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**Title:** A METHOD OF TREATING HYPOGLYCEMIA IN VERTEBRATES

**Abstract**

Method of raising blood glucose in vertebrates comprising administration of manganese-containing pharmaceutical preparations in appropriate ratios with isoleucine, methionine, phenylalanine, tyrosine and valine and in appropriate ratios as between the amino acids used to increase the blood glucose level of the vertebrate having the blood sugar level raised to within normal limits; these to be given in cumulative amounts appropriate to the individual patient in a schedule of treatment which varies in amount, frequency and the said ratios, all of which reflect the changing degrees of imbalance as the affected individual adjusts the glucose level to within normal limits.
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A METHOD OF TREATING HYPOGLYCEMIA IN VERTEBRATES

BACKGROUND OF THE INVENTION

1. Field of the invention

This invention relates to the raising of the blood sugar level in vertebrates. It relates to the compounds that can be used to avoid an excessive release of insulin by the beta cells of the islets of Langerhans in hypoglycemia, i.e., when the glucose levels fall below normal parameters of concentration.

The invention is directed to providing preparations that will prevent excessive release of insulin.

Hypoglycemia is an increasingly common condition throughout life. It is present at all ages, but is apt to become more common from the teens into the twenties and presents as a common problem in the thirties and forties.
2. Prior Art

"Monoamine oxidase is a flavoprotein oxidase of purported CENTRAL METABOLIC IMPORTANCE CONVERTING NEUROACTIVE AMINES INTO INACTIVE ALDEHYDES.... The flavin linked monoamine oxidase is localized in the OUTER MITOCHONDRIAL MEMBRANE OF ANIMAL CELLS. Walsh pp. 402, 403.

"Actions: Monoamine oxidase is a complex enzyme system widely distributed throughout the body. Drugs that inhibit monoamine oxidase in the laboratory are associated with a number of clinical effects. Thus, it is UNKNOWN WHETHER MAO INHIBITOR PER SE, OTHER PHARMACOLOGICAL ACTIONS, OR AN INTERACTION OF BOTH IS responsible for the clinical effects observed. Therefore, the physician should become familiar with all the effects produced by drugs in this class. PDR (Physicians' Desk Reference 1983) p. 1516.

Two classifications of amine oxidases were presented in 1959. That of Blashko, et al used the response to carbonyl inhibitors to distinguish between the activities of the various amine oxidase. That of Zeller, et al, used semicarbazide inhibitors. The use of inhibitors to classify the amine oxidases reflected difficulties encountered in purifying these enzymes and studying the structure of their active sites.

"A. Occurrence Monoamine oxidase (MAO) has been found in all classes of vertebrates so far examined (1970): mammals, birds, reptiles, amphibians and teleosts (161). The enzyme occurs in many different tissues, particularly in glands, plain muscle, and the nervous system (162).
In man the parotid and submaxillary glands seem to be the richest source of MAO (163). It also occurs in molluscs and plants (4)." Kapeller-Adler 31.

In 1957 iproniazid was introduced for the treatment of depression. New York Times article June 4, 1981, p. B9. It has been studied extensively and is a monoamine oxidase inhibitor. However, it has a variety of effects besides the effect on depression. These have frequently posed problems. The use of these drugs has continued to be empirical. Iproniazid was removed from the market because of severe liver toxicity. It is interesting to note that these drugs exert their beneficial effect in depressed patients anywhere from one to several weeks after treatment is begun.

"In some instances the improvement may progress to a state of euphoria, hypomania, or even mania. Central stimulatory effects are seen with these drugs in normal individuals as well as in depressed patients." Bevan. Other effects are orthostatic hypotension, allergic reactions affecting the liver, dizziness, and a number of anticholinergic type symptoms.

Disturbances in glucose level have been associated with a variety of diseases. The maintenance of the sugar within normal range is a matter of bringing the level down in hyperglycemia, and of bringing it up in various kinds of hypoglycemia. Some of the hypoglycemias are transient, others longstanding. Once developed the hyperglycemia of diabetes mellitus is usually permanent or becomes so. There are instances of hypoglycemia converting to hyperglycemia and diabetes mellitus. The latter disease is of universal distribution and a major cause of morbidity and death.
2. Prior Art

Diabetes mellitus has a wide variety of therapeutic approaches, of which diet is probably the principal one and of longest standing. It undoubtedly goes back to classical times and before the Roman Empire. It was described then in terms of polyphagia, polydipsia, and polyuria. It was named for the polyuria about 70 A.D. by the Greek physician Artaeus.

Banting and Best discovered insulin in 1922. It has been the standard in the treatment of the disease since its introduction. However, dietary control suffices for many mild cases for a considerable length of time. The use of oral hypoglycemic agents was initiated by Loubatieres in France. In 1955 Franke and Fuchs discovered the hypoglycemic action of carbutamide. Perhaps two-thirds of late-onset or adult-onset, or maturity-onset diabetes is controlled by their use. There are a number of names that might be equated with this type. Genetic (hereditary, idiopathic, primary, essential) diabetes have been used for cases, some of which undoubtedly overlap with adult-onset diabetes. Among the hereditary there are also those cases of juvenile diabetes in which failure to have adequate resistance to certain viral diseases results in destruction of beta cells of the Islets of Langerhans. Names overlap and even some juvenile or young-onset cases have the same slow onset of the disease seen in older individuals.

The oral agents vary. The sulfonylureas are
explained as facilitating insulin release. They are effective only when there are functioning beta cells present. Biquanides do not increase insulin release. Such ideas as blocking energy transfer at cytochrome b, decreased binding of insulin in plasma, and acting on receptor sites in membranes have been suggested.

Increased cardiovascular disease has been attributed to sulfonylureas. Insulin does not seem to prevent small blood vessel disease or retinal damage. Various forms of acidosis, such as lact acid acidosis, are contraindications for the use of oral hypoglycemic agents. There are difficulties adjusting dosage of insulin in unstable juvenile diabetes. The odd acidoses are an occasional cause of death in the diabetic.

Most of the efforts to control diabetes have centered around maintenance of the insulin supply and to balance the effect of insulin against other hormones, notably glucagon and adrenalin. Despite active efforts during the six decades since the introduction of insulin, all results have failed to provide a definitive explanation of the cause of the disease. Production of pure insulin of human type has been developed in bacteria through genetic engineering. Study of sustained release methods employing special implants continue to be studied. A general view of the disease as of 1970 is presented in Chapter 94 of Harrison's Principles of Internal Medicine 6th edition. See enclosure. Also see Addendum to Formulation, Section VII for further discussion of previous definitions of the cause of the disease.
CHEMICAL EFFECTS OF MONOAMINE OXIDASE

"SPECIFICITY"

"The enzyme isolated from a number of sources exhibits low specificity. In general, primary, secondary, and tertiary amines, tryptamine derivatives and catecholamines are oxidized (1,5). The enzyme isolated from human placenta, however, will only attack primary amines and with simple alkyl amines increase in chain length results in increased affinity (7)." Barman p. 180.

"Inhibition of MAO leads to a very pronounced increase in the levels of norepinephrine in the sympathetic nervous system and of the monoamines serotonin, norepinephrine, and dopamine in the monoamine-containing neurones of the CNS... Large amounts of amine now accumulate in the cytoplasm. The storage sites rapidly become filled to capacity with the transmitter. This enhanced accumulation of neuroamines within the neurones is presumed to be the basis for the antidepressant action of the MAO inhibitors... It should be added that the presence in the urine of large amounts of unmetabolized serotonin and 3-O-methylated catedholamines is characteristic of patients on MAO inhibitor antidepressants." Bevan pp. 183, 184.

These urinary compounds indicate clearance of the above amines from the blood and is consistent with an increased turnover rate of increased amounts of each amine.

"The flavoprotein responsible for the oxidative deamination of the catecholamine (monoamine oxidase) is found in a wide variety of tissues and is located primarily in the outer membrane of mitochondria." Frisell p. 628.
CHEMICAL EFFECTS ON MONOAMINE OXIDASE

Halogenated compounds enter the body frequently from the environment. The anaesthetics halothane and methoxyflurane are cases in point.

"Incubation of the volatile general anaesthetics halothane or methoxyflurane (labelled with $^{16}$Cl) with hepatic microsomes, NADPH, and oxygen is accompanied by extensive DECHLORINATION.

"Similarly thyroxine and triiodothyronine undergo deiodination by hepatic microsomal enzymes (8)." Bacq p. 577.

"Dimino and Hoch (1972) found a considerable enrichment of iodine in liver mitochondria of rats injected with $T_4$. These mitochondria were more dense than those of untreated animals and appeared to contain iodine TIGHTLY BOUND TO THEIR INNER MEMBRANES (9). ...Direct effects of $T_4$ on isolated mitochondria have been known for some time, but they occur only at HIGH, UNPHYSIOLOGICAL CONCENTRATIONS and their significance is doubtful. (9)." Lash p. 332.

"The actual biochemical mechanism of thyroid hormone action on neural tissue is poorly understood."

"It is evident that a single regulatory reaction has not been found to explain the multiple effects of thyroid hormones.

"Although the activities of more than 100 enzymes have been shown to be affected by thyroxine administration it appears that all are not influenced to the same degree. (10)." Frisell p. 608.
MANGANESE METABOLISM

"The early studies of Greenberg (65) with radiomanganese indicated only 3–4% of an orally administered dose is absorbed in rats. The absorbed manganese quickly appeared in the bile and was excreted in the feces. Experiments since that time with several species including man indicate that manganese is almost totally excreted via the intestinal wall by several routes. These routes are interdependent and combine to provide the body with an efficient homeostatic mechanism regulating the manganese levels in the tissues (16,90,129). The relative stability of manganese concentrations in the tissues to which earlier reference was made is due to such controlled excretion rather than to regulated absorption. (27)." Underwood p. 184.

It is important to realize that each of these tissues in the intestinal tract are actually using the same system to take in and to dispose of manganese. Whis is being described above is the flow of manganese into mitochondria and out again. It is a reflection of the mitochondrial pool, which is a very labile pool. Manganese is carried in the plasma bound to protein. Very little of it is cleared by the kidneys.

"Injected radiomanganese disappears rapidly from the bloodstream (23,90). Borg and Cotzias (28) have resolved this clearance into three phases. The first and fastest of these is identical to the CLEARANCE RATE OF OTHER SMALL IONS, SUGGESTING THE NORMAL TRANSCAPILLARY MOVEMENT, the second can be identified with the ENTRANCE
OF THE MANGANESE INTO THE MITOCHONDRIA OF THE TISSUES, AND
THE THIRD AND SLOWEST COMPONENT COULD INDICATE THE RATE OF
NUCLEAR ACCUMULATION OF THE ELEMENT....The kinetic patterns
for blood clearance and for liver uptake of manganese are
almost identical indicating that the two manganese pools-
BLOOD MANGANESE AND LIVER MITOCHONDRIAL MANGANESE - RAPIDLY
ENTER EQUILIBRIUM. A high proportion of body manganese
must, therefore, be in a dynamic mobile state. Underwood
p. 185.

"The turnover of parenterally administered $^{54}$Mn has
been directly related to the level of stable manganese in
the diet of mice over a wide range (27). A linear rela-
tionship between the rate of excretion of the tracer and
the level of manganese in the diet was observed and the con-
centration of $^{54}$Mn in the tissues was directly related to
the level of the stable manganese in the diet. THIS PRO-
VIDES FURTHER SUPPORT FOR THE CONTENTION THAT VARIABLE
EXCRETION RATHER THAN VARIABLE ABSORPTION REGULATES THE
CONCENTRATION OF THE METAL IN TISSUES." Underwood p. 185.

"Little is known of the mechanism of absorption of
manganese from the gastrointestinal tract, or of the means
by which excess dietary calcium and phosphorus reduce man-
ganese availability....The effect of variations in dietary
calcium and phosphorus on the metabolism of $^{54}$Mn in rats
has been studied further by Lassiter and associates (100).
These workers found that the fecal excretion of parenteral-
ly administered $^{54}$Mn was much higher and the liver reten-
tion lower on a 1.0% calcium diet than on a 0.64 calcium
diet. It appears, therefore, that calcium can influence manganese metabolism by affecting retention of absorbed manganese as well as by affecting manganese absorption. Variations in dietary phosphorus had no comparable effects on the excretion of intraperitoneally administered $^{54}$Mn, but the absorption of orally administered $^{54}$Mn was impaired. Underwood. p. 186.

During 1970 a rash of books drew attention to energized translocation or transport and to the changes in conformation of the membranes of the mitochondria. There were extensive correlations devised with the mitochondrial oxidative phosphorylations. By 1975 some of this was discounted by claims that many solutes crossed the mitochondrial membrane without active transport. A number of postulates evolved including proton, phosphate and other mechanisms for these transfers.

In muscle and nervous tissue there are differences of sixty millivolts or more between the inner and outer surfaces of cell membranes. A Ca/Mg pump explains a wide variety of data. There seemed initially to be good data for high resonant phosphate compounds activating the cation pumps of mitochondria. Such a pump is affected by changes in concentration of calcium and it is also modulated by magnesium. Mn goes in and out of mitochondria readily. It does so by active translocation and in the company of alkaline earth metal cations. Other metals participate but to a lesser degree. A Ca/Mg pump operating in tandem with Na/K ATPase pumps not only fits the cell membrane, but it also would have a place in the mitochondrial scheme of things.
It has long been suggested that mitochondria represent primitive bacteria originally ingested when cells developed phagocytic functions. The effective oxidation processes of the ingested cells are cited as the cause of the symbiosis developing. The corollary of that suggestion is the need that developed to correlate flow of high resonant compounds between the original cell and the mitochondria. This theory suggests that metabolic disease might well occur at the site of such a complex metabolic adjustment between the metabolism of two different cells. This mechanism of regulation is consistent with that theory.

The added point must be made that the high efficiency ascribed to mitochondria as sources of high resonant bonds highlights the need for a central control mechanism. Such a mechanism must collate the energy production of the mitochondria with the energy metabolism of the cells, organs, and indeed the entire organism. Calcium would seem a logical choice as the modulator of a system interactive between eukaryotic cells and mitochondria. This is consistent with the present presentation.

This mechanism or system of control has been called a mechanism of regulation. Listing the sequence of components described includes cation, ATPase pump, Mn, deiodinase, thyroid hormones, monoamine oxidase and amines. ALL ARE FOUND IN CLOSE PROXIMITY IN THE MITOCHONDRIA.
Diabetes mellitus was named for its frequency of urination (polyuria) by a Greek physician Aretaeus about 70 A.D. The disease was apparently written about by the ancient Chinese. It is a major cause of death throughout the world.

It has been described as due to inability to burn glucose. It has been explained as due to burning fat instead of sugar. Organic acids accumulate to produce the acidosis characteristic of the disease. Not being able to burn all the fat needed to meet the needs of the body has been given as an explanation of the accumulation of acetoacetic acid, beta-hydroxybutyric acid, and acetone when acidosis or ketosis develops.

Of course, these explanations are facile. In order to know which have merit, it would be necessary to identify the specific chemical reaction(s) that are abnormal in the disease.

In the cytosol, breakdown of glucose (glucolysis) produces pyruvate. Products of pyruvate enter the citrate synthase system in the tricarboxylic acid cycle of the mitochondria. Either oxaloacetate or 'active acetate' are formed from pyruvate. These are the two compounds necessary to form citrate. "...the cycle is at the center of aerobic metabolism,..." Frisell, p. 530. It is the citrate synthase reaction that is slowest of all in the cycle. It is, therefore, the rate-limiting step. Such a reaction qualifies as a controlling reaction in a metabolic cycle.
Besides the citrate synthetase or condensing enzyme, the tricarboxylate cycle has some seven other enzyme reactions. Three different forms of tricarboxylic acid exist in the cycle. The first of these, citric acid, is formed by the citrate synthase system. Citric acid is part of a three-member tautomeric isomerization, which includes aconitate and isocitric acid. The interconversions are assisted enzymatically by aconitase. Isocitrater is drained off by isocitrater dehydrogenase for forming of alpha-ketoglutarate. The latter in turn is converted into succinyl CoA by alpha-ketoglutarate dehydrogenase.

The succinyl CoA is acted upon by succinyl CoA synthetase. While it is producing a molecule of guanosine triphosphate (GTP), succinyl CoA synthetase releases the succinic acid from the succinyl CoA. The resonance of the CoA sulfur bond is thus transferred to GDP in forming the GTP.

From the succinic acid two hydrogen atoms are removed by succinate dehydrogenase to produce a double bond. The compound formed is fumarate with hydrogen atoms trans to one another at the double bond. A water molecule is added across the double bond. This is achieved by the activity of fumarate hydratase (fumarase) and forms malate. Finally, when two hydrogens are removed from malate by malate dehydrogenase we have oxaloacetate again and have completed a full cycle around the Krebs (TCA) cycle. We have traversed a tautomeric isomerization with a hydratase and two dehydrogenases followed by a synthetase, a dehydrogenase, another hydratase and a final dehydrogenase.
Somewhere in this aerobic oxidation system exists the means of preserving the chemical balance of the many metabolic pathways necessary to maintain vital functions of the organism. Diabetes, then, can be defined as a disease in which proper proportions of products of the TCA cycle are not maintained. The question is where in the myriad possibilities for imbalance does the derangement in a chemical reaction occur which results in diabetes mellitus?

Pyruvate from the breakdown of glucose provides oxaloacetate and acetyl CoA for the citrate synthase step of the Krebs cycle. There is a pool of each of these substrates. In addition, of course, there are pools of citrate molecules, alpha-ketoglutarate molecules and of molecules of succinyl CoA, succinate, fumarate, and malate.

The size of each of these pools is determined in part by the preceding enzyme substrate in the cycle, BUT ONLY IN PART.

For instance, the oxaloacetate pool is directly increased by the breakdown of asparagine and aspartic acid. The fumarate is increased by the degradation of the aromatic amino acids phenylalanine and tyrosine. The pyruvate from the breakdown of alanine, threonine, glycine, serine, and cysteine becomes available for formation of both the oxaloacetate pool and the acetyl CoA pool. The great portion of the acetyl CoA pool, however, is derived from the breakdown of fatty acid chains by beta oxidation in the lipolytic cycle.
"Amino acids whose carbon chains can be oxidized more or less directly to acetyl-CoA can also yield acetoacetate, a KETONE BODY. Formation of acetoacetate from such amino acids is particularly evident in fasting animals and accordingly, they are labeled KETOSTERIC. These amino acids are leucine, lysine, isoleucine, tryptophan, phenylalanine, and tyrosine. If we define a ketogenic acid as one whose carbon chain can give rise only to acetoacetate, there are only two ketogenic amino acids—leucine and lysine. The other four— isoleucine, tryptophan, phenylalanine, and tyrosine—can also give rise to glucose precursors and are, therefore, both ketogenic and glucogenic.

"Amino acids that can serve as precursors of phosphoenolpyruvate (therefore, of glucose) are called GLYCOGENIC OR GLUCOGENIC. These amino acids are:

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<td>Asparagine</td>
<td>Glycine</td>
<td>Hydroxyproline</td>
<td>Valine</td>
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p. 245 Frisell (1982)

We can realize from this that the acetyl CoA pool is a crossroads substrate for the TCA cycle and that it enters at the rate-limiting citrate synthase step in the cycle.

With this by way of introduction, we have two more substrates of the TCA cycle to consider. One of these is the alpha-ketoglutarate substrate and the other is its product from the action of alpha-ketoglutarate dehydrogenase. That product is succinyl CoA, the next substrate in the Krebs cycle.
When glutamate dehydrogenase is inhibited, less transamination occurs. This results in less breakdown of amino acids, those which have transaminase enzymes. Although this is true of the inhibition of the polymeric form of glutamate dehydrogenase, the monomeric forms proceed to oxidatively deaminate the three branched chain amino acids. Deamination of the methionine chain presumably occurs at the L-homoserine hydro-lyase (deaminating) conversion to 2-oxo-butyrate. This enzyme is also named homoserine dehydratase with HÖH being added and NH₃ and HÖH being products of the reaction. However, methionine and also 2-aminobutyrate are listed as substrates of glutamate dehydrogenase, presumably in the monomeric form. This raises some question as to the route of degradation of methionine in its ultimate production of succinyl CoA. The cytosol is a thick suspension. The matrix of the mitochondria may be an even thicker suspension with a very high protein content. In view of the monomers of glutamate dehydrogenase occurring in such milieu, it would be consistent to think of methionine breakdown not involving the B-6 assisted step in this context.

Lysine and threonine are the two essential amino acids not transaminated. In fact, they are not broken down readily. Lysine is used for forming organic electrolytes, the polyamines, and for the synthesis of carnitine, necessary for carnitine acyl transferase activity needed to transfer branched chain fatty acids into the mitochondria.
The overall effect of the above enzymatic steps presumably would be to increase the demand for the branched chain amino acids. Thus, the two aromatic amino acids, phenylalanine and tryptophan are spared by the inhibition of glutamate dehydrogenase. On the other hand, the initial sparing of valine, isoleucine, leucine and methionine is altered subsequently by the monomers of the enzyme oxidatively deaminating them (methionine). Under those circumstances the sparing effect gives way to increased breakdown and increased need for these amino acids. For the present at least, although threonine does degrade to some extent to succinyl CoA, that amino acid does not seem to be involved in either the sparing effect of the polymeric form or the increased degradation effect of the monomeric form of the inhibited glutamate dehydrogenase. The lysine is purely ketogenic. Its contribution to the acetyl CoA pool would seem to be unaltered under these circumstances.

Alpha-ketoglutarate is formed by isocitrate dehydrogenase in the TCA cycle. This pool is enlarged by the action of glutamate dehydrogenase. Alpha-ketoglutarate dehydrogenase promptly converts it into succinyl CoA. The succinyl CoA in turn is converted to succinic acid by succinyl CoA synthetase and a molecule of GTP is formed at the same time from GDP and P_i.

Discussing the rate-limiting citrate synthase step in the TCA cycle, Frisell has said: "The synthase reaction is accepted, therefore, as a nonequilibrium
reaction and becomes a major control reaction for the cycle. It is reasonable that the rate of the synthase reaction should be sensitive to the availability of acetyl CoA. IN ADDITION, HOWEVER, AN INTERMEDIATE OF THE CYCLE ITSELF, SUCCINYL COA, can INHIBIT CITRATE SYNTHESIS BY COMPETING WITH ACTIVE ACETATE."

This suggests that the size of the pools of acetyl CoA and succinyl CoA can assume prime importance in determining the overall rate of the TCA cycle. A number of substances are degraded to succinyl CoA These include:

1. isoleucine and valine via methymalonyl CoA;
2. branched chain fatty acids via propionyl CoA which in turn is changed into methymalonyl CoA;
3. methionine and tryptophan via alpha-keto-glutarate to the propionyl CoA.

The methymalonyl CoA rearranges through the action of a mutase to form succinyl CoA. This conversion requires B-12 for the enzyme to be active. The reaction on propionyl CoA itself requires biotin.

4. pyrmindine breakdown products including those from thymine also contribute to the succinyl CoA pool. The point has been made in detail before that the polymeric glutamate dehydrogenase feeds into this pool indirectly through the alpha-ketoglutarate (2-oxo-glutarate). When the polymeric form is inhibited, the monomeric forms increase breakdown of the above amino acids to produce succinyl CoA as if replacing that which was lost when the polymeric form was inhibited.
However, as demonstrated, alpha-ketoglutarate is formed readily as a part of the TCA cycle by isocitrate dehydrogenase. In fact, alpha-ketoglutarate from glutamate dehydrogenase can be thought of stoichiometrically in the above context as a return of the original product to the TCA cycle.

Lehninger, that brilliant analyzer and expositor of the mitochondrion, speaks of glutamate dehydrogenase on p. 439 (1970) as follows: "Glutamate dehydrogenase, probably because of its central role in the transfer of amino groups, is an allosteric enzyme. The beef liver enzyme has a molecular weight of 280,000 and contains a number of apparently identical subunits. The enzyme ASSOCIATED INTO LARGER AGGREGATES OF PARTICLE WEIGHT 2.2 MILLION WHICH ARE ROD-SHAPED. THE EQUILIBRIUM BETWEEN THE MONOMERIC AND POLYVALENT FORMS IS SHIFTED IN ONE DIRECTION OR THE OTHER BY VARIOUS EFFECTORS. The enzyme is inhibited by the effectors ATP, GTP, NADH AND IS ACTIVATED by ADP AND CERTAIN AMINO ACIDS. It is also influenced by the PRESENCE OF THE THYROID HORMONE THYROXINE and CERTAIN STEROID HORMONES."

In this regard, Frisell on page 240 (1982) remarks: "The thyroid hormone, thyroxine, can also influence the activity of glutamate dehydrogenase but the significance of this action is not yet established."
Just before that, he had said. "With regard to its macromolecular structure, glutamate dehydrogenase consists of SIX IDENTICAL SUBUNITS. As would be surmised, this subunit character of the enzyme gives it the possibility of being subject to allosteric control. Indeed, the dehydrogenase is inhibited by NADH, ATP, and GTP, and is stimulated by ADP, GDP, and SOME AMINO ACIDS.

Barman, _Vol. 1_ (1970) pp. 170-171. 1.4.13 is somewhat more specific:

"With enzyme isolated from beef liver, GTP and diethylstilbestrol stimulate the oxidation of the following monocarboxylic amino acids: alanine, leucine, isoleucine, methionine valine, norleucine, norvaline and 2-aminobutyric acid.

ADP inhibits these oxidations. However, the oxidation of glutamate is inhibited by GTP and diethylstilbestrol but is stimulated by ADP.

These data can be explained in terms of an equilibrium between different forms of the enzyme WHICH HAVE DIFFERENT RELATIVE SUBSTRATE SPECIFICITIES and it is thought that the position of this equilibrium is influenced by modifiers."
It can be seen that oxidation of the monocarboxylic amino acids occurs when the dicarboxylic amino acid glutamate is not being oxidased. The glutamate is being oxidized when the monocarboxylic amino acids are not being oxidized.

In table form, Barman's data can be presented as follows:

<table>
<thead>
<tr>
<th>Oxidation</th>
<th>Beef liver</th>
<th>Stimulate +</th>
<th>GTP &amp; DES</th>
<th>Inhibit 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>+</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>+</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>+</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Methionine</td>
<td>+</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Valine</td>
<td>+</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Norleucine</td>
<td>+</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2-aminobutyrate</td>
<td>+</td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

L-glutamate: 0 +

Note: For the reverse reaction pyruvate is utilized by frog liver and beef liver enzymes.

In order to understand the 'different forms', we will use as standard nomenclature that described by Lehninger (1970) pp. 58-59. It is as follows:

'Specific terms are commonly used to refer to different aspects or levels of protein structure. The term primary structure refers to the covalent backbone of the polypeptide chain and specifically denotes the sequence of its amino acid residues. Secondary structure polypeptide chains, particularly as they occur in fibrous proteins.'
The term tertiary structure refers to the manner in which the polypeptide chain is bent or folded to form compact, tightly folded structure of globular proteins (Figure 3-2). The more general term conformation is used to refer to the combined secondary and tertiary structure of the peptide chain in proteins. The term quaternary structure denotes the manner in which the individual polypeptide chains of a protein having more than one chain are arranged or clustered in space. Most larger proteins, whether fibrous or globular, contain two or more polypeptide chains, between which THERE MAY BE NO COVALENT LINKAGES (Fig. 2-2). In general, the polypeptide chains of proteins usually have between 100 to 300 amino acid units (mol wt 12,000 to 36,000). A few proteins have longer chains, such as serum albumin (about 550 residues) and myosin (about 1,800 residues). However, any protein having a molecular weight exceeding 50,000 can be suspected to have two or more chains.

"Proteins possessing more than one chain are known as oligomeric proteins; their component chains are called protomers.

A well-known example of an oligomeric protein is hemoglobin, which consists of four polypeptide chains, two identical alpha-chains and two identical beta-chains. Each chain has about 140 amino acids. The four chains fit together tightly to form a globular assembly OF GREAT STABILITY, despite the fact that THERE ARE NO COVALENT LINKAGES. Oligomeric proteins usually contain an even number of peptide chains."
There may be anywhere from two to twelve subunit chains among the smaller oligomeric proteins to dozens or even hundreds among the larger proteins. Tobacco mosaic virus particles have over 2,000 peptide chains.

"Since oligomeric proteins contain two or more polypeptide chains, which are usually not covalently attached to each other, it may appear improper or at least ambiguous to refer to oligomeric proteins as "molecules" and to speak of their "molecular weight. However, in most oligomeric proteins, the separate chains are so tightly associated that the complete particle usually behaves in solution like a simple molecule. Moreover, ALL THE COMPONENT CHAINS OR SUBUNITS OF OLIGOMERIC PROTEINS ARE USUALLY NECESSARY FOR THEIR FUNCTIONS."

To bring this further into perspective in physiological terms and enable us to match structural details with observed changes in vital functions, we had best enlarge still further upon the "subunits." This is discussed on pp. 184-185 of Lehninger as follows:

"This mechanism for hemoglobin oxygenation is directly applicable to regulatory enzymes. The binding of the first substrate molecule to one subunit of a homotropic enzyme enhances the binding of a second substrate molecule to a second subunit because there is a conformational change in the first subunit which is transmitted mechanically or sterically to the second subunit. In all cases studied to date, regulatory enzymes have been found to be rather large molecules containing subunits; presumably, the existence of
interacting units is necessary for their function.

"Note that the term "subunit" IS AMBIGUOUS and may have TWO DIFFERENT MEANINGS WHEN APPLIED TO OLIGOMERI PROTEINS. Hemo-
globin contains four structural subunits or protomers, i.e., the two alpha and two beta chains, but two functional sub-
units, i.e., the two alphabeta half molecules.

**ISOXYMES**

"Recent research has revealed another way in which the activi-
ty of some enzymes may be controlled THROUGH FEATURES OF THEIR MOLECULAR STRUCTURE. A number of different enzymes have been found to exist in multiple molecular forms WITHIN A SINGLE SPECIES, or EVEN WITHIN A SINGLE CELL. Such multiple forms can be detected and separated by gel electrophoresis of cell extracts; they are therefore distinct molecular species differ-
ing in net electrical charge. Multiple forms within a single species or cell are called **isozymes** (or **isoenzymes**).

"Lactate dehydrogenase, one of the first enzymes in this class to have been studied extensively, exists in five dif-
ferent major forms, or isozymes, in the tissues of the rat (Figure 9-12). These have been obtained in pure form. Al-
though all five isozymes of lactate dehydrogenase catalyze the same reaction overall, they have DISTINCTLY DIFFERENT Kₐ VALUES for their substrates; the biological significance of these differences will be described in Chapter 15 and 18. The five isozymes all have the same particle weight, about 134,000, and all contain four polypeptide chains, each of mol wt 33,500.
"On the 18th of February, 1969, Paul Langerhans defended his thesis about the cell clusters in the pancreas which later came to bear his name. Exactly 100 years after that remarkable observation, representatives from the main centers of islet research met in Umea, Sweden, for a second international symposium on "The Structure and Metabolism of the Pancreatic Islets."

The hypoglycemic effects of leucine and of arginine discussed at that symposium were reported in Vol. 16 of Wenner-Gren Center International Symposium Series (1970) Pergamon Press Limited, ed. Falkner, Mellman, and Taljedal. This was some fifteen years ago.

Unger, et al discussed the regulation of glucagon release in vivo. On page 147 they state, "In man arginine infusion elicits a parallel rise in insulin and glucagon during which glucose concentration rises to an average peak of 16mg%." Then, they further remark, "In genetic diabetes, however, the glucagon response is intact but the beta-cell response is reduced; the result is hyperglycemia with an average peak rise of 45 mg%. One could postulate that if alpha-cell function were reduced without symmetrical impairment of beta-cell function, the administration of arginine would result in hypoglycemia, and, indeed, experiments in dogs in which all of the pancreas save the uncinate process had been resected support this supposition..."
Hellerstrom, et al discuss the effects of amino acids on oxygen consumption of the beta-cell in relation to insulin release. Valine and arginine were without effect, 
"...whereas a marked respiratory enhancement was noted with either alanine or leucine."

Stork, et al studied beta-cell respiration in the presence of sulfonylureas. The latter "...strongly depressed" leucine degradation in the beta-cells.

Malaisse, et al demonstrated that gut extract "...also significantly enhanced the stimulant effect of leucine," on insulin secretion.

Another paper by Unger, et al led them to conclude: "This suggests that the magnitude in the hyperglycemic response to arginine is determined by the glucagon concentration in relation to the concomitant insulin level, rather than to glucagon concentration alone."

The last paper of the symposium was "The Pancreatic Beta-Cells in the Pathogenesis of Human Diabetes Mellitus" by Cerasi and Luft. Their summary reads as follows:

"The findings of a delayed and decreased insulin response to glucose infusion in healthy monozygotic twin sibs of patients with diabetes mellitus makes it very likely that this defect in insulin release is a major, genetically determined pathogenetic factor involved in the development of diabetes mellitus. In a non-selected population of subjects with normal glucose tolerance, the
frequency of such a defective insulin response to glucose is around 20%, indicating that the prediabetic state is rather common in a general population.

"The biochemical abnormality responsible for the defective insulin release in prediabetics is not known. Evidence is presented in the present work indicating that the adenyl cyclase system of the beta-cell might be involved in this abnormality, since insulin response can be normalized in prediabetics by pretreatment of the subjects either with theophylline or with large doses of human growth hormone, both agents known to result in an elevation of the intracellular level of cyclic 3',5'-AMP."

The conference is summarized by B.A. Houssay. This gives the most advanced status of the study of diabetes mellitus and the lowering of blood glucose at that time.

Despite this knowledge and the continuing study of it, a dependable, continuing modulation of insulin release in NIDDM (Type II) diabetes mellitus by the use of these naturally occurring substances was not achieved.

From these observations of the effects of under-secretion of insulin in producing diabetes mellitus, it is easy to conceive of the opposite situation in which there exists a lowered blood sugar from a relative excess of insulin secretion with a resulting excessive drop in blood glucose, i.e., hypoglycemia.
SUMMARY OF THE INVENTION

The present invention provides a method for raising blood glucose in vertebrates with hypoglycemia. The use of amino acids having hyperglycemic actions in various ratios each with the other and each with manganese in effective ratios decreases insulin release in chemical hypoglycemia.

The present invention differs in its relation to effective amounts given in that these amounts are constantly changing, so that there is a pattern of changing requirements as to frequency, amount and individual requirements.
DESCRIPTION OF THE PREFERRED EMBODIMENT

In accordance with this invention, patients with hypoglycemia have usually been treated with exercise and with a diet such as one low in sugars and high in protein. In addition, a variety of hormonal and other agents claimed to produce a rise in the blood sugar level have also been used. The above program and medication can for the most part be continued as this method is initiated. It will, however, be desirable to discontinue any hormone used in the previous treatment in order to determine base values before initiating treatment.

Following an introductory period of observation, the hyperglycemic agents named in the invention may be used stepwise. The introductory period permits evaluation of the fasting blood sugar.

A fasting blood sugar is taken when the hyperglycemic agent is to be introduced. The desired amount of the agent is then given with food. A day later or after a suitable time period a subsequent fasting blood sugar determination is made to detect any change in blood glucose.

For example, isoleucine in the amount of 125 mg may be given initially. If there is no change, this should be increased stepwise to 250 and 500 mg or higher values until a drop in the blood glucose can be correlated with the amount given.
The less direct actions of valine, methionine, phenylalanine and tyrosine are best explored after the treatment schedule has been undertaken.

A small dose of manganese of one or two mg (calculated as manganese content in manganese gluconate) can be given separately. Both the glucose level and the subjective response of the patient are monitored. If these initial amounts are well tolerated, the amount can be increased by increments of two to three mg at a time up to doses of five and ten mg or more. Once the upper amount tolerated has been determined, these levels are likely to decrease rapidly. There is no need to activate special methods of disposing of manganese by giving much large amounts.

The amount of manganese reached for positive correlation with a drop in the glucose level is likely to fall progressively over a few weeks or days. The amounts required sometimes decrease rapidly and are spaced out over longer and longer intervals. It is best to be prepared to promptly drop to lower amounts at the earliest indication that the amount required is decreasing.

Combinations of manganese and isoleucine may appear to have synergistic effects. This reflects the different mode of action of each. The manganese may appear to have a modulating effect on the action of isoleucine.
As amounts are adjusted downwards, increase the interval between treatments as well as decreasing the amounts given. This is the 'cumulative' effect of pharmaceutical agents, which may be described as being similar to 'filling a hole'. This use of the word cumulative must be distinguished from that in legal phraseology. The legal definition follows the heaping up or piling up definition of the word cumulative.

The clinician must be aware of the implications of the above. It is best that the substances be administered personally during the initial period of treatment. The period of adjustment varies from patient to patient. It is unwise to provide the patient initially with a standard daily dosage on the assumption that the effects will be predictable.

As the blood sugar rises, any other agents used may be discontinued. No effort should be made to force the glucose level above the normal range, which may be estimated in the human at 110 to 140 milligrams/100 milliliters of blood, i.e., between 110 to 140 mg\%

Hyperglycemic effects produced in such patients by the use of agents which cause a stress reaction, e.g., cortisol, which causes the breakdown of protein should be avoided. The overall effects of these substances may prove to be quite adverse and are to be avoided. In accordance with this, any form of stress, such as injury and infection, are prone to produce variations in the amounts of isoleucine and/or manganese required. No manganese should be given during fever.
Example # 1

Patient M.V.

Clinical Status: The patient has a long history of a low, flat glucose tolerance test. Values were especially low a number of years ago. However, they were remaining in the 50 mg range and an attempt was made to change the low level towards the normal range of blood glucose values.

Treatment periods: Initially a weekend in which two days of treatment were undertaken. Then at various times the next three weeks.

Treatment period interval: Ranged from one week to two days.

Objective findings:

I Blood glucose: Ranged upwards from about 50 milligrams/100 milliliters (mg%) to 100 mg%.

II Blood pressure:
   A Systolic pressure: ranged from 150 to 124 mg Hg (millimeters mercury pressure)
   B Diastolic pressure: ranged from 86 to 110 to 75 mm Hg in that order
   C Pulse pressure: ranged from ninety to 34 mm Hg

III Pulse: Ranged from 70 to 76

Range of medication:

   Ratios: Manganese (mg in manganese gluconate) 2mg+ at one to ten day intervals.
   Isoleucine in quarter, half and whole tablet, 500 mg/tablet in time between meals to ten days.
   (1.6 to 6.7 mg/kg body weight)
   (0.007 to 0.27

Objective of Treatment: To bring glucose level to normal range of (100-110) to 140-150 mg%

Subjective findings: The patient was anxious and upset during part of period associated with a viral upper respiratory infection. During this interval anxiety was prominent.

Clinical response: An episode of labile blood pressure occurred during the time involved and then drifted down to 124/75 over a two week period. The change in blood glucose level occurred easily. The most striking observation was the level of 90 mg% still present six months later. The patient continued throughout to feel considerably better from day to day than was his usual pattern.
Example # 2

Patient T.S. midlife History of headaches Recurrent peptic ulcer with occasional activity treated with tagamet.

Treatment Periods: varied from one to two weeks to two consecutive days.

Treatment: After fbs (fasting blood sugar) and blood sugar before meals, manganese gluconate (manganese calculated in milligrams, mg.) Days 5, 15, 16, 18, 39 and 54 1mg+ to 2mg+ given. Isoleucine separately or with manganese gluconate in amounts of one-fourth to one tablet, i.e., 125 to 500 mg.

Treatment period interval: Initially at two to ten days; then two to fifteen days thereafter.

Objective findings:
I Blood glucose: Increased from about 60 milligrams/100 milliliters, i.e., 60, to 100 mg% (milligrams per cent) over a seven week period.

II Blood pressure:
A Systolic (upper) from 154 to 116 mm Hg (millimeters mercury)
B Diastolic from 92 to 78
C Pulse pressure = difference between systolic and diastolic = 62 to 40

III Pulse: from 66 to 80/minute

Range of Medication:
Ratios: isoleucine to manganese = 63/1 in mg to 250/1 About 2mg/kg to 8mg/kg isoleucine About 0.03 mg/kg to 0.09 mg/kg body weight

Objective of treatment: To restore fasting blood sugar (fbs) to normal range of values (100 to 110) to (140 to 150) mg% without headaches developing

Subjective findings: Patient appeared to feel better subsequent to the course of treatment. Clinical course correlated with a slow drop of 25 to 30 mg% over about a four month period. Affect good.

Clinical response: Satisfactory Course of treatment uneventful
Example 3

Patient M.A., mid-thirties

Returned after ten year interval. Under dietary treatment for reactive hypoglycemia.

Avoided crowded areas.

Did only local driving.

Treatment periods: Each for one or two days consecutively.

Treatment: Blood sugar drawn fasting and between meals and hyperglycemic agent given 2 to 3 times/day with food

Manganese gluconate 1 - 2 mg/treatment period.

Treatment period interval: increased from 2-3 weeks to 3 – 5 months.

Blood sugar: increased from 65-70 mg% to 85-90 mg%.

Objective of treatment: to restore blood sugar to normal range of fasting values, i.e., 90 to 110 mg%.

Clinical Response: Normal range of blood sugar - patient symptom-free

Full driving schedule

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SUBSTITUTE SHEET
1. A method of treating hypoglycemia in vertebrates and other organism comprising administering to the subject an antihypoglycemically effective amount therefor of at least one compound of (a) comprising L-valine, D-valine, L-methionine, D-methionine, L-isoleucine, D-isoleucine, their alpha-keto and alpha-hydroxy analogs, and the di- and tripeptides of the aminoacids or the pharmaceutically acceptable acid addition salts thereof and an effective amount therefor of at least one compound of (b) comprising L-phenylalanine, L-tyrosine, D-phenylalanine, D-tyrosine, their alpha-keto and alpha-hydroxy analogs, and the di- and tripeptides of the aminoacids or the pharmaceutically acceptable acid addition salts thereof in an antihypoglycemically effective ratio with an effective nonlethal amount therefore of (c) a preparation consisting essentially of a manganese compound.
1. A method of treating hypoglycemia in vertebrates comprising administering to the subject an antihypoglycemically effective amount therefor of at least one compound of (a) comprising L-methionine, D-methionine, L-isoleucine, D-isoleucine, their alpha-keto and alpha-hydroxy analogs, and the di- and tripeptides of the said aminoacids or the pharmaceutically acceptable acid addition salts thereof and an effective amount therefor of at least one compound of (b) comprising L-phenylalanine, L-tyrosine, D-phenylalanine, D-tyrosine, their alpha-keto and alpha-hydroxy analogs, and the di- and tripeptides of the said aminoacids or the pharmaceutically acceptable acid addition salts thereof in an antihypoglycemically effective ratio with an effective nonlethal, physiologically-tolerated, pharmacokinetically-appropriate, pharmaceutically-acceptable amount therefor of (c) a preparation consisting essentially of a manganese compound.
**INTERNATIONAL SEARCH REPORT**

**I. CLASSIFICATION OF SUBJECT MATTER**

According to International Patent Classification (IPC) or to both National Classification and IPC

<table>
<thead>
<tr>
<th>Int. Cl</th>
<th>US Cl</th>
<th>Classification System</th>
<th>Classification Symbols</th>
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</table>

**II. FIELDS SEARCHED**

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Classification Symbol</th>
</tr>
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<tbody>
<tr>
<td>424/177 424/287</td>
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</tr>
</tbody>
</table>

**III. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>U S, A, 4,298,601 (Howard) 03 November 1981</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>U S, A, 4,340,592 (Adibi) 20 July 1982</td>
<td>1</td>
</tr>
<tr>
<td>X,P</td>
<td>U S, A, 4,435,424 (Wurtman) 6 March 1984</td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>U S, A, 4,218,474 (Barnish) 19 August 1980</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>U S, A, 3,873,296 (Ashmead) 25 March 1975</td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>N, Chemical Abstracts, Vol. 77, 1972, Spisni, Effects of L-leucine, L-lucyl-L-lucine, and L-leucinamide on blood sugar in rabbits. abst. no. 43849r</td>
<td>1</td>
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<tr>
<td>X</td>
<td>N, Chemical Abstracts, Vol. 69, 1968, Worbe, Changes in glucose metabolism following the administration of single doses of L-lysine and L-tyrosine to the rat, abst. no. 104945c</td>
<td>1</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "F" document member of the same patent family

**IV. CERTIFICATION**

Date of the Actual Completion of the International Search: 6 May 1985

Date of Mailing of this International Search Report: 10 May 1985

International Searching Authority: ISA/US

Signature of Authorized Officer: [Signature]
<table>
<thead>
<tr>
<th>X</th>
<th>Chemical Abstracts, Vol. 98, 1983 Mogre, Effect of manganese (2+) on blood sugar level in rats, abst. no. 52301s</th>
</tr>
</thead>
</table>

### VI. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(3) (a) for the following reasons:

1. Claim numbers 1-10, because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim numbers 11-20, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

### VII. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

**Remark on Protest**

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.