"Plasma Insulin Activity" in Human Diabetes During Hypoglycemic Response to Tolbutamide and Indole-3-Acetic Acid. (24562)

HOLBROOKE S. SELTZER AND WALTER L. SMITH
(Introduced by Carleton B. Chapman)
Dept. of Internal Medicine, University of Texas Southwestern Medical School, and Vet. Admin. Hospital, Dallas.

The principal action of tolbutamide (Orinase) is probably direct stimulation of beta cells of the islets of Langerhans to secrete insulin(1). Conversely, Mirsky's pioneering studies with indole-3-acetic acid indicate that the latter's hypoglycemic effect is due to extra-pancreatic inhibition of insulinase activity(2). After administration of either substance, one might expect depression of circulating glucose levels to be paralleled by increased "insulin activity"* in peripheral blood. It was found, however, that the hypoglycemic responses of normal subjects and mild diabetics to both compounds was not accompanied by enhanced plasma insulin activity.

Materials and methods. Clinical material included 5 metabolically normal subjects, 7 elderly diabetic patients whose fasting blood sugars were easily controlled on diet plus daily tolbutamide, and 5 labile or juvenile diabetics who promptly developed severe ketonuria when exogenous insulin was reduced. All patients were males. Mean age of each group was, respectively, 46, 57, and 39 years (Table I). No elderly patient received insulin within a week before beginning the tests. One patient with juvenile diabetes tolerated complete withdrawal of insulin, but the others required periodic small injections of regular insulin daily to prevent development of frank ketoacidosis. No insulin was given later than noon of day preceding each test. All subjects consumed 300 g of carbohydrate and maintenance calories for 3 days prior to testing. On first test day, samples were obtained for blood glucose and for "plasma insulin activity" in the fasting state, and again 1 hour after taking 100 g of glucose orally. The next day similar fasting samples were drawn, the individual given 100 mg/kg of indole-3-acetic acid† in capsules, and specimens for blood glucose obtained hourly for 5 hours. Between 2nd and 5th hours, a second plasma sample was drawn for insulin assay. Two or 3 days later the procedure was repeated, except that the person received 3 g of tolbutamide‡ orally after drawing fasting specimens, and the second sample for insulin assay was obtained between 1st and 4th hours. Blood glucose was determined in duplicate by the Somogyi-Nelson method(3). Vallance-Owen's rat-diaphragm technic(4) was used to determine "insulin activity" of plasma. The latter was expressed as net glucose uptake from plasma, in mg % per 10 mg dried diaphragm, *i.e.*, total glucose uptake from test plasma by hemidiaphragm minus baseline uptake from buffered glucose solution by control hemidiaphragm. Paired fasting and post-treatment plasmas were always assayed simultaneously.

Results. Tolbutamide and indole-3-acetic acid demonstrated quantitatively parallel hypoglycemic effects throughout (Table I), the latter being slight in normal subjects, impressive in elderly diabetics and essentially zero in juvenile diabetics, respectively. Maximal depression of fasting glucose in normal individuals was 22 ± 4% (mean ± S.E.M.) after tolbutamide, and 11 ± 4% after indole-3-acetic acid. Respective values in mild adult diabetics were 44 ± 4% after tolbutamide and 35 ± 6% after indole-3-acetic acid. Negligible blood sugar changes of 1 ± 1% after tolbutamide, and 7 ± 3% after indole-

* "Plasma insulin activity" refers to biologically effective insulin concentration, *i.e.*, total insulin content minus effect of insulin antagonists in undiluted plasma.

† Purchased from Nutritional Biochemicals Corp., Cleveland.

‡ Generously provided by C. J. O'Donovan, Upjohn Co.

TABLE I. Maximal Depression of Blood Glucose Concentration after Oral Administration of 3 g of Tolbutamide or 100 mg/kg of Indole-3-Acetic Acid to Normal Subjects and Diabetic Patients (Mean ± S.E.M.).

Clinical status of patients (No.)	Age	Tolbutamide		Indole-3-acetic acid	
		Fasting blood glucose (mg %)	Max fall from fasting level (%)	Fasting blood glucose (mg %)	Max fall from fasting level (%)
Normal (5)	46 ± 5	82 ± 2	22 ± 4	87 ± 3	11 ± 4
Adult (stable) diabetes (7)	57 ± 4	187 ± 20	44 ± 4	195 ± 16	35 ± 6
Juvenile (labile) diabetes (5)	39 ± 3	390 ± 49	1 ± 1	420 ± 45	7 ± 3

3-acetic acid, were exhibited by juvenile diabetics.

The data on "plasma insulin activity" (Table II) is subdivided as follows:

(1) *Insulin activity in fasting state.* Rat diaphragm incubated in fasting plasma from either normal subjects or elderly diabetics extracted significantly more glucose than did control diaphragm incubated in buffered glucose solution. In contrast, rat diaphragm showed no net uptake of glucose from fasting plasma of uncontrolled juvenile diabetics. In normal subjects the all-inclusive, average net glucose uptake (mean of all 3 groups of fasting values) was 8.9 ± .9 mg %/10 mg dried diaphragm; this increment above baseline uptake was highly significant ($p<.001$). Comparable net fasting uptakes were 7.1 ± 0.7 ($p<.001$) in elderly diabetics, but only 0.6 ± 0.6 ($p<.7$) in the juvenile group.

(2) *Insulin activity after glucose.* Both normal individuals and mild diabetics also showed statistically significant increases in glucose uptake, above fasting values, 1 hour after ingesting glucose. Respective mean rises above fasting levels were 7.4 ± 1.4 mg %/10 mg diaphragm ($p<.001$) in normals, and 4.8 ± 1.1 ($p<.02$) in elderly diabetics. In uncontrolled juvenile diabetics, circulating insulin activity remained undetectable (0.7

TABLE II. "Plasma Insulin Activity," Expressed as Glucose Uptake by Rat Diaphragm from Plasma of Normal and Diabetic Individuals, after Ingestion of Glucose, Tolbutamide and Indole-3-Acetic Acid.

Clinical status of patients	Net glucose uptake,* in mg %/10 mg dried diaphragm (mean ± S.E.M.)			
	Fasting (A)	Post-treatment (B)	Post-treatment increase above fasting (B − A)	p
(G)		Glucose (100 g)		
Normal	6.4 ± .4	13.8 ± 1.6	7.4 ± 1.4	<.001
Adult diabetes	10.1 ± .9	14.9 ± 1.2	4.8 ± 1.1	<.02
Juvenile "	.8 ± .7	1.5 ± 1.6	.7 ± 1.3	<.5
(T)		Tolbutamide (3 g)		
Normal	7.9 ± 1.1	7.7 ± 1.0	− .2 ± .6	<.9
Adult diabetes	6.1 ± .8	6.0 ± 1.3	− .1 ± 1.3	>.9
Juvenile "	1.7 ± 1.0	−1.0 ± 1.2	−2.7 ± 1.5	<.2
(I)		Indole-3-acetic acid (100 mg/kg)		
Normal	12.4 ± 1.7	10.7 ± 1.6	−1.7 ± 1.3	<.5
Adult diabetes	6.3 ± 1.6	7.6 ± 1.8	1.3 ± 1.1	<.6
Juvenile "	− .3 ± 1.2	− .4 ± 1.8	− .1 ± 1.5	>.9
(G + T + I)	"Grand mean" of corresponding fasting uptake values (A)			
Normal	8.9 ± .9			<.001†
Adult diabetes	7.1 ± .7			<.001
Juvenile "	.6 ± .6			<.7

* Net glucose uptake = uptake from plasma minus baseline uptake from buffered glucose solution.

† Significance of net fasting glucose uptake above baseline uptake.

± 1.3 mg %/10 mg diaphragm) following glucose load.

(3) *Insulin activity after tolbutamide and indole-3-acetic acid.* In contrast to increased glucose uptake by rat diaphragm after glucose, normal subjects and mild diabetics showed no enhancement of insulin activity in peripheral blood after either tolbutamide or indole-3-acetic acid. Mean variation from fasting glucose uptake in normals was –0.2 ± 0.6 mg %/10 mg diaphragm after tolbutamide, and –1.7 ± 1.3 after indole-3-acetic acid. Respective values in mild diabetics were –0.1 ± 1.3 after tolbutamide, and 1.3 ±1.1 after indole-3-acetic acid. None of these minor deviations was numerically significant. In the juvenile group, neither substance improved the already negligible fasting glucose uptake, the mean change being –2.7 ± 1.5 mg %/10 mg diaphragm after tolbutamade and –0.1 ± 1.5 after indole-3-acetic acid.

Discussion. The magnitude of average maximal hypoglycemic responses to indole-3-acetic acid in normal subjects (11%) and mild diabetics (35%) agrees closely with respective mean values of 16% and 38% reported by Mirsky(5). Similarly, the demonstration of "insulin activity" in fasting plasma of normal individuals and mild diabetics, its enhancement in each group 1 hour after glucose ingestion, and its undetectability in juvenile diabetics both before and after glucose, all duplicate the findings of Vallance-Owen (4,6).

The closely comparable hypoglycemic effects of indole-3-acetic acid and tolbutamide in normal subjects and diabetics indicate that the tryptophan metabolite, like sulfonylurea, requires insulin-secreting capacity of the patient. Moreover, failure of the marked fall in circulating glucose shown by mild diabetics, to be accompanied by elevated "plasma insulin activity," suggests that insulin-dependent hepatic mechanisms may underlie the acute hypoglycemic response to both substances. Ricketts(7) proposed that only small, "permissive" amounts of insulin are needed to catalyze predominantly intra-hepatic effects of sulfonylureas, since tolbutamide caused marked lowering of blood sugar in totally depancreatized dogs maintained on constant but minimal exogenous insulin dosage. However, Pfeiffer(8) reported increased insulinlike activity in portal vein blood after administering tolbutamide; and Madison(9) found 54% of endoportally injected insulin-I^{131} bound within the liver of fasting subjects during its first transhepatic circulation. These reports imply that tolbutamide does stimulate beta cells to release insulin, which is largely bound to liver cells in post-absorptive individuals. Insulin-binding in turn may necessarily precede the reduced hepatic output of glucose following administration of either insulin itself (10) or tolbutamide(11). Regarding indole-3-acetic acid, and accepting its postulated role as non-competitive inhibitor of insulinase activity, the highest concentratons of insulinase reside in the liver. Decreased destruction of insulin by insulinase would increase net hepatic content of insulin, which might similarly be bound to liver cells and shut off release of glucose by that organ. Administration of either tolbutamide or indole-3-acetic acid, therefore, might augment intra-hepatic insulin activity and cause hypoglycemia without being paralleled by enhanced insulin activity in peripheral blood. Renold likewise did not find increased plasma insulinlike activity during the acute hypoglycemic response of normal subjects to intravenous tolbutamide(12). The foregoing analysis remains compatible with the demonstration of increased peripheral insulin activity after oral glucose loading. Not only may hyperglycemia be a quantitatively stronger insulogenic stimulus *per se* than sulfonylureas, but the liver may bind progressively less insulin as the fasting state yields to that of sustained hyperglycemia with its increased rate of insulin secretion(13).

Finally, although the findings fully support Mirsky's view that indole-3-acetic acid acts essentially as an insulinase-inhibitor, other interpretations have not been eliminated. Indole-3-acetic acid is also a potent plant growth hormone. Other hypoglycemic mechanisms *via* known or postulated effects of animal growth hormone therefore warrant investigation, such as decreased gluconeogenesis dur-

ing protein synthesis or direct stimulation of beta cells to anatomical and functional hypertrophy(14).

Summary. Hypoglycemic responses of normal and diabetic men to tolbutamide and indole-3-acetic acid were similar both qualitatively and quantitatively. These substances, respectively, depressed fasting glucose levels 22% and 11% in normal subjects; 44% and 35% in elderly patients with mild diabetes; and 1% and 7% in uncontrolled juvenile diabetics. Normal individuals and mild diabetics demonstrated significant "insulin activity" in their fasting plasmas, and both groups exhibited sharp increases in insulin activity after ingestion of glucose. Insulin activity was not detectable in blood of juvenile diabetics either before or after glucose. In no individual, however, whether normal or diabetic, was the hypoglycemic response to tolbutamide or indole-3-acetic acid accompanied by increased "plasma insulin activity" in peripheral blood.

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Metabolism of Sulfobromophthalein Sodium (BSP) in Dog and Man.*† (24563)

JAY I. MELTZER, HENRY O. WHEELER,‡ AND WILLIAM I. CRANSTON§
(Introduced by Stanley E. Bradley)
Dept. of Medicine, Columbia University, College of Physicians and Surgeons, and Presbyterian Hospital, N. Y. City

Incomplete recovery of sulfobromophthalein sodium (BSP) from bile following intravenous administration has been attributed either to extrahepatic removal(1) or to BSP metabolism by the liver(2). Brauer and his associates(3,4,5) clarified this problem by a variety of technics including column chromatographic analysis with S^{35} labelled dye as tracer. They have shown that BSP is altered by liver and that the products in bile are colored compounds resembling BSP in their absorption spectra, but apparently differing with respect to extinction coefficients. As a result, colorimetric determinations of BSP in bile are affected by an error leading to systematic underestimation of concentration. Recent studies(6,7) indicate that BSP is transferred into bile by a rate-limited transport system and that it accumulates in hepatic cells in some fixed concentration gradient with respect to plasma. Further understanding of these

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‡ John and Mary R. Markle Scholar in Medical Science.

§ Rockefeller Travelling Fellow in Medicine.