiments must be carried out with longer periods of fasting compared with low carbohydrate diets restricted to less than 1000 calories.

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