Plasma Concentrations of A and B Chains of Insulin in Nondiabetic, Diabetic and High Risk Potential Diabetic Subjects

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SUMMARY

Radioimmunoassays for the measurement of the A chain and B chain of insulin in the presence of insulin are described. The assays are sensitive to 0.3 ng./ml. each of A chain and B chain. The results of the immunoassays of human plasma indicate that both A and B chains are present in human plasma. The criteria for this evidence were (a) quantitative recovery of added A and B chains from plasma and (b) parallel relationships between the standard curves of A and B chains and results upon dilution of plasma. The mean concentrations of A chain and B chain in plasma from nondiabetic subjects were 3.7 ± 1.0 and 3.4 ± 1.1 ng./ml., respectively. The concentrations of A chain were markedly higher in diabetic and high risk potential diabetic subjects. It appears possible that an elevated level of A chain may be used as a diagnostic criterion for prediabetes. There was no apparent effect of oral glucose on the concentration of A and B chains in the plasma of nondiabetic subjects as that observed on the concentration of insulin.

Studies on the immunospecificity of the cysteine residues of the two chains showed that thiol, S-sulfonated, oxidized and S-carboxymethylated chains reacted similarly; this indicates that S-sulfonated cysteine residues are probably not involved in the antigen-binding reaction. DIABETES 17:547-56, September, 1968.

The insulin molecule is composed of two polypeptide chains; the chain with glycine at the N-terminus has been designated A chain and the chain with phenylalanine at the N-terminus has been designated B chain. It is not known whether A chain and B chain occur in the pancreas, blood or anywhere else in the body, or whether their presence might be of significance. Recently, we reported on the production of antibodies to A and B chains of bovine insulin.[1] It was shown that there are species differences in the reaction of A chain and B chain with their respective antibodies but that the chains derived from pork insulin probably react like those derived from human insulin. In the present paper, we have made use of these antibodies for the measurement of the amounts of A and B chains in the plasma of nondiabetic, diabetic, and high risk potential diabetic subjects. Preliminary notes of this work have appeared.[2,3]

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EXPERIMENTAL

Details of the preparation of antibodies to insulin, A chain and B chain[1]; S-sulfonated, reduced and S-carboxymethylated A and B chains[4] and I-125 labeled insulin and S-sulfonated A and B chains[1] have been described. Oxidized A and B chains were prepared by bubbling oxygen through solutions of reduced chains, until no sulfhydryl groups could be detected with Ellman's reaction.[5]

Clinical material: Information about the subjects studied is listed in tables 1, 2 and 3. The nondiabetic subjects were chosen from laboratory personnel and were not known to be suffering from any disease. They all showed a normal oral glucose tolerance test and had no known family history of diabetes (table 1). The diabetic subjects had illnesses of varying degrees of severity and duration and received the indicated antidiabetic therapies (table 2).

The subjects with a normal glucose tolerance test but who had close relatives with diabetes[6] are listed as high risk potential diabetic subjects (table 3). The exact relation to diabetic relatives and other signs of prediabetes, the bearing of large babies[7] and the occurrence of hypoglycemia,[8] if present, are also noted.

Blood samples were taken with heparinized vacutainers in the morning after an overnight fast. The persons with diabetes were asked not to take their hypoglycemic medication on the day blood was drawn. Plasma was separated immediately after blood was drawn and was kept cold in a refrigerator. The assays for insulin, A chain and B chain were usually started within one to three hours after the withdrawal of blood. In a few cases for which the assays could not be started the

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TABLE 1

Clinical details of the normal subjects studied and their plasma levels of insulin, A chain and B chain

Subject no.	Sex	Age (yrs.)	Height (in.)	Body weight (lbs.)	Plasma Levels Insulin ng./ml.	A chain ng./ml.	B chain ng./ml.	$\log_{10} \frac{A+B \text{ chains*}}{\text{Insulin}}$
1	M	47	67	140	0.36	3.65	3.21	1.6003
2	M	53	70	155	0.38	5.56	2.77	1.6861
3	M	31	71	145	0.52	4.10	3.71	1.5017
4	M	29	67½	150	0.35	3.50	—	—
5†	M	33	60	148	0.32	3.35	—	—
6	M	27	74½	170	0.22	2.10	4.10	1.7447
7‡	M	49	72	175	0.15	2.20	—	—
8	M	32	68	155	0.55	2.85	5.85	1.4867
9	M	23	75	180	0.5	4.27	2.87	1.5833
10	F	47	62	125	0.5	3.90	3.01	1.5639
11	M	23	73	185	0.5	4.30	2.20	1.5528
12	M	37	65½	120	0.5	4.32	2.85	1.5850
				Mean ± S.D.	.40±.13	3.68±.97	3.40±1.07	1.5894±0.0821

*Ratios of A + B chains to insulin are calculated on a molar basis, using molecular weights: 5733 for insulin, 2333 for A chain and 3400 for B chain. Logarithms of the ratios are presented; this was necessary to eliminate skewness and weighting of the ratios for the purposes of statistical analysis (test of significance).[30]
†Paternal grandfather developed diabetes after age fifty-five years.
‡Paternal grandmother developed diabetes at the age of eighty years.

TABLE 2

Clinical details of the diabetic subjects studied and their plasma levels of insulin, A chain and B chain

Subject no.	Sex	Age (yrs.)	Height (in.)	Body weight (lbs.)	Duration of diabetes*	Family history of diabetes†	Hypoglycemic therapy	Plasma levels Insulin ng./ml.	A chain ng./ml.	B chain ng./ml.	$\log_{10} \frac{A+B \text{ chains‡}}{\text{Insulin}}$
13	M	43	71¼	149	8y	—	Diet	2.8	5.75	3.15	0.8439
14	F	66	62½	187	12y	—	Diet	1.85	4.23	2.78	0.9149
15	M	62	72½	182	30y	—	Diet	2.96	5.17	4.68	0.8448
16	M	84	74	174.5	21y	—	Diet	2.61	6.78	2.58	0.9079
17	F	74	65	143	23y	+	Diet	2.59	5.91	6.27	0.9877
18	M	74	63	156	10d	—	Diet	2.15	4.23	4.02	0.9047
19	M	57	70	204	5d	+	Diet	2.22	6.57	5.57	1.0615
20	M	63	71	165	4y	+	Dymelor	2.90	5.51	7.50	0.9571
21	M	67	68.5	201	6y	+	Dymelor	4.19	5.38	6.79	0.7716
22	M	56	73	155	6y	+	Orinase	2.15	4.54	6.88	1.0265
23	M	22	73	181	4y	+	Orinase	2.39	—	6.46	—
24	F	61	70	245	5y	+	Orinase	4.25	6.55	0.76	0.6138
25	M	79	69	150	2y	—	Orinase	0.54	6.58	0.3	1.5652
26	F	68	64.5	240	3-4y	—	Orinase	0.35	11.14	3.26	1.9746
27	F	69	61	115	?	+	Orinase	0.67	5.48	5.08	1.5171
28	M	48	75	249	1y	—	DBI	3.06	5.33	4.33	0.8261
							Mean ± S.D.	2.36±1.12	5.94±1.66	4.40±2.14	1.0478±0.3599

*y = years; d = days
†(+) indicates positive family history of diabetes and (—) indicates no known family history of diabetes.
‡For explanation, see footnote(*) to table 1.

same day the plasma was stored at —15° C.; in no case were the assays begun later than three days after blood collection.

Preparation of I-125 labeled antigens: The iodination procedure used was the previously reported modification[1] of Greenwood et al.[9] It should be noted that bovine serum albumin was used only during the elution from Sephadex G-25 column.

Whenever a decrease of 1 to 2 per cent in the binding of labeled antigens with their respective antibodies occurred, the labeled antigens were purified. This took place after about three weeks. The purification was carried out on a Sephadex G-75 column. In each case (A chain, B chain or insulin), three radioactive peaks were obtained.

The material present in the second peak showed the

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TABLE 3

Clinical details of the high risk potential diabetic subjects studied and their plasma levels of insulin, A chain and B chain

Subject no.	Sex	Age (yrs.)	Height (in.)	Body weight (lbs.)	Relatives with diabetes*	Other signs†	Plasma levels Insulin ng./ml.	A chain ng./ml.	B chain ng./ml.	$Log_{10}\frac{A+B \text{ chains}}{\text{Insulin}}$§
29	F	43	60	110	F	LB,HG	0.4	7.91	4.43	1.8273
30	M	14	61	95	MGF,M	BLB,HG	0.48	9.51	4.57	1.8097
31	F	19	64	115	MGF	SLB,HG	0.70	6.73	3.93	1.5214
32	M	4	—	40	F,GM,MU		0.45	6.33	3.53	1.6831
33	F	11	—	63	F,GM,MU		0.79	8.81	5.20	1.5884
34	F	35	65.5	145	M,B		0.42	4.31	4.49	1.6382
35	M	41	67	165	M,A	HG	0.4	4.67	4.48	1.6767
36	F	30	62	95	C‡	HG	0.45	5.44	3.55	1.6372
37	M	25	72.5	265	M,PGM	PU,PP,PD	—	5.34	6.38	
						Mean ± S.D.	.51±.14	6.56±1.84	4.50±.88	1.6728±.1037

*F = Father, M = Mother, MGF = Maternal Grandfather, MU = Maternal uncle, GM = Grandmother, B = Brother, A = Aunt, C = Cousin

†Other signs suggesting potentiality for developing diabetes: LB = gave birth to large baby, BLB = Large Baby at Birth, SLB = Sib of Large Baby, HG = occasional hypoglycemic attacks, PU = Polyuria, PP = polyphagia, PD = polydipsia

‡This subject is included because in addition to having a close relative with diabetes she has history of hypoglycemic attacks.

§For explanation, see footnote(*) to table 1.

optimal binding with its corresponding antibody.

Determination of insulin, A chain and B chain in plasma: For these measurements two-antibody immunoassay systems capable of measuring insulin, A chain and B chain in the presence of each other were devised. The assay depends on the specific inhibition of the immunological reaction between I-125 antigen and its antibody either by standard or by endogenous antigen in plasma. We have shown previously[1] that antisera to insulin are specific for insulin and those for A chain are specific for A chain, so insulin and A chain were measured directly using anti-insulin and anti-A chain antibodies, respectively. Antisera to B chain react with B chain and also with insulin. So, for the determination of B chain, insulin present in plasma samples was first neutralized by reaction with anti-insulin serum, and the assay was then carried out in the usual manner using B chain antibodies. The procedure used was as previously described[1] except that (a) I-125-labeled and unlabeled (used as standards) insulin, S-sulfonated A chain and S-sulfonated B chain were pork instead of beef, (b) the systems contained disodium ethylene diaminetetraacetate (EDTA) in 0.01 M final concentration[10] and (c) for the determination of B chain, insulin was first neutralized by incubating it with anti-insulin antibody. Briefly, the procedures were as follows: Reactions were carried out in 75 × 12 mm. disposable plastic culture tubes (Lab-Tek Products). All solutions and dilutions were made with sodium chloride-borate buffer, pH 8.5, containing 5 per cent bovine serum albumin fraction V; for the solution of labeled antigens and the dilution of No. U-3 serum, the buffer also contained 0.14 M EDTA. The first antibody reactions were carried out in the following manner: For the assay of insulin and A chain, mixtures of standards or of plasma (in a volume of 1 ml.) and the appropriate I-125-labeled antigens (0.1 ml.) were incubated with 0.1 ml. of appropriately diluted corresponding antisera in the manner previously described. For the assay of B chain, standards or plasma specimen (in a volume of 1 ml.) were incubated first with 0.05 ml.* of 1:300 diluted anti-insulin serum No. U-3, then 0.05 ml. of I-125-B chain was added and incubated with 0.1 ml. of appropriately diluted B chain antibody. The second antibody reactions were carried out in the same manner as previously described.[1] For each assay, a plasma specimen was estimated at a minimum of two dilutions in duplicate together with an appropriate series of standards. Throughout the studies, antisera used for each set of assays were from single animal.

RESULTS AND DISCUSSION

Effect of structural changes in the cysteine sulfhydryl groups on immunospecificity: If the A chain and B

*This amount was capable of neutralizing 2 ng. of insulin. Levels of B chain in plasma were high enough that only 0.2—0.4 ml. of plasma were necessary for the assay. Therefore the amount of No. U-3 serum used was sufficient to neutralize 5-10 ng. (125-250 μU./ml.) insulin.

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chain of insulin were to be present in plasma, they would be expected to occur either as the free thiol or S-sulfonated* chains, or bound (probably linked through their sulfhydryl groups) to plasma proteins. Since the chains used as antigens were in the S-sulfonated form, the effect of structural changes in the cysteine sulfhydryl groups of the chains on their immunospecificity was investigated. In figure 1 is shown the competitive inhibition of the binding I-125-sulfonated A chain to its antibodies by unlabeled S-sulfonated, S-carboxymethylated, reduced and oxidized

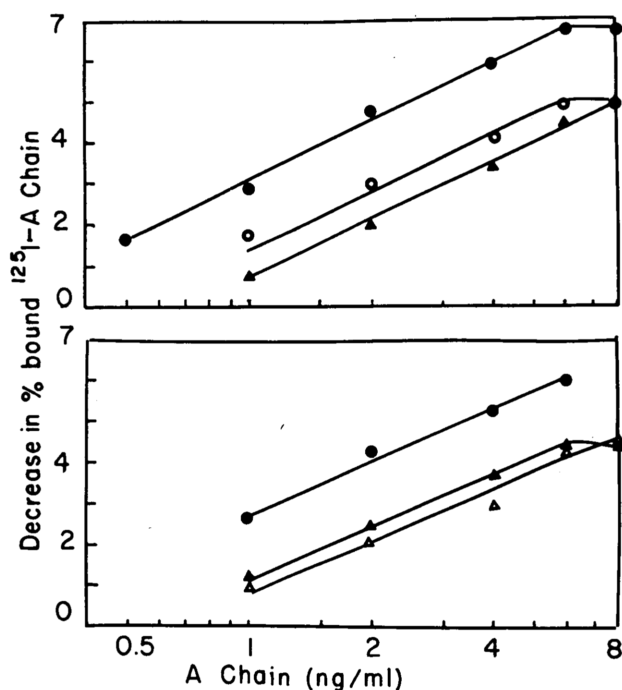

FIG. 1. Cross-reactivity of various derivatives of cysteine residues of A chain. Inhibition of binding of I-125-labeled S-sulfonated A chains to its antibody by unlabeled S-sulfonated (●), S-carboxymethylated (○), reduced (▲) and oxidized (△) A chains. The dilution of antiserum used was 1/100. The binding of I-125-S-sulfonated A chain in the absence of any unlabeled S-sulfonated A chain was about 11 per cent. For details, see text. Each figure represents dose-response curves carried out simultaneously with different preparations of I-125-A chain.

A chains. The inhibition was greater with S-sulfonated derivative than that with the remaining three derivatives which reacted about the same. Dose-response curves for all the derivatives yielded curves with the same slope.

In figure 2 are shown the results of similar experiments with B chain. Again, the slopes of the inhibition curves with all the derivatives of B chain were the same. S-carboxymethylated and oxidized derivatives reacted slightly lower and reduced chain reacted the same or better than S-sulfonated derivative.

Since parallel slopes of inhibition curves are indicative of immunologically identical structures,[14,15] it appears that cysteine sulfhydryl groups of the two chains are either not involved in their antigenicity or, if they are, they are involved to such a smaller extent that their chemical modification does not alter their immunological properties.

In agreement with the results of Yagi et al.,[12] we also observed that with B chain antibodies, S-sulfonated and

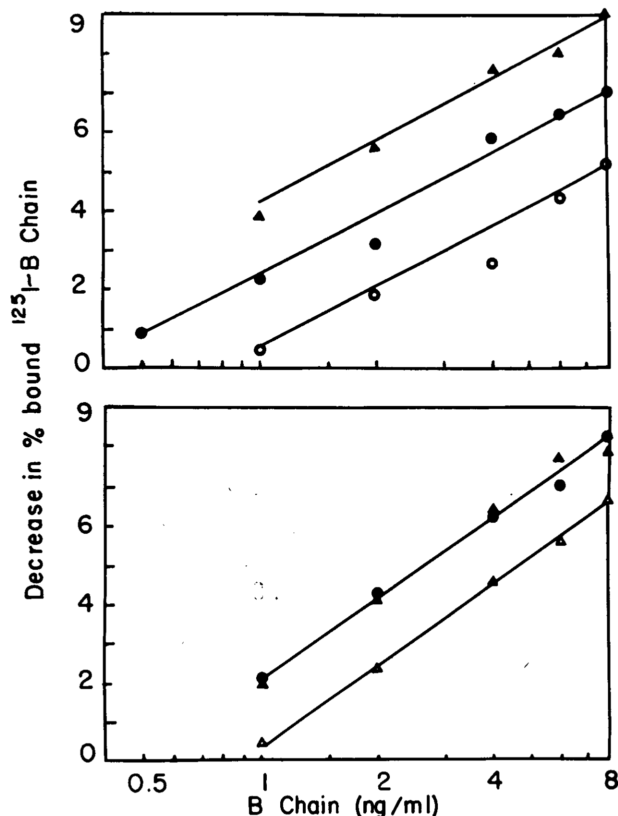

FIG. 2. Cross-reactivity of various derivatives of cysteine residues of B chain. Inhibition of binding of I-125-labeled S-sulfonated B chain to its antibody by unlabeled S-sulfonated (●), S-carboxymethylated (○), reduced (▲) and oxidized (△) B chains. The dilution of antiserum used was 1/1600. The binding of I-125-S-sulfonated B chain in the absence of any unlabeled B chain was about 18 per cent. For details, see text. Each figure represents dose-response curves carried out simultaneously with different preparations of I-125-A chain. For the sake of clarity, only one line is drawn for the reactions of S-sulfonated and reduced chains (lower figure).

*A possibility that the chains also could exist in S-sulfonated form is raised by the recent reports that S-sulfonated cysteine[11] and S-sulfonated glutathione[12,13] occur normally in mammals.

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S-carboxymethylated B chains reacted the same. However, in their experiments, S-carboxymethylated A chain reacted 6 to 25 per cent of S-sulfonated A chain with A chain antibodies. More recently, Blackard[17] reported that in the I-125-S-carboxymethylated A chain—its antibody system, S-carboxymethylated and S-sulfonated A chain reacted the same, but oxidized A chain did not react at all. These differences observed in the reaction with A chain antibodies in the three studies might occur because A chain antibodies obtained from different animals are directed to different regions of A chain; such differences have been reported for insulin antibodies.[18]

Immunoassay of A and B chains: Inhibition curves obtained with S-sulfonated A chain and S-sulfonated B chain as shown in figures 1 and 2, respectively, are representative of their respective typical standard curves. Linear relationships are observed over the range of 0.5 to 6 ng. per ml. in case of A chain and 0.5 to 8 ng. per ml. in the case of B chain. In other experiments, the systems have been found to be sensitive to 0.3 ng. per ml. in each case.

The precision of the assay systems, expressed as the standard deviations from their means, is shown in table 4. The factor which contributed greatly to the precision of the assay, besides the usual extreme care in the pipetting and the manipulation of the reagents, is the use of plastic tubes probably because of their nonwetting property.

Application of immunoassay of A and B chains to human plasma: Throughout the studies S-sulfonated derivatives of A and B chains were used as standard antigens. In figure 3 are shown a standard curve and

TABLE 4

Precision of the assay for A and B chains

Amount of chain (ng./ml.)	Decrease in per cent bound I-125-antigen Mean	S.D.
A chain		
0.5(8)*	2.65	1.05
1.0(8)	3.23	0.66
2.0(7)	4.57	0.48
4.0(8)	5.02	0.30
8.0(8)	5.88	0.35
B chain		
0.5(8)	1.16	0.23
1.0(8)	3.06	0.59
2.0(7)	5.12	0.41
4.0(8)	7.85	0.28
8.0(8)	9.55	0.49

*Number of assays are shown in parentheses.

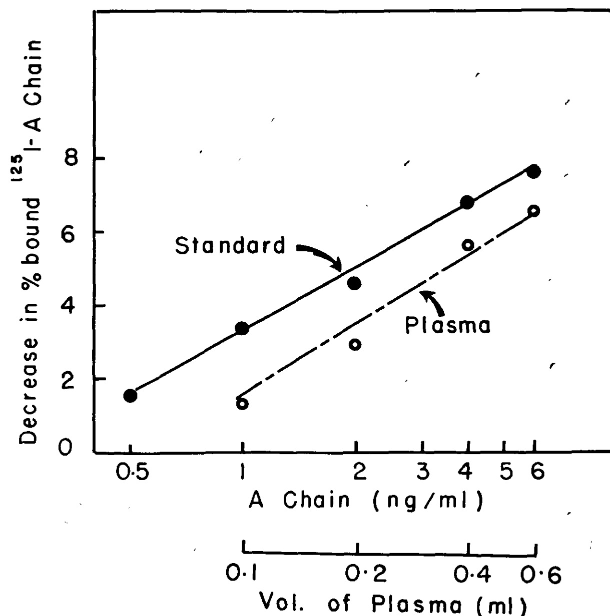

FIG. 3. Parallel inhibition of binding of I-125-labeled S-sulfonated A chain to its antibody between unlabeled A chain and plasma. The procedure used was as described for figure 1.

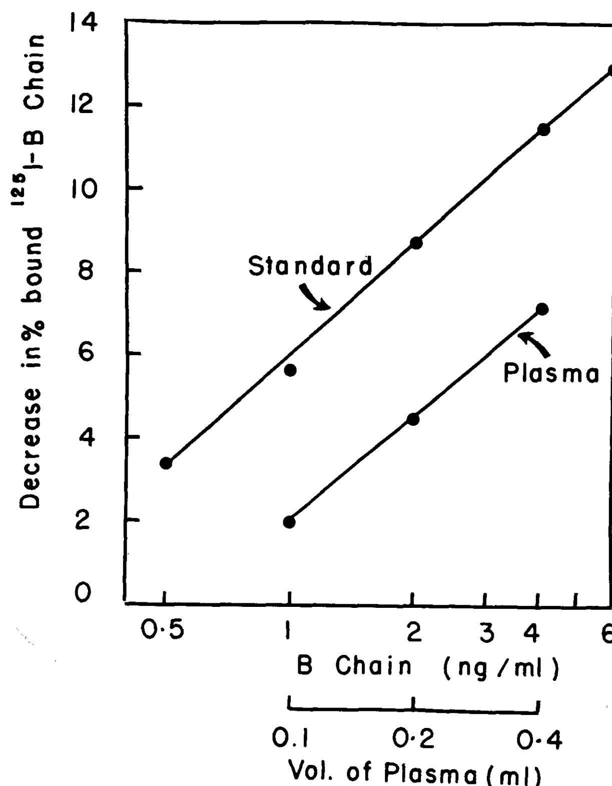

FIG. 4. Parallel inhibition of binding of I-125-labeled S-sulfonated B chain to its antibody between unlabeled B chain and plasma. The procedure used was as described for figure 2.

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a dilution curve of plasma for A chain, run simultanecusly. The apparent concentration of endogenous A chain decreases proportionately as the plasma is diluted and is parallel to the standard curve. Similar data were obtained with B chain (figure 4).

Results obtained in recovery experiments are shown in table 5. Both A chain and B chain added in vitro to plasma are recovered almost quantitatively. These data, together with the parallel relationships between the standard curves and the results upon dilution of plasma (figures 3 and 4), show that materials immunologically indistinguishable from pork A chain and pork B chain are present in human plasma.[14,15]

In control experiments with plasma, complete in every respect except that the antisera were omitted, negligible amounts of labeled antigens (~ 0.2 per cent) were bound. One plasma sample was assayed before freezing, three and six days after freezing; the values of A chain and B chain were within the limits of the experimental error. There was no difference in the values obtained with plasma and serum prepared from the same sample of blood.

TABLE 5
Recovery of added and endogenous A and B chains from plasma

Amount of chain added (ng.)	Chain (ng.) Endogenous	Total recovered	Recovery per cent
	A Chain		
1	0.61	1.43	89
2	0.61	2.08	80
3	0.61	3.13	87
1	1.40	2.45	102
2	1.40	2.75	81
3	1.40	3.25	74
	B Chain		
1	0.79	1.37	77
2	0.79	2.45	88
3	0.79	4.40	116

Concentrations of insulin, A chain and B chain in the plasma of nondiabetic, diabetic and potential diabetic subjects: In tables 1, 2 and 3 are given the clinical details and the concentrations of insulin, A chain and B chain, and the molar ratios of A plus B chains to insulin in plasma of nondiabetic, diabetic and potential diabetic subjects respectively; therapeutic agents used by the diabetic subjects also are listed. The differences among the various groups are more easily seen when the data are examined diagrametrically (figures 5, 6 and 7) and statistically (table 6). In figure 5 are presented

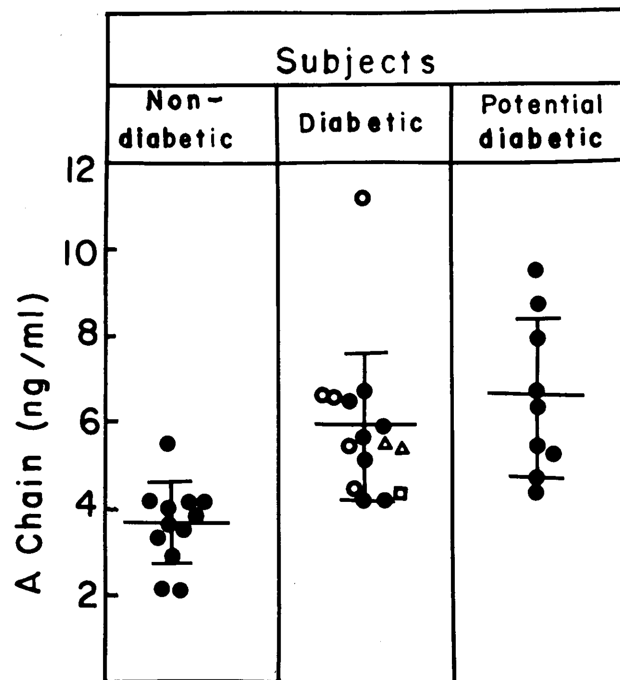

FIG. 5. Concentrations of A chain in the plasma of nondiabetic, diabetic and potential diabetic subjects. Data of tables 1, 2 and 3. Horizontal line represents the mean concentration ± S.D. For diabetic subjects, different symbols represent different antidiabetic therapies they were on: diet (●); Dymelor (△); Orinase (○); DBI (□).

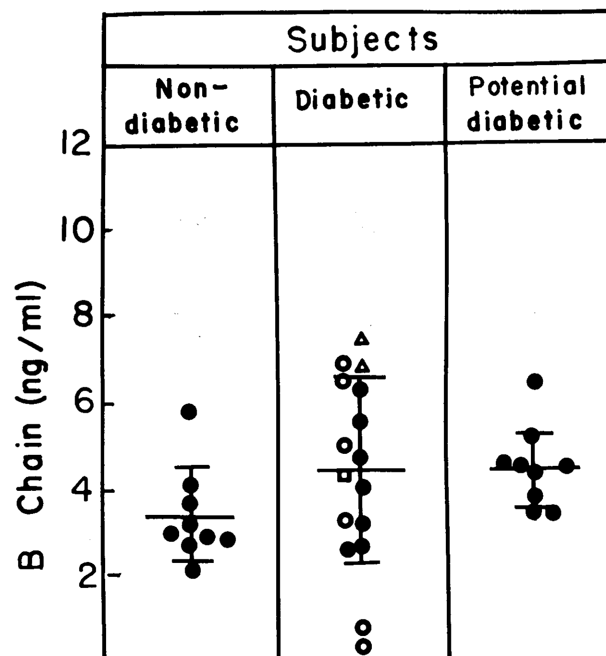

FIG. 6. Concentrations of B chain in the plasma of nondiabetic, diabetic and potential diabetic subjects. Data of tables 1, 2 and 3. Horizontal line represents mean concentration ± S.D. For diabetic subjects, different symbols represent different anti-diabetic therapies they were on: diet (●); Dymelor, (△); Orinase (○); DBI (□).

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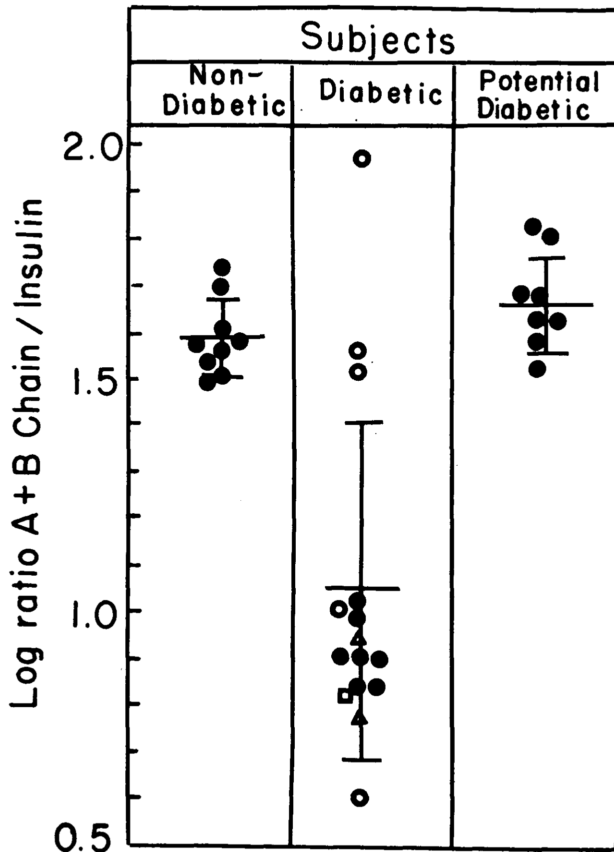

FIG. 7. Molar ratios of A + B chains/insulin in the plasma of nondiabetic, diabetic and potential diabetic subjects. Data of tables 1, 2 and 3. Horizontal line represents the mean ratio ± S.D. For diabetic subjects, different symbols represent different antidiabetic therapies they were on: diet (●); Dymelor (△); Orinase (○); DBI (□).

the concentrations of A chain; although there is a scatter of individual values, mean average values represented by horizontal lines are markedly higher for diabetic ($p < 0.001$) and potential diabetic ($p < 0.001$) subjects than those of nondiabetic subjects. In figure 6 are shown the concentrations of B chain in the same individuals. The B chain concentrations in the plasma of diabetic and potential diabetic subjects tend to be higher than those of nondiabetic subjects; the difference is not significant between the diabetic and nondiabetic subjects ($p < 0.3$) and is also probably not significant between the potential diabetic and nondiabetic subjects ($p < 0.05$).

It should also be noted that the concentrations of insulin were also higher in diabetic than those in nondiabetic subjects.

In figure 7 these data are expressed on a molar basis as ratios of combined A plus B chains to insulin. This method of presentation provides an indication of relative amounts of the chains and insulin in plasma and changes in the different groups. While the actual concentrations of the chains are higher in the diabetic than in the nondiabetic subjects, the amount of the chains relative to insulin is higher in nondiabetic than in diabetic subjects.

Levels of A and B chains during oral glucose tolerance test: Five normal adult human subjects who had fasted overnight were given 75 gm. of glucose (Glucola*) orally. The mean values of A chain, B chain

*Product of Ames Company, Elkhart, Indiana.

TABLE 6
Levels of significance
Comparison of plasma levels of insulin, A chain, B chain, and A plus B chains/insulin of normal, diabetic, and prediabetic subjects

Subjects compared	P values* A chain	Insulin	B chain	$\log_{10}\frac{[A+B \text{ chains}]}{[\text{insulin}]}$
Normal and diabetic	<0.001*	<0.001*	<0.3	≦0.002*
Normal and prediabetic	<0.2	≦0.001*	<0.05	<0.2
Prediabetic and diabetic	<0.001*	<0.7	<0.9	<0.001*

*significantly different

*Probability that the difference between the means of the two groups compared occurred by chance.

The level of significance (P) between the two groups compared was calculated by equation,

$$\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(N_1 \sigma_1^2 + N_2 \sigma_2^2)(N_1 + N_2)}{(N_1 + N_2 - 2) N_1 N_2}}}$$

where $\bar{x}$ = mean of insulin, A chain or B chain

σ = standard deviation, $\sqrt{\Sigma(\bar{x} - x)^2 / N - 1}$

N = total number of subjects

sub 1 and 2 = two different groups being compared.

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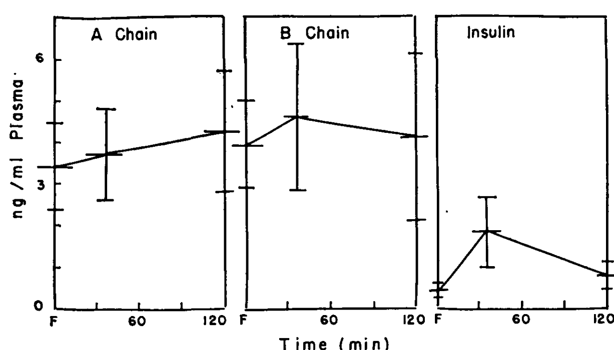

FIG. 8. Plasma concentrations of A chain, B chain and insulin before and during a 75 gm. oral glucose tolerance test in five normal adult human subjects. Each value represents the mean concentration ± S.D.

and insulin in the plasma of blood drawn at 0, 35 min., and 120 min. after the ingestion of glucose are shown in figure 8. There was no apparent effect of glucose on the plasma concentrations of A and B chains similar to that observed on the concentration of insulin.

COMMENTS

Because of the limited data and the difference in the age, body weight and treatment among the groups, any explanation of the results of this paper would be speculative. Nevertheless, in the interest of further research in this area, some comments to bring out the complexity of the problem may be useful. It remains to be shown whether the presence of A and B chains of insulin in blood indicates that the pancreas normally secretes these chains, that they constitute physiologically important components of blood, or, alternatively, that they represent catabolic products of insulin.

The molar ratio of the concentration of A chain to B chain is 1.58 in nondiabetic and 2.1 in diabetic subjects. This conceivably could occur because there is an asynchronous formation of A and B chains and in the development of diabetes there is an overproduction of A chain.* However, this explanation is improbable in view of the recent report that the precursor of insulin, proinsulin, is a single polypeptide chain molecule.[20] It is more probable that A and B chains arise from insulin. They are not in equimolar amounts either because some of the B chain is in a modified form, possibly albumin bound, which does not react with B chain antibody or is catabolized more rapidly than A chain.

The increased concentrations of the chains in diabetes could be the result of an increased rate of degradation of insulin due to increased activity (or amount) of glutathione-insulin transhydrogenase,[21-23] because of the production of an increased amount of insulin, or because of an increased secretion or formation of the chains by the pancreas in diabetes. In diabetes the fact that A chain has increased and B chain has not could indicate that there is either more of a modified form of B chain or, again, more rapid catabolism of B chain. Albumin-bound B chain* has been shown to act as an antagonist to the action of insulin.[24-28] It is possible that both A† and B chains in the circulation, either free or albumin-bound, may be capable of antagonizing the action of insulin; this would then explain the apparent unavailability of insulin to diabetic subjects, in whom it is present in higher amounts. On the basis of relationship of the two chains to insulin (figure 7) potential diabetic and normal subjects appear to be similar whereas diabetic subjects are distinctively different from either; this would suggest that alteration of the balance between the two chains and insulin is involved in the development of diabetes. Whether the potential diabetic subjects who have higher levels of A chain will develop diabetes or are carriers of the gene for diabetes (assuming recessive Mendelian inheritance) has yet to be seen.‡ Finally, the lack of effect of a glucose load on the levels of A and B chains is not

*An analogous situation is on record: Bence-Jones proteins which are excreted in multiple myeloma have been shown to be composed of light (L) chains of γ-globulin.[19] An explanation has been forwarded that possibly the formation of γ-globulin takes place by way of asynchronous synthesis of its L and H (heavy) chains and in pathological conditions of multiple myeloma, there is an overproduction of L-chains and this gives rise to Bence-Jones proteins.[19]

*Because circulating insulin antagonist (synalbumin) and albumin-bound B chain both are able to act as antagonists towards some of the effects of insulin there has been a tendency to assume that synalbumin is, in fact, bound B chain. There is, however, no direct experimental evidence to support this concept. The reports on the effect of synalbumin have presented conflicting results.[29-33] With the exception of Mirsky's report,[34] workers are in agreement that albumin-bound B chain is an insulin antagonist.[24-28] Details of Mirsky's report[34] are not available. However, it appears that he injected B chain in a free form and not as bound to albumin which, according to other workers,[24-28] is a prerequisite for the B chain to function as insulin antagonist.

†There is as yet no evidence that A chain antagonizes the action of insulin as has been reported to be the case with B chain.[24-28]

‡It is of interest to note that one of the potential diabetic subjects (No. 29) who had a normal tolerance to glucose in July 1967 but had a high level of A chain showed abnormal tolerance to glucose later in April 1968.

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understood. Experiments are in progress to answer some of the questions raised by the present studies.

ADDENDUM

Since this manuscript was prepared, Meek et al.[35] have reported the presence of A and B chains in human plasma. In agreement with our results, they also observe that A chain is present in higher amounts in the plasma of diabetic than that of nondiabetic subjects and that the administration of oral glucose has no effect on the concentration of A and B chains in the plasma of nondiabetic subjects. However, the mean fasting plasma concentration of A and B chains they reported, particularly that of A chain (22 ng./ml.), is much higher than we obtained. The reason for this difference in the two studies is not clear.

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Protein Quality in Groundnuts

The increased use of groundnuts, or peanuts as they are called in the United States, as human food has received emphasis in India in an attempt to increase the protein content of the diet. The quantity of protein in groundnuts is high compared with cereals, and defatted groundnut meal has a much higher protein content, often approaching 60 per cent. However, the quality is not as good as that of animal protein, primarily because of the lack of methionine.

Studies of cereal grains have shown that there is often a considerable difference between the protein content and quality in different varieties. Also, soil and climatic conditions sometimes result in a variety with a high protein content or a better quality protein.

A recent report by P. S. Cheema and G. S. Ranhotra (J. Nutrition Dietet. *4*:93, 1967) discusses the difference in protein quality and quantity in thirty varieties of groundnuts grown in Ludhiana, India, and in Tifton, Georgia. Samples were extracted with a fat solvent, and the defatted meal analyzed for protein and methionine content. A biological value was calculated by the chemical score method from the methionine content using whole egg as the reference protein.

No significant differences were found in varieties grown in the two locations. In a few cases a variety had 6 to 7 per cent greater protein in one location as compared with the other, but there was no consistent trend. The greatest variation occurred between varieties—the lowest protein content in a defatted groundnut meal was 43.8 per cent, the highest 65.3 per cent. Both the highest and lowest protein contents were reported from groundnuts grown in India.

Methionine content also varied, with the higher methionine levels reported for varieties with the lower protein contents. Methionine levels ranged from 0.62 gm. to 0.95 gm. per 16 gm. of nitrogen. The lowest level occurred in the sample grown in Georgia and the highest in a variety with low protein content. This latter variety had a high methionine content when grown in either location.

Since the calculation for biological value was directly related to methionine content, the biological value varied with that content. Most of the samples had calculated values of approximately fifty-five, with fifty-eight the highest value.

While the differences in protein and methionine content were found, none of the groundnut varieties produced a meal significantly higher in total protein or in methionine. Growing conditions in the two locations appeared to have very little effect on the composition of the groundnuts. A recent report on the amino acid composition and biological value of nine varieties of groundnuts suggests that variety difference is small (A. K. Chapra and G. S. Sidhur, Brit. J. Nutrition *21*:519, 583, 1967). Studies by these workers indicate that the biological value of peanut meal as determined by rat feeding trials was consistent with the values expected based on the amino acid content.

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