An Abnormal Insulin in Juvenile Diabetes Mellitus

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SUMMARY

A technic is described for the isolation of insulin from serum using a series of gel filtrations in combination with an interim complexing with guinea pig anti-insulin serum. Insulin thus derived from the serum of juvenile diabetic subjects was shown to be significantly more resistant to insulinase than that from normal persons. It is suggested that there is a genetically determined structural difference in the insulin of diabetic children which contributes importantly to the underlying biochemical disorders of the disease. DIABETES *14*:780-87, December 1965.

Recent studies have suggested that the inheritance of diabetes mellitus may be governed by a single autosomal gene showing incomplete dominance.[1-3] Although the precise molecular expression of this condition has not yet been defined, the possibility of it being in the form of an abnormal insulin is a logical and appealing hypothesis.

The purpose of this report is to describe certain observable differences in the rate of breakdown by insulinase between insulin from juvenile diabetics and normal serum insulin.

METHODS

The basic essentials of the methodology are set out in figure 1. Insulin is first separated from serum proteins by gel filtration. The crude peptide fraction is then reacted with guinea pig anti-insulin after which the insulin/anti-insulin complex is separated by a second gel filtration from the other peptides of normal human serum. In a final gel filtration the insulin is separated from anti-insulin and other guinea pig proteins. The rate of destruction of the normal and diabetic insulins by pork insulinase is then compared. More elaborate details are outlined below.

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OUTLINE FOR TECHNIC FOR ISOLATING INSULIN FROM SERUM

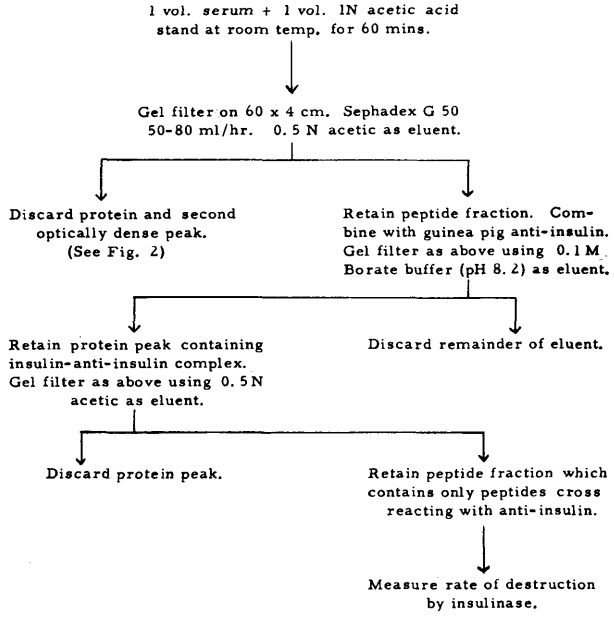

FIGURE 1

A. *Preparation of insulin*

Sera were obtained from healthy, normal young adults and from diabetic children under the conditions listed in table 1. The fasting levels of insulin in the untreated and diabetic children (Group A) ranged from 18 to 150 μU./ml. Four of these were in moderately severe ketoacidosis and had levels below 40 μU./ml. In the other three, symptoms were minimal, and the levels were 57 μU./ml., 150 μU./ml. and 132 μU./ml., respectively. The latter level was obtained following tolbutamide administration. All of these children subsequently required conventional doses of insulin for adequate control. Aliquots of 2 to 10 ml. of each sample were mixed with an equal volume of 1 N acetic acid at room temperature and gel filtered within two hours on a 60 x 4 cm. column of beaded, coarse, Sephadex G50 at 4° C., with a flow rate of 50 to 80 ml./hr. of 0.5 N acetic acid as eluent. The column effluent was collected in 5 ml. fractions and the O.D.

TABLE 1
Subjects studied

Group	Number	Conditions
Normal adults	8	No family history of clinical diabetes in grandparents, parents, cousins or siblings.
Diabetic children Group A	7	Children on first admission in diabetic acidosis to whom no insulin had been administered.
Diabetic children Group B	4	Fasting samples from insulin-treated children* from whom all insulin had been withdrawn for not less than forty-eight hours and not more than sixty hours.
Diabetic children Group C	5	Fasting samples from insulin-treated diabetic children* taken between twelve and twenty-four hours after the last dose of insulin. One-milliliter samples for thirty children were pooled to form five 6-ml. aliquots.
Diabetic children Group D	9	Nonfasting samples from insulin-treated diabetic children within twelve hours after the administration of Semilente/Ultralente mixtures and diluted with pure porcine insulin (see table 4).

*Treated with Regular, NPH and Lente insulins in varying combinations

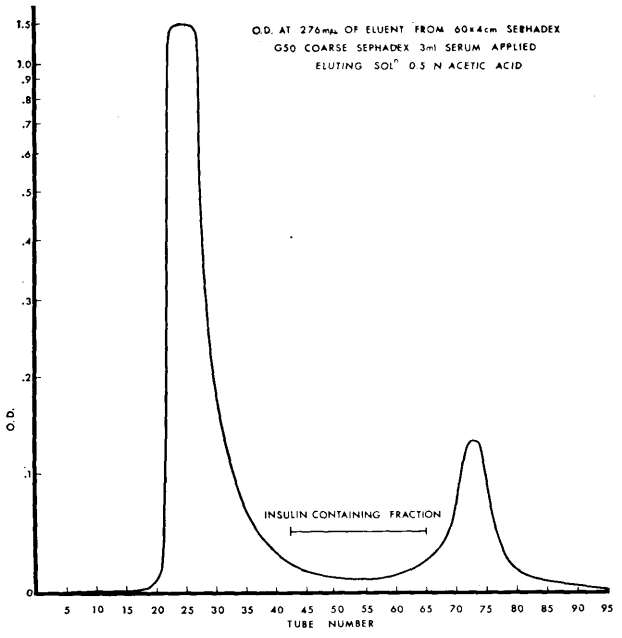

FIGURE 2

at 276 mμ was continuously recorded, as shown in figure 2. A large protein peak appeared first containing small amounts of insulin. This was followed by fractions of low optical density containing the greater part of the insulin and other peptides, and a further optically dense peak. The location of the insulin-containing fractions was determined by immunoassay. The protein-free insulin-containing fractions were pooled and flash evaporated to dryness at 37° C. The residue was then dissolved in 20 ml. of 0.1 M borate buffer (*p*H 8.2) at 30° C. and the *p*H adjusted to 8.2 when necessary with 0.1 N NaOH. An amount of guinea pig anti-insulin serum (see Section C) sufficient to combine with approximately 20 milliunits of I-125 insulin (mean specific activity 0.25 mc./U.) was then added and the whole stood at 4° C. for 48 to 72 hrs.

The mixture, which now contained the insulin as a soluble insulin/anti-insulin complex, was refiltered in an identical manner using 0.1 M borate buffer (*p*H 8.2) as the eluent. The protein fraction, including the insulin/anti-insulin complex and other guinea pig proteins was collected and the remaining fractions discarded. The eluent was again flash evaporated at 37° to dryness and after dissolving in 10 ml. of 0.5 N acetic acid to dissociate the insulin/anti-insulin complex it was gel filtered once more, using 0.5 N acetic acid as eluent. The final peptide fraction was presumed to contain only insulin or other peptides crossreacting with anti-insulin. This fraction was again dried in vacuo, redissolved in 3 ml. of 0.1 N acetic acid, and assayed for insulin by a modification of the double antibody assay.[4] Slight contamination (<0.1 per cent) with other substances absorbing at 276 mμ was anticipated, there being an especial technical risk from the inclusion of any guinea pig anti-insulin which would invalidate the measurement of insulin and its rate of degradation by insulinase. For this reason, the final fraction was also assayed for guinea pig anti-insulin content by substituting an aliquot of this fraction for the added guinea pig anti-insulin serum in the conventional insulin assay.[4] In the event that 0.1 ml. of the final 3 ml. sample bound more than 0.1 per cent of the 5 μU. of added 0.25 mc./U. I-125-labeled insulin, the sample was discarded. The uncontaminated samples were lyophilized and taken up in 1.5 ml. 2 per cent bovine serum albumin (BSA) in 0.1 M phosphate buffer (*p*H 7.3). After readjustment of the *p*H to 7.3 with 0.1 N NaOH the volume was made up to 2.0 ml. The resulting insulin concentrations, as measured in Section D at the start of the insulinase resistance test, ranged between 60 and 652 μU./ml. These values, however, do not correlate accurately with the initial serum levels because of the variable recoveries from the over-all separation procedure. It should be recognized also that no special attempt was made to discern the efficacy of 1.0 N acetic acid on eluting "bound" or "atypical" forms of insulin.

B. *Preparation of insulinase*

Insulinase was prepared from fresh rat skeletal muscle by homogenizing 30 gm. of tissue in 100 ml. of 0.1 M phosphate buffer (*p*H 7.3) and centrifuging at 1 x 10^5 G. for thirty minutes. The clear intermediate layer between the supernatant fat and the particulate deposit was drawn off and stored at −20° C. in individual 1 ml. aliquots. These were thawed for use as required, the solution being then diluted by the same *p*H 7.3 phosphate buffer in such a manner that 0.1 ml. of the final solution was sufficient to degrade 30 to 60 per cent of 300 μU. of porcine insulin dissolved in 2 ml. of 2 per cent bovine serum albumin in 0.1 M phosphate buffer (*p*H 7.3) at 38° C. in thirty minutes (see Section D).

C. *Preparation of guinea pig anti-insulin serum and the assay of potency*

Twenty units of Protamine Zinc Insulin (Lilly) was administered subcutaneously to adult guinea pigs at three-week intervals. Two weeks after the sixth injection, blood was withdrawn from the heart, and the serum separated. Following this, 0.05 ml. of serial dilutions from 1/250 to 1/5000 of the serum in 0.1 M *p*H 8.2 borate buffer in 5 per cent BSA was added to tubes containing 0.9 ml. of the same buffer. Subsequently, 0.05 ml. of this buffer containing 5 μU. of the I-125-labeled insulin was then added, and the tubes mixed and stood at 4° C. for twenty-four hours. Undiluted rabbit anti-guinea pig serum[4] in a volume between 0.05 and 0.10 ml. which was sufficient to pre-

TABLE 2 (*Continued on page* 783)

The destruction of pure porcine, pure human, and commercial pork/beef insulins

Type of insulin		Initial insulin concentration μU./ml.	Per cent destroyed in first thirty-minute interval	Per cent destroyed in second thirty-minute interval	Mean per cent destruction per thirty-minute period	Mean as per cent of porcine insulin ± I.S.D.
Pure porcine		60	61.7 (23)*	60.8 (9)	61.2	104.5
		77	66.2 (26)	46.2 (14)	56.2	96.0
		147	63.3 (54)	61.1 (21)	62.2	106.2
		173	67.6 (55)	50.9 (27)	59.2	101.1
		193	62.6 (72)	47.2 (38)	54.9	93.7
		218	50.4 (105)	64.8 (37)	57.6	98.4
		410	59.7 (165)	63.6 (60)	61.6	105.2
		448	55.3 (200)	56.0 (88)	55.6	94.9
	Means		60.8	56.3	58.6	100 ±5%
Commercial pork/beef		100	80 (20)	35 (13)	57.5	98.2
		150	72 (42)	37.7 (25)	54.8	93.6
		190	66.8 (63)	65.9 (22)	66.3	113.2
		265	62.2 (100)	54.5 (40)	58.3	99.6
		300	58.3 (125)	65.6 (43)	61.9	105.5
	Means		67.9	51.7	59.8	102 ±8%
Pure human		130	55.4 (58)	55.1 (26)	56.8	97.0
		190	57.8 (80)	56.2 (35)	57.0	97.3
		210	47.6 (110)	59.0 (45)	53.3	91.0
		257	53.3 (120)	53.3 (56)	53.3	91.0
		307	52.7 (145)	55.1 (65)	53.9	92.0
		398	58.2 (178)	46.6 (95)	52.4	89.5
		445	50.5 (212)	55.1 (95)	52.8	90.2
	Means		54.1	54.3	54.2	93 ±3.4%
Normal human serum derived insulin		60	45 (33)	45.4 (18)	45.2	108.3
		80	29.9 (57)	29.9 (40)	29.9	77.2
		112	40.2 (67)	17.9 (55)	29.1	86.3
		186	31.7 (127)	30.4 (90)	31	78.5
		290	29.3 (205)	35.4 (120)	32.3	91.4
		297	40.4 (177)	26.5 (130)	33.4	71.4
		358	35.7 (230)	37.4 (140)	36.6	96.6
		430	31.4 (295)	31.8 (200)	31.6	82.1
	Means		35.4	31.8	33.6	86.5 ±12%

*Figures in parentheses are actual insulin concentrations in the medium at end of first and second thirty-minute periods.

cipitate maximally the insulin/anti-insulin complex, and 0.05 ml. of 1:130 dilution of normal guinea pig serum were added and mixed. The tubes were allowed to stand for a further twenty-four hours in the cold to allow maximal interaction of the reagents. The precipitate resulting from the centrifugation at 3,000 rpm for fifteen minutes in an International Model C50, was washed once with 0.5 ml. 0.1 M borate and respun; the first and wash supernatants from each tube were then pooled. The supernatants and precipitates were then counted sequentially in a Packard gamma spectrometer. A guinea pig serum which in a 1:1000 dilution was capable of combining with 60 per cent of 5 μU. of I-125-labeled insulin with a specific activity of 0.25 mc./U. was deemed suitable for use in the preparative procedure.

D. *Assay of resistance of insulinase*

A solution of 0.1 ml. of insulinase (Section B) was added to 2 ml. aliquots of the serum insulin fractions (Section A) in 5 ml. glass-stoppered tubes as above, and triplicate 0.1 ml. aliquots of the mixture were withdrawn for insulin assay at zero, thirty, and sixty minutes during incubation in a water bath at 38° C. The reaction in the assay system was stopped by immediate dilution of the aliquot in 0.9 ml. 5 per cent BSA in borate buffer, *p*H 8.2, at 0° C. The fall in insulin concentration over the two consecutive thirty-minute intervals was expressed as a percentage of the concentrations at zero and thirty minutes, respectively, and averaged. The presence of BSA in excess insured

TABLE 2 (*Continued from page* 782)

The destruction of pure porcine, pure human, and commercial pork/beef insulins

Type of insulin	Initial insulin concentration μU./ml.	Per cent destroyed in first thirty-minute interval	Per cent destroyed in second thirty-minute interval	Mean per cent destruction per thirty-minute period	Mean as per cent of porcine insulin ± I.S.D.
Diabetic children Group A	80	28.7 (57)	3.5 (55)	16.1	37.9
	69	2.9 (67)	0 (67)	1.4	3.1
	65	10.8 (58)	−29.3 (75)	0	0
	95	0 (105)	0 (110)	0	0
	390	10.2 (350)	28.6 (250)	19.4	26.2
	95	0 (110)	0 (110)	0	0
	75	13.3 (65)	0 (65)	6.6	8.9
Means		9.4		6.2	10.9 ±15%
Diabetic children Group B	280	5.35 (265)	11.3 (235)	8.3	39.8
	246	17.4 (203)	13.3 (176)	15.3	33.7
	275	2.5 (267)	15.7 (225)	9.1	20.5
	266	51.1 (130)	15.3 (110)	33.2	58
Means		19.1	13.9	16.5	38 ±16%
Diabetic children Group C	137	34.3 (90)	36.6 (57)	35.5	78
	390	20.5 (310)	4.8 (295)	12.6	27.7
	375	24 (285)	37.1 (179)	30.5	67.1
	455	28.5 (325)	16.9 (270)	22.7	50
	545	33.3 (363)	6.3 (340)	19.8	43.6
Means		28.1	20.3	24.2	53.2 ±20%
Diabetic children Group D	307	30.3 (200)	25 (150)	27.6	78.1
	652	15.6 (550)	13.6 (475)	14.6	40.8
	292	39.3 (177)	0 (195)	19.6	72.6
	485	18.1 (397)	6.8 (370)	12.4	46.2
	330	13.6 (285)	12.3 (240)	12.9	25.9
	574	9.4 (520)	4.6 (496)	7.0	47.9
	140	53.5 (65)	10.8 (58)	32.1	86
	218	24.7 (164)	33.5 (109)	29.1	78
	163	41.7 (95)	12.6 (83)	27.1	62.4
Means		27.3	13.3	20.3	59.8 ±21%

*Figures in parentheses are actual insulin concentrations in the medium at end of first and second thirty-minute periods.

TABLE 3

Summary of rates of insulin destruction expressed as a percentage of porcine controls

Group	Number	Mean		S.D.*		p†
1. Pure porcine insulin	8‡	100%	101%	±5%	±6%	No significant difference between 1 and 2. <0.01 for pure porcine viz. human insulin from serum
2. Commercial bovine/porcine insulin mixture	5‡	102%		±8%		
3. Purified human pancreatic insulin	7‡	93%	89%	±3.4%	±9%	No significant difference between 3 and 4. <0.01 for combined human insulins (3 and 4) viz. combined porcine and bovine insulins (1 and 2) or porcine only (1)
4. Normal human insulin derived from serum	8	87%		±12%		
5. Diabetic children Group A (no insulin treatment)	7	11%	21%	±15%	±20%	<0.01 for diabetic children with minimal exogenous insulin (5 and 6) viz. combined human insulins (3 and 4) and diabetic insulins admixed in vitro or in vivo with porcine/bovine insulin (7 and 8)
6. Diabetic children Group B (off insulin forty-eight hours)	4	38%		—		
7. Diabetic children Group C (insulin treated)	5	53%	57%	±20%	±20%	<0.01 for diabetic children with bovine and/or porcine insulin (7 and 8) viz. combined human insulins (3 and 4) and combined porcine and porcine/bovine insulins (1 and 2)
8. Diabetic children Group D (insulin treated plus added porcine insulin)	9	60%		±21%		
9. Normal human insulin (plus added porcine insulin)	7	103%		±14%		<0.001 viz. diabetic children Group D

*S.D. = standard deviation with Bessel correction for small sample bias.

†p = probability of being normal as calculated from the Student *t* test where the variance ratio is at <5 per cent level and in other instances by the Aspin-Welch test of Trickett, W. M., Welch, B. L., and James, G. S.: Biometrica *43*:203, 1956.

‡ represents number of estimations on the same pool.

against the measurement of nonspecific peptidases and maintained the insulin in solution.

Pure porcine insulin, pure human insulin and a commercial mixture of beef and pork insulins were incubated under similar conditions, over a range of concentrations between 60 and 448 μU./ml. These latter studies were all carried out on the same day and with the same sample of insulinase.

The effect of A and B chains of porcine insulin on the system was ascertained by adding 0.1 ml. of insulinase solution (Section B) to 2 ml. aliquots of 600 μU./ml. of porcine insulin in 2 per cent BSA in *p*H 7.3 0.1 M phosphate buffer containing A or B chain in the concentrations shown in table 5. Simultaneous controls were run without the addition of A and B chains.

RESULTS

The percentage destruction of all groups of insulin for each of the consecutive thirty-minute intervals as described in Section D under Methods is shown in table 2. A correction for the slowly decreasing potency of stored insulinase had to be made in the studies on normal and diabetic sera by running two pure insulin standards of 150 and 300 μU./ml. with each experiment and expressing the final results as a percentage of the destruction of these porcine standards.

The percentage destruction of the pure insulins and the commercial porcine/bovine mixture did not differ significantly either as between the two consecutive thirty-minute periods, or in relation to the absolute insulin concentrations (table 3). Nor could any difference be found between the destruction of pure porcine and commercial insulins, but human insulin derived from serum was significantly ($p=<0.01$) more slowly destroyed than porcine insulin. While no difference could be found between the pure human insulin and the column derived normal serum insulin, there

TABLE 4

Rates of insulin destruction expressed as percentage of porcine controls

Group	Amount of derived insulin (μU./ml.)	Amount of added porcine insulin (μU./ml.)	Rate of destruction as porcine insulin + 1 S.D.
Group D diabetics	16	147	62.4
	81	59	86.0
	92	215	78.1
	159	59	78.0
	180	102	72.6
	228	102	47.9
	373	102	46.2
	437	215	40.8
	472	102	25.9
		Mean	59.8*±21%
Normals	12	147	97.6
	16	147	111.7
	36	147	116.3
	47	147	86.9
	59	59	104.2
	69	59	121.3
	100	100	85.4
		Mean	103.34±14.1%

*Significantly less than the normals (Groups 3 and 4, table 3), p = 0.001.

was a very significant difference between the combined human insulins derived from pancreas and serum; and both pure porcine insulin (p=<0.01), and the combined pool of porcine and porcine/bovine samples (p=<0.01).

The results in the cases of insulins derived from diabetic patients were as follows. In seven diabetic children in Group A who had received no treatment, the destruction of insulin ranged from zero to 37.9 per cent (mean 11 per cent) in comparison with pure pork insulin. In another group (B) of four children, insulin had been withheld for a period in excess of forty-eight hours. Here the mean rate of destruction of insulin was 38 per cent of pork insulin (39.8 per cent, 33.7 per cent, 20.5 per cent, 58.0 per cent). Together, this group of eleven children might be supposed to represent a sample in which there was little or no contamination with porcine/bovine insulin or its breakdown products. The mean destruction of insulin in the combined sample was 21 per cent of that of pork insulin.

TABLE 5

The effects of normal reduced A and B chains of insulin on insulinase

Concentration	Per cent destruction related to porcine standards: A chain (Per cent)	B chain (Per cent)
0.10 μgm./ml.*	114	94.2
100 μgm./ml.*	72	57.3

*One microgram of insulin = 24,000 μU. insulin.

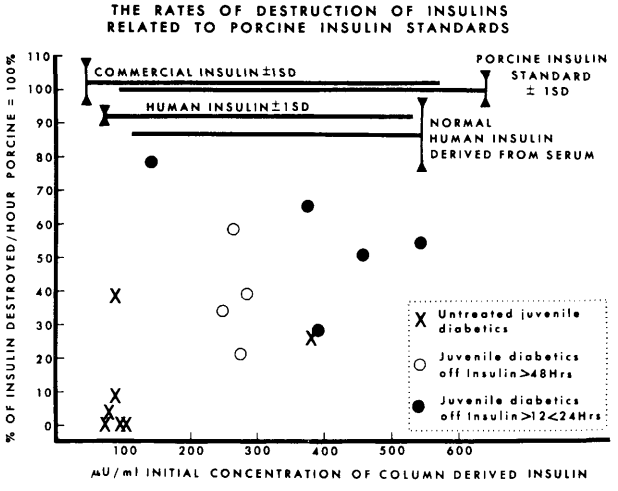

FIGURE 3

Groups C and D were diabetic children who were on treatment with Ultralente and Semilente insulins. In these children the insulin fraction derived from the columns presumably contained diabetic human insulin variably diluted with previously administered porcine and bovine insulin. In the case of Group D, there was a further in vitro admixture of porcine insulin. If these two groups are combined to represent a sample in which diabetic human insulin has been diluted in vivo or in vitro with porcine and bovine insulin, the mean destruction of the pooled insulin is 58 per cent ± 19 per cent S.D., a figure that, as might be expected, lies between that for Groups A and B combined and the means for bovine, porcine and human insulins.

The rate of destruction of plasma insulin from children in Groups A and B combined, namely those in whom the diabetic human insulin is minimally contaminated with pork or beef insulin, is significantly less than that of both normal human serum insulin, and of the combined samples of serum and pancreas derived human insulin and of the combined porcine and porcine/bovine samples.

Similarly the rate of destruction of insulin in Groups C and D combined, that is in those cases where the diabetic human insulin is mixed with bovine and porcine insulin, is again significantly lower than that of porcine and porcine/bovine samples and combined normal human samples.

The derived insulins from Group D diabetics which were further mixed in vitro in varying proportions with porcine insulin, were more slowly destroyed still than insulin derived from normal sera, similarly admixed (table 4). This is evidence that the resistance to insulinase of the insulin from diabetic children was not due to any addition of porcine insulin in vitro or derivatives thereof. There remains a possibility, however, that such inhibition could have resulted from some in vivo breakdown product of porcine/bovine insulin which was capable of crossreacting with anti-insulin. This again is improbable in view of the fact that resistance to insulinase was significantly greater in those diabetics with minimal contamination with exogenous insulin (Groups A and B) as compared to those (Groups C and D) where such admixture had occurred to a greater extent in vivo or in vitro.

A summary of the statistical relationships between the various groups is shown in table 3.

DISCUSSION

The data just described show that insulin isolated from the serum of children with diabetes is significantly more resistant to destruction by a crude rat insulinase preparation than is normal insulin derived from either serum or pancreas. It is possible that this effect might have been achieved by the presence in the diabetic serum of excessive amounts of an insulinase inhibitor.[5,6] Such inhibitors as vasopressin and oxytocin[5] and other sulphydril-containing peptides would not have reacted significantly with guinea pig anti-insulin and therefore would not have been retained in the protein fraction of the second gel filtration. In the same way kynurenine derivatives of tryptophane[6] would also have been discarded.

It is also possible that immunologically cross reacting breakdown products of administered insulin might have produced these results. However, the observation that insulinase resistance is greater in the insulin from those diabetic children who had either had no insulin or in whom insulin had been withdrawn for forty-eight hours or more (table 1, Groups A and B) than in the groups in whom the insulin was admixed with beef and pork insulin either endogenously or exogenously is a forceful argument against this. As additional evidence a small study (table 5) showed that reduced A and B chains were only inhibitory to insulinase in amounts greatly in excess of any recorded insulin concentration. The fact that A and B chains of insulins fail to explain insulinase resistance does not discount a possible role in the phenomena of clinical diabetes.[7]

Other antagonists to insulin itself[8] such as insulin binding antibodies, lipoproteins and serum albumin would likewise not effect the response of insulin to insulinase in this system because they would have been discarded with the protein fraction in the first gel filtration.

It would therefore appear that the resistance to insulinase of the insulin from diabetic children is a function of some difference in the insulin itself. Superficially this might imply a biological advantage in that insulin would have a longer effective biological half life. More probably, however, it indicates some structural change in the diabetic insulin molecule, which does not involve the anti-insulin antibody binding sites, but does affect the active site for insulin-glutathione transhydrogenase. The further implication is that this same structural change may be responsible for impaired function in the more important roles of insulin in glucose transport and in such enzymatic activities as glucokinase induction.[9]

The concept of an abnormal insulin also offers an acceptable explanation for the acid ethanol extractable substance[10] in diabetic sera, which inhibits glucose uptake by isolated tissues, as well as for the inhibitor found in the serum of cases of lipoatrophic diabetes.[11] It is also probable that the previously described heritable inhibitor of insulinase[3] was in fact this same abnormal insulin. If this was indeed so, it is evidence that the inheritance of diabetes may be mediated through a structurally abnormal insulin. Although clearly conjecture at this stage, it may well be that there is more than one kind of abnormal insulin and that such variants may be associated with a variety of clinical entities such as lipoatrophic diabetes and the Prader-Willi syndrome, in much the same manner as the hemoglobinopathies.

ACKNOWLEDGMENT

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REFERENCES

[1] Fajans, S. S., and Conn, J. W.: The early recognition of diabetes mellitus. Ann. N.Y. Acad. Sci. *82*:208, 1959.

[2] Vallance-Owen, J., and Ashton, W. L.: Inheritance of essential diabetes mellitus from studies of the synalbumin insulin antagonist. Program of the American Diabetes Association, p. 25, June 1963.

[3] Stimmler, L., and Elliott, R. B.: Inheritance of a diabetic-serum factor inhibiting normal utilization of insulin. Lancet *1*:956, 1964.

[4] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody system. Diabetes *12*:115, 1963.

[5] Katzen, H. M., and Stetten, D.: Hepatic glutathione-insulin transhydrogenase. Diabetes *11*:271, 1962.

[6] Mirsky, I. A., Perisutti, G., and Jinks, R.: The hypoglycemic action of metabolic derivatives of L-tryptophan by mouth. Endocrinology *60*:318, 1956.

[7] Ensinck, J., and Vallance-Owen, J.: Antagonism of insulin by the albumin-bound "B" chain of insulin. Program of the American Diabetes Association, p. 17, June 1963.

[8] Salas, M., Vinuela, E., and Sols, A.: Insulin-dependent synthesis of liver glucokinase in the rat. J. Biol. Chem. *238*: 3535, 1963.

[9] Vallance-Owen, J.: Synalbumin insulin antagonism. Diabetes *13*:241, 1964.

[10] Louis, L. H., Conn, J. W., and Minick, M. C.: Lipoatrophic diabetes: isolation and characterization of an insulin antagonist from urine. Metabolism *12*:861, 1963.

Impaired Renal Excretion of Urate During Hyperketonemia

S. Goldfinger, J. R. Klinenberg, and J. E. Seegmiller (New Eng. J. Med. *272*:351, 1965) investigated the effect of intravenously administered sodium beta-hydroxybutyrate, sodium acetoacetate, and acetone on the renal clearance of urate in four normal human volunteers—height, weight, and sex unspecified. None was classified as obese. Glomerular filtration rate was measured by inulin clearance. From seven to ten one-half-hour clearance periods comprised each study. Only one ketone body was infused in a given study. Plasma ketone bodies were measured by the method of S. S. Chernick (Proceedings of the Second Applied Seminar of the Association of Clinical Scientists, F. W. Sunderman and F. W. Sunderman, Jr., Editors, p. 144. Philadelphia, Lippincott, 1961) which allows separate quantification of each of the three ketone bodies.

When sodium beta-hydroxybutyrate was infused, a fall in the *C urate/C inulin* ratio inversely related to the level of ketonemia occurred. The ketonemia produced by the sodium beta-hydroxybutyrate consisted mainly of an increased beta-hydroxybutyrate concentration, with a slight increase in the concentration of acetoacetate. Sodium acetoacetate infusions resulted in a fall in the *C urate/C inulin* ratio and in a rise in plasma, both in acetoacetate and in beta-hydroxybutyrate levels.

Glucose infusion in the final clearance period resulted in a prompt return of the clearance ratio to normal. However, hypoglycemia as a cause of the reduced ratio was ruled out by the finding of unchanged blood glucose concentrations during infusion of any ketone. Similarly, hyperlacticacidemia was ruled out, since this condition did not occur during infusion of any ketone.

Acetone infusion produced a modest decline in one subject in the ratio C *urate/C inulin* associated with an increase in both acetone and beta-hydroxybutyrate plasma concentrations. In another subject, however, in whom an even higher acetone concentration was achieved, but in whom no increase in beta-hydroxybutyrate or acetoacetate concentrations occurred, no depression in the ratio *C urate/C inulin* was observed.

These studies suggest that the three ketone bodies normally elevated in ketosis do not have the same capacity to depress the ratio *C urate/C inulin.* Apparently, increased acetonemia without an increased concentration of beta-hydroxybutyrate will not reduce this ratio. Since sodium acetoacetate infusion always produced an increased beta-hydroxybutyrate concentration in plasma, the independent effect of acetoacetate on the ratio could not be measured. Beta-hydroxybutyrate had a definite depressive effect.

Despite the additional information which this investigation provides, the exact mechanism by which urate clearance is reduced in the presence of beta-hydroxybutyrate and/or acetoacetate remains unclear. The authors suggest that interference with transport sites, substrates, or cofactors required in energy production at transport sites or other unknown factors may be involved.

The results of these two investigations provide a clear explanation for the hyperuricemia noted in patients treated for obesity by complete fasting. They suggest

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