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METABOLIC EFFECTS OF MONOMETHYL HYDRAZINE

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13. ABSTRACT The pattern of utilization of glucose specifically labeled with ^{14}C at various carbon atoms by animals under subacute MMH intoxication indicates that MMH produces a profound depression of glycolytic metabolism, possibly associated with failure of insulin release. Functionally, the deficit appears to be located immediately prior to formation of triose phosphate, although assay of enzyme activity shows that pyruvate kinase is also severely inhibited. Blood glucose levels in intoxicated rats with adequate glucose available but low glycogen rise precipitously and remain high for as long as 12 hours. Similar but less extensive increase was induced in absolutely fasted rats. The observed metabolic defects respond to pyridoxine HCl and to insulin therapy.			

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FOREWORD

This study was initiated by the Aerospace Medical Research Laboratory, Aerospace Medical Division, Wright-Patterson AFB, Ohio 45433. The research was performed under contract F 33615-71-C-1516. Drs. F. N. Dost and C. H. Wang were principal investigators for Oregon State University, Corvallis, Oregon 97331. Dr. K. C. Back, Chief, Toxicology Branch, Toxic Hazards Division, was the contract monitor for the Aerospace Medical Research Laboratory. The research included in this report was initiated 1 January 1971 and completed 31 December 1972.

This technical report has been reviewed and is approved.

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INTRODUCTION

The pioneering observations on the effects of hydrazines on carbohydrate metabolism were probably those of Underhill (1911, 1915) and his students, who demonstrated a hypoglycemic effect of hydrazine itself, and attempted to find similar changes after intoxication with monomethylhydrazine (MMH) and other substituted hydrazines (Underhill, 1914). Evidence gathered since has indicated that hydrazine interferes with various components of the gluconeogenic sequence (Izume and Lewis, 1926; Amenta and Johnson, 1963; Amenta and Dominguez, 1965; Fortney, et al., 1967; Ray, et al., 1970).

Hydrazine, on the other hand, has been reported to increase mobilization of liver glycogen in dogs, with concurrent increase in blood glucose (Taylor, 1966). As liver glycogen approached exhaustion, blood glucose concentration dropped below normal, and muscle glycogen loss became marked. Fortney (1966), in more detailed studies, showed that in anesthetized dogs which had been depleted in liver glycogen, the blood glucose did not increase after hydrazine treatment but instead immediately decreased in concentration. Studies by Potter et al. (1969) have shown in rats that single doses of hydrazine in the lethal range and of phenelzine (a hydrazine derivative monoamine oxidase inhibitor) decrease blood glucose and insulin levels in 18 hour fasted rats.

The existing evidence on the effects of alkylhydrazines on carbohydrate metabolism is somewhat erratic, possibly because a variety of preexperimental dietary preparations have been used. Much of the data is based on measurements of blood glucose and liver glycogen. O'Brien et al., (1964) found that acute LD₅₀ doses of hydrazine, MMH and 1,1 dimethylhydrazine (¹¹UDMH) caused immediate hyperglycemia of several hours duration in fed rats. MMH in high acute doses caused transient hypoglycemia and glycogen depletion in anesthetized dogs in studies by Fortney and Clark (1967). These observations were secondary to other work, however, and the time course was not clearly indicated. We have found substantial increases in blood glucose at the end of a ten-hour infusion of 0.043 mmole MMH/kg/hour (Dost et al., 1971a) and have since determined the time course of this change. Bitter et al. (1967) observed a decreased respiratory quotient in fasted rats treated with MMH and nicotinic acid but not with MMH alone, and suggested that this effect was due to decreased glucose utilization. Minard et al. (1965) measured brain glycogen and phosphorylase in fed rats after UDMH-induced convulsions and found that glycogen content in nervous tissue was decreased by the convulsions but not by UDMH itself. Hepatic glycogen also was lowered during this process. Increases in brain glycogen have also been reported after

hydrazine treatment, according to an abstract of work by Musyalkovska, et al. (1969). In rats fasted for 48 hours, Bitter et al. (1967) were unable to further alter glycogen concentration by treatment with MMH at doses of 0.3 and 0.22 mmole/kg.

UDMH at high nonlethal single doses has been shown to decrease oxidation of glycine-1- ^{14}C by rats (Amenta and Dominguez, 1965), and causes decreased oxidation of glucose- ^{14}C (Amenta and Dominguez, 1965; Dost et al., 1971a). Smith (1964) incubated rat brain homogenates with various simple hydrazines and found that MMH has less effect on glucose-1- ^{14}C metabolism than on the metabolism of other glucose labels. Under some conditions MMH stimulated glucose-1- ^{14}C metabolism. The metabolism of γ -aminobutyric acid (GABA), which may represent a significant fraction of total energy metabolism was found to be temporarily delayed by MMH and UDMH, but subsequently proceeded at a normal rate (Dost et al., 1971b). Hydrazine treatment almost completely blocked GABA turnover, however. Those findings and the inability of UDMH to impair glutamic acid metabolism (Reed et al., 1963) indicate that UDMH and MMH do not impair tricarboxylic acid cycle activity.

In probing the effects of hydrazine on glucose and glycogen, Fortney (1966) found increased blood pyruvic and lactic acid concentrations, which he attributed to either increased glucose utilization or depressed glucose synthesis. In part his reasoning was based on the presumed sensitivity of transaminases to hydrazine, and the necessity of transamination to the function of the Cori cycle. He also noted that the initial effects of hydrazine resembled sympathetic stimulation: increased free fatty acid concentrations, lactate accumulation and glycogen depletion. In liver homogenates, 5×10^{-3} - 5×10^{-2} M hydrazine has decreased oxidation of α -ketoglutarate, pyruvate and fumarate, with little effect on succinate or glucose oxidation (Krulik, 1966). For comparison with these concentrations, the LD_{50} of hydrazine for the rat is 2×10^{-3} mole/kg.

Other substituted hydrazines have been shown to affect glucose uptake or catabolism under various conditions. Most of this collateral work is related either to effects of acetyl phenylhydrazine (APH) on red blood cells, or to the mechanism of action of monoamine oxidase inhibitors. The varied structural character of these compounds may or may not make comparison with procarbazine inappropriate, but it appears that at least some of these comparisons are valid.

APH has been found useful in detection of glucose-6-phosphate dehydrogenase (G6PD) deficiency because it causes a massive fall of reduced glutathione (GSH) in erythrocytes deficient in G6PD (Beutler, 1957). GSH levels depend on adequate formation of reduced NADPH, a product of the

G6PD reaction. In G6PD deficient humans, GSH concentrations are lower than normal (Kosower, 1963) but are apparently maintained at a workable level by other reducing systems. APH lowers but does not eliminate GSH in normal cells and in most cases completely eliminates GSH from G6PD deficient cells (Kosower, 1963). Since the only routes of glucose metabolism in red cells are glycolysis and at least a portion of the pentose phosphate pathway, it seems reasonable to assume that the alternate systems to maintain glutathione in a reduced state (or concurrent in normal subjects) are generated by glycolysis. A similar effect on GSH has recently been shown in liver of rats treated with 0.5 mmole/kg hydrazine HCl (Krulik and Brezinova, 1966). The pathway common to both normal and G6PD deficient human cells is glycolysis which is apparently highly vulnerable to APH since all activity in deficient patients ceases.

The effect of APH blockade on transit of specific carbons of glucose in erythrocytes was examined by Szeinberg and Mark's (1961), who found a 6 fold increase in conversion of C-1 of glucose and a 15 fold increase in conversion of C-2 to $^{14}\text{CO}_2$. C-2 conversion is normally only about 15% of C-1 oxidation. Under APH influence not only was an increased flow through the pentose phosphate pathway initiated, but the total recycling of glucose-2- ^{14}C through the pentose pathway was also elevated, presumably because of a blockage prior to formation of fructose-1,6 diphosphate. Kosower (1963) and her associates found evidence that glycolytic activity was in fact reduced by APH, although activities of lactic acid dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were not affected. Mills and Buell (1964) subsequently also supported the evidence that glycolysis was decreased by APH. The generation of NADPH by G6PD is not affected by high concentrations of MMH in vitro. (D. J. Reed, personal communication).

The effect of the MMH derivative procarbazine on carbohydrate metabolism has apparently been the subject of very little study. Mortensen (1965) studied the metabolism of erythrocytes treated with 2.5×10^{-2} M procarbazine and found no change in ATP or GSH levels and no interference with glucose utilization. There was also no change in glucose-6-phosphate dehydrogenase and pyruvate kinase. In limited experiments Gale et al. (1967) found that 2×10^{-4} M procarbazine did not affect glucose utilization of ascites tumor cells, even though DNA synthesis was inhibited. This study employed glucose-1- ^{14}C , however, which may not reflect modest changes in glycolysis if pentose phosphate pathway activity is not impaired. Oxygen consumption of yeast, diaphragm and liver cells also did not change under procarbazine treatment although respiration in glucose-rich yeast cells was depressed. (Romo et al., 1969).

The relationship between hydrazines and insulin activity may be a significant component of the toxicity of the hydrazines. The work of Potter

et al. (1969) has already been alluded to, in which hydrazine and phenelzine were shown to decrease plasma glucose and insulin. Earlier clinical use of both hydrazine-based and nonhydrazine monoamine oxidase inhibitors (MAOI) had caused serious hypoglycemic consequences in some cases, prompting a detailed editorial in the journal Diabetes by Cooper and Ashcroft (1967), and a number of efforts to establish the mechanism of the MAOI induced hypoglycemia.

Chronic administration to rats of hydrazine MAOI (Adnitt, 1968a, Barrett, 1965) and nonhydrazine MAOI (Adnitt, 1968a) increased sensitivity to insulin in glucose tolerance tests. Both studies showed that single doses of the agents could not potentiate insulin effect. Similar evidence has been obtained in human patients treated with mebanazine and phenelzine (Adnitt, 1968b). The mechanism apparently is related to intracellular replacement of norepinephrine by the false transmitter octopamine following MAO inhibition; the suggested result of such replacement will be a diminution of the sympathetic hyperglycemic response (Barrett, 1970). The obvious consequence of this change is the removal of a major potential influence antagonizing insulin hypoglycemia.

In contrast to potentiating the response to insulin, Aleyassine and Lee (1971) have shown that hydrazine can impede secretion of insulin in response to an administered glucose load, and can also inhibit the production of insulin from pancreatic tissue in vitro. The mechanism as they evaluate it also appears to be based on intracellular monoamine oxidase inhibition which results in accumulation of biogenic amines, which are capable of inhibiting insulin release.

Alyessine and Lee (1971) point out that adrenaline is known to abolish in vitro glucose stimulation of insulin release from pancreatic tissue (Coore and Randle, 1964) and quote histochemical evidence that monoamines accumulate in cells of rat and mouse pancreas after administration of MAO inhibitors (Cegrell et al., 1964).

In this report, we present evidence that MMH causes a blockade of glycolysis which appears to be located primarily at the phosphofructokinase step, although other key glycolytic enzymes may also have been inhibited. Pentose phosphate pathway activity is apparently unimpaired, as are triose metabolism and citric acid cycle activity. MMH caused a marked gluconeogenic response, characterized by a substantial rise in blood glucose, even in glycogen depleted rats with no glucose input. Pyridoxine and crystalline insulin are both capable of counteracting the observed metabolic effects. The responses to antidotes, alterations in pathway participation, blood glucose responses and enzymatic changes thus far observed are consistent with failure of insulin release as a consequence of MMH intoxication.

METHODS AND APPROACHES

ANIMALS AND ANIMAL MAINTENANCE

Male Sprague-Dawley rats were used throughout these studies. (Horton Laboratories, Oakland, Calif.; formerly BioScience Laboratories.) Animals were delivered at 200 grams, maintained on Purina Laboratory Chow and water ad libitum to a weight of 280-300 grams at which time they were surgically prepared. Post-surgical animals were maintained on the same diet, in a separate facility.

The experiments comprising this research were conducted according to standards described in "Principles of Laboratory Animal Care" of the National Society for Medical Research.

SURGICAL PREPARATION

Indwelling cannulae were used in almost all metabolic studies of this program. Animals were usually operated at a weight of about 280 grams and were ready for experiment after regaining presurgical weight, usually in five days. Slow weight recovery or other evidence of inadequacy disqualified a prepared animal. Sodium pentobarbital was used for anesthesia.

Several locations for indwelling cannulae have been used routinely. Labeled substrates were usually infused into the upper 1.5 cm of the duodenum, or in some cases into the fundus of the stomach. Unless interfered with by some aspect of the experiment these are the physiological entry routes for glucose and are the only acceptable routes for high rates of substrate infusion. Blood sampling and drug injection cannulae were placed in the dorsal aorta or posterior vena cava. In all cases polyvinyl tubing, size PV2 or PV3 is used.

All cannulae were installed through an abdominal incision, leading from the insertion through the body wall and passing subcutaneously to emerge ahead of the withers. A good deal of slack was left in the tubing throughout its course to prevent pulling. The pliable vinyl tubing causes less discomfort than polyethylene and does not promote clotting as does silicone rubber. The intestinal cannulae were anchored with #4 silk to the wall of the pylorus; gastric cannulae are anchored to the stomach serosa close to the point of insertion. Postcaval and aortic cannulas were stabilized by a ligature on the tube, which is then anchored somewhat forward of the insertion point, in the dorsal retroperitoneal musculature.

Our technique for venous or arterial cannulation is unique in that we can insert a tube in the vena cava or aorta without occluding flow and usually

without blood loss. We find that 70-80 percent of these preparations are successful and many remain open for sampling for a month or more. Blockage to injection is extremely rare.

At present the insertion device must be made by hand. It is simply a curved, atraumatic needle, fitted with a tapered guard formed from a drop of solder just ahead of the eye, in the manner of the hand guard of a medieval lance. The eye is clipped off, leaving a short stub behind the guard. Prior to installation, the cannula is filled with heparin solution and the end of the cannula is sleeved over the stub and moved snugly against the tapered guard. After exposure of the vessel, the needle is inserted into the lumen, and brought out again a few mm up or down stream as desired. The entire cannula is drawn through until the square cut distal end pulls into the vessel. The tube can then be passed along the vessel to any desired sampling point. Leakage from veins usually does not occur; when cannulating an artery, a small piece of Surgicel (Johnson and Johnson Co.) should be held over the entry site as the cannula is pulled into the vessel and held in position for a short time until clotting is established.

It is our practice to suture body wall and skin with #4 silk. This is more time consuming than the use of wound clips, but provides a much more comfortable subject with less wound infection over the extended experimental periods that are required.

RADIORESPIROMETRY

The radiorespirometry system is capable of continuously measuring the $^{14}\text{CO}_2$ output from four animals simultaneously. Animals are held in plastic restraining cages, with the head confined in a "helmet" fashioned of a six ounce plastic bottle. Room air is drawn past the head of each subject at 500 ml/min, dried in a Drierite column and passed through a flow ionization chamber coupled to a Cary Model 31 vibrating reed electrometer. Each air stream then passes through a flow meter, metering valve and pump, and finally is transferred into an exhaust.

The instruments are empirically calibrated with $^{14}\text{CO}_2$ gas obtained commercially and standardized in our laboratory. Air flow rates, which are also critical, are checked by comparison with a standard flow meter which is absolutely calibrated with a constant head displacement bottle.

The data acquisition system scans the four electrometers and feeds the analog information from each into a digital volt meter, the output of which is recorded on punch tape. The tape is translated onto magnetic tape and data conversion carried out by the O.S.U. Computer Center. Conversion from analog to digital form and integration are incorporated in the calculation process.

The sensitivity of the radiorespirometer at usual settings (300 mv input, 500 ml/min airflow) for whole animal studies is such that a continuous input of 1 m μ Ci/min will cause a signal about 10 times background. In practical terms this means that if a single 1 μ Ci dose of a labeled substrate were metabolized over a 1,000 minute period, the average output would be 10 times background. If infused at 0.1 μ Ci/hr, 100% conversion to $^{14}\text{CO}_2$ would provide 150 times the background output. The capacity of the system at usual settings is about twice this output. The range of inputs acceptable to the electrometer range from 1 mv to 30 volts, which means that sensitivity theoretically could be increased 300 fold or decreased 100 fold from the usual range.

RATIONALE OF CONTINUOUS SUBSTRATE INFUSION EXPERIMENTS

A major obstacle in the study of metabolic kinetics in vivo with tracers lies in the physical and metabolic compartmentation of the intact organism. If an animal is injected with a single dose of a substrate which already exists in substantial quantity in the body, an inordinate time must elapse before the exogenous material equilibrates with body pools, and if turnover or excretion is at all active, equilibration may never occur. The observed parameter, whether it be the output of the labeled $^{14}\text{CO}_2$ as a terminal product or isolation of an intermediate, has full meaning only when the entire time course has been run, and integrated data representing the entire experiment are available. The information gained is still compromised, however, because concentrations and reaction rates are continually variable throughout the experiments. No useful information about transport or transformation rates can be derived unless distribution is instantaneous. To overcome this disadvantage we have adopted the procedure of continuous infusion of substrate to provide an extended period in which a metabolic steady state with respect to infused substrate may prevail.

When glucose- ^{14}C is infused intravenously or intraintraintestinally at 150 mg/hr to a 300 gm rat, a period of up to 6 hours of infusion is required before the steady state is established. If unlabeled glucose is infused instead at the same rate for six hours and then labeled glucose is added while the glucose flow continues, the same period is still required to reach isotopic equilibrium. The sequence is no faster if trace levels of glucose only are infused.

Substrates such as sodium acetate- ^{14}C or L-alanine-1- ^{14}C require very little time for equilibration, presumably because the residual amount of each in intra- and extra-cellular spaces is limited.

The nature of the prolonged metabolic equilibrium period for glucose should not be confused with equilibria related to more directly observable parameters, such as the immediate elevation of blood glucose after refeeding or infusion. In a matter of minutes circulating glucose rises, and presumably

the fraction of energy metabolism attributable to glucose in a normal animal rises almost as quickly. These events have no relation to pool distribution of isotope because exogenous unlabeled glucose cannot be distinguished from endogenous glucose. Overall availability increases immediately, through factors as simple in some cases as mass action.

Whether the time required to achieve equilibrium reflects in part the time required for metabolic degradation of the molecule is unknown. We can demonstrate that the equilibrium time for glucose-3,4- ^{14}C is shorter than for other glucose labels, which should be expected if decarboxylation of pyruvate occurs appreciably before citric acid cycle oxidation. Significance of this difference is not our present concern however.

Though it takes an extended period to distribute and metabolize a complex substrate, any acute depression in metabolism is quickly reflected in decreased $^{14}\text{CO}_2$ output, if it occurs after equilibrium has been achieved. The reason for this is that the entire reaction sequence, represented by the labeled substrate and its products, is proceeding at the same governed rate, and allowing for loss into anabolic processes, an interference at any point will be reflected in immediately decreased delivery of intermediates to subsequent steps. We are often able to see, even with so well protected a system as glucose catabolism, immediate effects of large acute doses of intoxicant administered during the equilibrium state. Other more sensitive parameters may respond to minute degrees of intoxication almost instantaneously.

When intoxication has been established prior to metabolic observation, the steady-state output provides a direct index of the rate of turnover of the substrate, for comparison with that of normal animals.

In most radiorespirometric experiments reported here, labeled substrate was infused continuously through a cannula in the small intestine over a 20-hour period, usually beginning four hours after infusion of the intoxicant was started. The time schedule of such experiments was: T = 0, start MMH; T = 4 hr., start substrate; T = 14 hr., stop MMH; T = 24 hr., stop substrate.

Glucose- ^{14}C was infused at a rate of 150 mg/hr., approximately equivalent to the caloric demands of the rats under study. Substrates which are not present at high concentration in the circulation or other pools were infused at low concentration and accompanied by 150 mg glucose/hr. The usual substrate infusion volume was about 1.5 mg/hour. Infusions were driven by a Harvard 600-2-200 variable speed syringe pump, or by a similar pump fabricated in the OSU Department of Physics Instrument Shop, driven by a Harvard power unit. The usual rate of MMH administration was 0.036 mMole MMH in 0.1-0.5 ml/hour, with precise dose rate adjusted by dilution.

BLOOD GLUCOSE

Determinations of glucose in 10 μ l plasma samples were made with a Beckman glucose analyzer. Blood was usually drawn from an indwelling cannula in the posterior vena cava. The procedure used enabled almost unlimited sampling from each rat; 50-60 samples were obtained from each animal over a course of several hours. Each cannula had a known volume and was refilled with heparin with a microsyringe, taking care to fill the cannula but not to inject significant amounts of heparin into the circulation. The tube was closed with a blunted common pin and was clamped during attachment of the syringe with a fine forceps shod with rubber tubing. When sampling, the heparin was withdrawn until undiluted blood appeared, 30-40 μ l blood was removed, and the heparin replaced. Blood was placed in a centrifuge tube formed from 1/2 of a microhematocrit tube with the end flame sealed; the tube was placed in a small plastic centrifuge tube of a Beckman Microfuge, spun for one minute, the hematocrit measured and 10 μ l of plasma removed for glucose assay.

The cycling time of the glucose analyzer is about one minute, allowing observation of rapid changes in blood glucose and if necessary in an experiment it is possible to respond almost immediately to these observed changes.

CHEMICALS AND RADIOCHEMICALS

^{14}C -labeled substrates were obtained from New England Nuclear Corp., Boston, Mass. except for fructose-6- ^{14}C , which was purchased from Nuclear Research Chemicals Inc., Orlando, Florida.

Stated specific activities at receipt in mCi/mMole were: glucose-1- ^{14}C , 6.42; glucose-2- ^{14}C , 2.36; glucose-3,4- ^{14}C , 13.8; fructose-6- ^{14}C , 1.16; glycerol-2- ^{14}C , 16.1; Na pyruvate-2- ^{14}C , 8.1; Na acetate-1- ^{14}C , 2.0; Na acetate-2- ^{14}C , 2.0.

Radioactivity of labeled substrates was standardized by liquid scintillation counting. A measured volume (usually 10 μ l) of substrate solution was placed in 7 ml of ethanol-ethanolamine (2:1, v/v) and 10 ml toluene containing 0.3% (w/v) terphenyl and 0.003% (w/v) POPOP (1,4-bis-2(5-phenyl oxazole)-1 benzene). The samples so prepared were counted in a liquid scintillation spectrometer (model 314 EX-2, Packard Instrument Company, La Grange, Illinois). A sufficient number of counts were collected to ensure that the relative standard deviation of the counting rate was no greater than 2 per cent. Counting efficiency was established by internal standardization.

Monomethylhydrazine (MMH), BP 97-98 was obtained from Matheson, Coleman and Bell Division of Matheson Co., E. Rutherford, N.J. MMH was

diluted before each use in glass distilled water equilibrated with room air to pH 5.5. Injection volume was adjusted to less than 0.5 ml/hr.

ENZYME ASSAYS

Activities of glucokinase, pyruvate kinase and hexokinase were measured in several animals which had been continuously infused with 0.036 mMole MMH/kg/hr for 13 hours and with 150 mg glucose/hr for 9 hours.

Glucokinase was measured by the method of DiPietro and Weinhouse (1960). The glucokinase and/or hexokinase phosphorylation of glucose is coupled in this assay to the oxidation of glucose-6-phosphate formed in the reaction and simultaneous reduction of NADP^+ to NADPH by glucose-6-phosphate dehydrogenase. Because the K_m for glucokinase is about 10^{-2}M glucose and is about 10^{-4}M glucose for hexokinase, determination in the presence of high glucose concentration will provide the total activity of both enzymes. At low glucose concentration only hexokinase is appreciably active, and glucokinase activity is obtained by difference. Activity is proportional to NADPH formation and is expressed as change in $\text{OD}_{340}/\text{mg protein/minute}$, during the initial stages of the reaction.

Pyruvate kinase activity was measured according to Weber et al., (1965). The common naming for this enzyme refers to the reverse reaction, which is not of physiological significance; in glycolysis ADP is the component phosphorylated. The reaction forms pyruvate from phosphoenol-pyruvate, and in vitro the subsequent lactic dehydrogenase reaction with accompanying oxidation of NADH_2 provides the measurement index. In this case disappearance of NADH_2 is expressed as decrease in $\text{OD}_{340}/\text{mg protein/minute}$.

Results of each series of assays were analyzed by application of a one-tailed 't' test to ascertain differences between data from treated and untreated animals.

RESULTS

GLUCOSE CATABOLISM DURING ACUTE MMH INTOXICATION

Figure 1* shows the effect of acute severe MMH intoxication upon the catabolism of glucose- $\mu\text{-}^{14}\text{C}$. Similar effects have been described in earlier reports; this figure is included for comparison with subacute experiments to be discussed. In each experiment, the animal illustrated survived the intoxicant dose while a companion subject treated identically at the same time died. The labeled glucose was continuously infused intraintestinally

*Figures are located on pages 22-27.

at a rate of 150 mg/hour; when the metabolic steady state was reached after about seven hours of infusion the rats were given a single intraperitoneal injection of 0.45 mmole MMH/kg. The substrate infusion was continued for several additional hours. In both cases the emergence of $^{14}\text{CO}_2$ from labeled glucose fluctuated sharply after intoxication, then decreased substantially and remained low through the remaining hour of glucose infusion. Under such vigorous acute treatment, we have found depression in catabolism of all carbons of glucose but the great variation which characterizes these experiments with acute doses prohibits a quantitative evaluation of specific effect upon the catabolism of individual labeled carbon atoms.

Such acute experiments as these are of some value, but the high dose required causes difficulty in distinguishing specific effects from general damage due to massive intoxication. In the cases illustrated the survival of the animals provided some assurance that the damage was confined to a few systems, including that represented by the observed data.

Under similar acute intoxication no decrease in $^{14}\text{CO}_2$ production was found in animals infused with sodium acetate-1- or -2- ^{14}C , indicating that MMH caused little or no interference with activity of the citric acid cycle. Earlier studies, in which metabolism of single doses of glutamic acid- ^{14}C were unaffected by the alkylhydrazines also suggested that the citric acid cycle, as well as glutamate transaminase, was not vulnerable to survival doses of MMH.

These limited observations suggested that the alteration of glucose catabolism caused by MMH would probably involve changes in the glycolysis sequence, and the studies discussed in the next section were directed toward identifying such changes.

CATABOLISM OF GLUCOSE- ^{14}C AND OTHER LABELED SUBSTRATES IN RATS DURING SUBACUTE MMH INTOXICATION

In the usual procedure, MMH infusion began four hours prior to beginning of the substrate infusion. The dose rate in a typical experiment was 0.036 mMole MMH/kg/hr, or 0.5 mMole during the usual 14 hours of infusion. Infusion of substrate proceeded for 20 hours, continuing 10 hours beyond the end of MMH administration. This schedule allowed observation of the early effects of lower doses, the increasing effect of cumulated toxicity, acute episodes, and a substantial period for observing a recovery phase if it is to occur. This approach also provides much more reproducible data than that arising from acute experiments.

Treatment of rats with 0.036 mmole of MMH/kg/hr for 14 hours caused a sharply diminished turnover of glucose-6- ^{14}C (Fig. 2, 3, 4), which reached a maximum after the end of MMH infusion. Glucose-2- ^{14}C (Fig. 5) and -3,4- ^{14}C (Fig. 6) were similarly impeded. Oxidation of glucose-1- ^{14}C was much less depressed however (Fig. 7), with an apparent minor depression late in the experiment. In the experiments with glucose-6- ^{14}C (Fig. 2, 3, 4) and glucose-1- ^{14}C (Fig. 7, 8) the data from intoxicated animals have been presented both as averages and as groups of individuals to illustrate the character of the data and the degree of reproducibility which can be expected in treated and untreated animals. It is evident in these experiments that MMH causes major fluctuation in $^{14}\text{CO}_2$ output even when the average rate of turnover is not depressed. The same oscillations in output are also characteristic of treated animals utilizing other metabolic substrates, regardless of the presence or absence of depressed metabolism (see Fig. 12, 13). Observation of total CO_2 output over the course of these experiments showed in most cases a series of sharp increases, during the period of $^{14}\text{CO}_2$ variation in which CO_2 output rose in some cases to three times normal levels over a 10-20 minute period. We have not been able to establish a precise correlation in time with variations in $^{14}\text{CO}_2$ output or with preconvulsive or convulsive activity, but at present we conclude that the sharp variations in $^{14}\text{CO}_2$ output are related to general respiratory activity.

The available data indicate that turnover of fructose-6- ^{14}C is much less depressed (Fig. 9) than that of glucose-6- ^{14}C (Fig. 2). The data in figure 9 describe experiments at very low levels of fructose, accompanied by 150 mg glucose/hour. At higher levels of fructose (50 mg/hr with 100 mg glucose/hour) the relationship was similar except that some intoxicated animals died 6-7 hours after the beginning of substrate infusion, and those which died were apparently converting fructose- ^{14}C at a higher rate than did the surviving intoxicated rats. The metabolism of glycerol-2- ^{14}C , which may be expected to enter the glycolytic scheme as glyceraldehyde-3-phosphate, was severely inhibited by MMH (Fig. 10).

Sodium pyruvate-2- ^{14}C , which is decarboxylated to acetate and is subsequently catabolized in the TCA cycle, was metabolized at a normal rate (Fig. 11) indicating that neither the loss of carbon-1 of pyruvate nor entry of acetate into the TCA cycle was impeded. A similar absence of effect on sodium acetate-1- ^{14}C (Fig. 12, 13), confirmed that the effect of MMH is not subsequent to pyruvate formation. The metabolism of acetate-2- ^{14}C is also unaffected by MMH.

We have attempted to observe the effect of MMH upon metabolism of long and short chain fatty acid metabolism. In these experiments, the labeled substrate was usually introduced at a chemical level of about 10 mg/hr., and accompanied by 150 mg glucose/hour or an isocaloric amount of vegetable oil. The observations were inconclusive in part because the animals were not acclimated to a specific dietary lipid level for some time prior to experiment.

EFFECTS OF MMH ON REGULATION OF BLOOD GLUCOSE

We have recently begun observations of changes in blood glucose during extended intoxication with the hydrazines, with particular reference to availability of exogenous glucose and/or glycogen. In some cases these changes are very rapid, and appear to be associated with convulsive or preconvulsive activity. These data have been correlated in some cases with simultaneous observation of $^{14}\text{CO}_2$ output from labeled glucose. The experiments completed thus far suggest that careful use of this rather simple parameter may contribute effectively to our studies.

In the example shown in Fig. 14 and 15, the animal received 150 mg glucose-6- ^{14}C /hr, and MMH was introduced over a seven hour period at a rate of 0.05 mmole/kg/hr, beginning after equilibration of $^{14}\text{CO}_2$ output. Blood glucose concentration began to rise 3 hours after MMH was started and rose steadily for about 4 hours until administration of MMH was terminated at the time of the first convulsion. Blood glucose then climbed abruptly to 280-290 mg/100 ml. (Fig. 14). The blood glucose level at the onset of convulsions was about 170 mg/100 ml and remained above that level for nearly 4 hours. The entire hyperglycemic episode was evident for more than eight hours.

It appears that the beginning of the very fast rise in blood glucose and the end of the MMH infusion (or onset of convulsions) are coincidental, but evidence relating to this relationship is as yet lacking. Preconvulsive behavior was evident about an hour before convulsions and about two hours after blood glucose had obviously begun to rise.

A log plot of blood glucose data was made to learn whether the entire rising phase would translate into a plot with a single slope. The objective was to find whether convulsive activity occurred at some point during a sustained rate of increase, and could thereby be considered tentatively as dependent on that change, rather than being responsible for a change in the rate of increase. The latter relationship could result from as straightforward an effect as massive epinephrine release. This plot provided grounds for both possibilities (Fig. 15), with an apparent constant rate of change until convulsion, and an apparent major breakdown of regulation following the convulsion.

For comparison, $^{14}\text{CO}_2$ production by a rat treated identically is also shown in Fig. 14. It is evident that a few hours after the end of MMH infusion $^{14}\text{CO}_2$ production from glucose-6- ^{14}C was about 1/2 of that found in control experiments.

In experiments such as that illustrated in Figure 14 the output of $^{14}\text{CO}_2$ did not rise toward normal rates after MMH was ended, unlike the experiments shown in Figures 2, 5, 6 and 7. The principal difference lies in the timing of MMH administration; MMH was started long after the metabolic equilibrium was established in the former case, and was started before glucose infusion in the radiorespirometric experiments of Figure 2, 5, 6 and 7. This difference in behavior was characteristic of all similar experiments. The MMH dose rate was also about 50% higher in the experiments exemplified by that in Figure 14. The mechanism of the difference in response is not understood.

In most experiments in this series, a glucose infusion rate of 150 mg/hr has been maintained throughout. In an initially fasted animal this input provides approximately the entire theoretical glucose requirement and will not cause appreciable glycogen deposition (unpublished observations, this laboratory). With this adequate supply, a source for the excessive circulating glucose pool is easy to postulate.

When the experiment was carried out with 36 hour fasted rats and with glucose- $\text{U-}^{14}\text{C}$ infused at trace levels only, blood glucose again rose sharply, though to a lesser degree (Fig. 16). Although not shown in Fig. 16, the oxidation of glucose to $^{14}\text{CO}_2$ was also again modestly depressed as the blood glucose level rose.

ALTERATION IN GLYCOLYTIC ENZYME ACTIVITY BY MMH

Subacute treatment with MMH caused a significant decrease in the activity of pyruvate kinase, glucokinase and hexokinase (Table 1). Studies of phosphofructokinase and fructose diphosphatase have been initiated but a number of difficulties have appeared in translation of published methodology into effective analyses.

Table 1

Effect of subacute MMH (0.036 mMole/kg/hr for 13 hours prior to sacrifice) upon glycolytic enzyme activities. Glucose (150 mg/rat/hour) infused intraintestinally for 9 hours prior to sacrifice.

	$\Delta\text{OD}_{340}/\text{mg protein}/\text{min.}$		Diff.	% Δ	P
	Control	MMH Treated			
Pyruvate Kinase	2.84 $\pm$ 0.35 (N = 7)	1.65 $\pm$ 0.58 (N = 6)	1.19	-34%	> 0.99
Glucokinase	0.146 $\pm$ 0.054 (N = 6)	0.099 $\pm$ 0.036 (N = 6)	0.047	-32%	> 0.90
Hexokinase	0.134 $\pm$ 0.034 (N = 7)	0.069 $\pm$ 0.035 (N = 6)	0.065	-49%	> 0.975

POTENTIAL ANTIDOTAL THERAPY

In seeking the mechanism of the dramatic changes we have observed in blood glucose and in turnover of labeled glucose, we have treated some animals with two prospective antidotes on the basis of previous exploratory experiments. We observed during earlier work that pyridoxine, which is an established life saving antidote for MMH intoxication in the rat, will partially reverse the effect of acute MMH intoxication on glucose- ^{14}C catabolism. We have also found that pyridoxine will antagonize MMH interference with glycogen mobilization. In non-intoxicated animals, we have observed that insulin can cause increased turnover of labeled glucose, which is not a surprising finding. More to the point of the present experiments, the massive and sustained elevations in blood glucose due to subacute MMH have demanded that the possibilities of inhibited insulin release and inhibited target response be examined.

Pyridoxine HCl has proven to be effective in reversing both decreased oxidation of glucose- ^{14}C (Fig. 17) and the increased blood glucose caused by MMH (Fig. 18). Radiorespirometric experiments with glucose-6- ^{14}C , which is subject to a more intense depression than other labeled glucoses, indicate that 3 mg pyridoxine HCl/kg/hr administered with the glucose infusion enabled an almost normal rate of turnover. When pyridoxine administration was started four hours after glucose infusion was begun, an intermediate corrective effect was obtained. The results of this treatment are compared in Fig. 17 with the glucose-6- ^{14}C data from control and treated animals which was previously shown in Fig. 2.

The pyridoxine effect upon MMH hyperglycemia was also substantial. In the two cases illustrated in Fig. 18, B_6 was administered to each rat about 30 minutes after the initial seizure; the hyperglycemic effect of MMH usually has begun to develop by this time. In one of the rats in this experiment the incipient hyperglycemia was arrested and the blood glucose concentration promptly dropped. In the other, in which the tendency to increase blood glucose was apparently delayed, no appreciable hyperglycemia developed.

The employment of insulin in these experiments is of possibly even greater significance. When MMH was administered at a rate of 0.1 mmole/kg/hr, for three hours, a pattern of blood glucose elevation emerged which was similar to that already discussed. When 0.05 units of crystalline insulin was administered intravenously near the peak of glucose concentration a precipitous, but brief, drop in blood glucose occurred (Fig. 19). Successive doses caused a similar response. Not only were the decreases after individual doses very rapid, but when compared with the non-insulin-treated animal the recovery phase was somewhat foreshortened. In one similar experiment in which insulin was initiated at a slightly earlier point, a good response developed initially but subsequent treatment required increasingly larger doses for much less dramatic effect.

We have been unsuccessful, thus far, in preventing death or convulsions with insulin therapy, but the timing and dosage of this treatment deserve a more thorough investigation.

DISCUSSION

MMH has been shown to depress catabolism of glucose labeled in the -2-, -3, 4-, and -6- positions, but causes only a modest decrease in turnover of carbon-1 of glucose. The metabolism of fructose-1-¹⁴C is slightly obstructed and the metabolism of pyruvate-2-¹⁴C, acetate-1- and -2-¹⁴C, and butyric acid-1-¹⁴C appear to proceed at a normal rate after MMH treatment. MMH also causes severe inhibition of metabolism of glycerol-2-¹⁴C, but this effect apparently occurs prior to entry into the triose pool and is not a function of impaired glycolytic activity.

MMH also causes spectacular increases in blood glucose in animals which have an adequate carbohydrate supply and substantial increases in 36 hour fasted animals with no glycogen and no glucose input. The hyperglycemia resulting from MMH is responsive to both pyridoxine and insulin therapy. The defect in glucose oxidation can apparently be corrected by pyridoxine therapy; insulin as an antidote has not been fully examined.

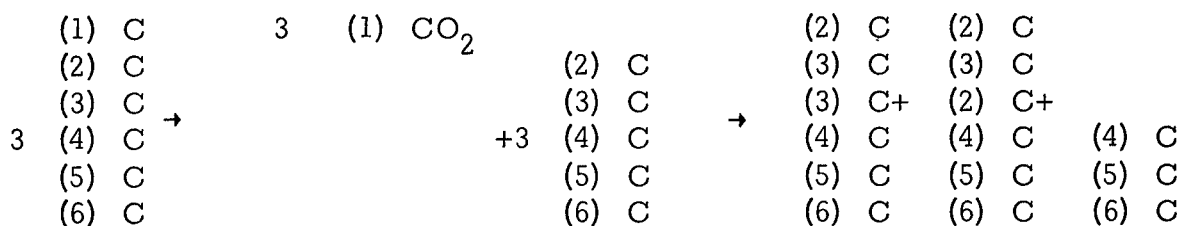
The tentative conclusion to be drawn from this information is that MMH ultimately causes a blockage in glycolysis at the phosphohexose isomerase or phosphofructokinase steps. The response to both pyridoxine and insulin suggest that inhibition of insulin release may be primary to the block in glycolysis. The effect on insulin may in turn depend on monamine oxidase inhibition and derangement of adrenergic activity in the pancreatic cell (Aleyassine and Lee, 1971). Much confirmatory evidence is needed to firmly establish this sequence of events, but the existing data is consistent with the contention of impaired insulin release.

The interference with turnover of carbons 2 through 6 shows clearly that a major blockade exists, and the unimpaired metabolism of pyruvate and acetate show that the citric acid cycle is not affected. The relatively free passage of the first glucose carbon suggests that at least the initial reactions of the pentose cycle are operative. Fructose-6-¹⁴C, which is converted to glyceraldehyde and dihydroxyacetone by fructokinase without mediation of the initial steps of glycolysis, (Sillero *et al.*, 1969), is converted to ¹⁴CO₂ with little change, locating the defect somewhere in the hexose component of glycolysis.

The pentose phosphate pathway, which apparently turns over most of the glucose-1-¹⁴C in an intoxicated animal, normally carries only a small

part of glucose metabolic traffic in most organs. In erythrocytes this pathway appears to have a much greater capacity when other routes are obstructed (Szeinberg and Marks, 1951), and the same adjustment may occur in liver and other tissues.

The pentose pathway will produce, for each three entering glucose molecules, three molecules of CO_2 , two of fructose-6-phosphate and one of glyceraldehyde-3-phosphate. The activity of the pentose pathway, and the labeling pattern of glucose processed by it, is important in understanding the observations we have made. The labeling distribution may be summarized in this diagram:



Note particularly that the two hexose molecules formed in the pentose pathway retain carbons 4, 5 and 6 in their original positions, and that carbons 1, 2, and 3 of the newly formed hexoses, the first three carbons, are assembled from the 2 and 3 carbons of the three parent glucose molecules. Carbons 4, 5 and 6 of the "third" glucose form glyceraldehyde. As such, it is the only carbon 6 of the three parent glucose molecules that can emerge as CO_2 without passage through the phosphofructokinase step of glycolysis. Of the other labels used, carbon 2 will recycle in the pentose cycle to emerge as the first glucose carbon in the two new hexose molecules, and half of the -3,4-label used will emerge as the first carbon in succeeding cycles.

Quantitatively, we should expect that in impairment of hexose glycolysis, turnover of glucose-6- ^{14}C would be inhibited to the greatest extent because it will be converted to CO_2 through pentose cycle activity to the least extent. The difference from normal therefore represents almost the total degree of inhibition, but of course, gives no information about the location of the blockade. Glucose-3,4 metabolism should be somewhat less inhibited because carbon 3 will emerge as pentose cycle $^{14}\text{CO}_2$ during subsequent turns of the cycle, while carbon 4 can only emerge as glyceraldehyde at 1/3 of that rate. Carbon 2 emerges through glycolysis to the same extent as carbon 6 plus recycling through the pentose cycle. As a result we should see inhibition of turnover of various glucose carbons in the order $6 > 3, 4 > 2 > 1$ which is demonstrated in Fig. 2-5. Under normal conditions of metabolic demand, the recycled hexose enters the glycolytic scheme as fructose-6-phosphate and proceeds to fructose-1,6 diphosphate and is then split to triose. If

glucose is in excess the molecule may recycle through glucose-6-phosphate to be incorporated in the glucose pool or in liver glycogen.

If glycolysis cannot proceed effectively, the result should be accumulation of glucose derived both from exogenous glucose and probably by reverse flow of glycolytic intermediates. The observed consequences of glucose catabolic interference by MMH intoxication are consistent with blockade of this kind. It is perhaps useful to compare interference at the hexose phase of glycolysis with impairment of triose metabolism. If blockage of triose metabolism did occur it would no doubt interfere with oxidation of all carbons of glucose, as occurred in studies of the metabolic effects of fluoride intoxication in this laboratory. In that work utilization of all glucose labels, including carbon-1, was depressed in intact surviving animals when tissue fluoride concentrations (measured as ^{18}F) were equivalent to those reported to block the enolase reaction *in vitro* (Knaus *et al.*, 1971). If the fluoride effect on glucose catabolism was indeed at enolase, the differentiation we observe further indicates that MMH must be effective at some point early in glycolysis.

The absence of MMH effect upon the catabolism of labeled fructose is direct evidence that triose metabolism was not disordered. There is apparently a high capacity for the reaction of fructose through fructokinase, and of fructose-6-phosphate through aldolase to form dihydroxyacetone and glyceraldehyde-3-phosphate. The rate of $^{14}\text{CO}_2$ production from fructose-6- ^{14}C in untreated animals was similar at trace concentrations when accompanied by 150 mg glucose per hour, and at an infusion rate of 50 mg per hour accompanied by 100 mg glucose. The normal catabolic rate for fructose in treated rats also implies that there is little "leakage" through the fructaldolase reaction of fructose-6-phosphate formed by isomerization of glucose-6-phosphate.

The repeatable and rapid response of MMH hyperglycemia to small doses of intravenous crystalline insulin suggests that insulin was not released during the high glucose phase, and that target organ response was not impaired. This supposition is supported by Aleyassine and Lee's finding (1971), that hydrazine interferes with insulin release.

It is constructive to review some of the metabolic effects of insulin in the context of observed MMH effects. Insulin depresses the activity of fructose diphosphatase (FDP), a key gluconeogenic regulating enzyme. High activity of FDP results in suppression of glucokinase and phosphofructokinase activity in the glycolytic sequence. In the absence of insulin, fructose diphosphatase will predominate, the glycolytic enzymes will become less active, and the reaction equilibrium will favor formation of glucose. The increased blood glucose concentration in MMH intoxication probably arises

partly from glucose synthesized as a result of this relationship. An absence of insulin created by administering anti-insulin serum (AIS) also increases hepatic gluconeogenesis (Stern et al., 1963). In considering the hyperglycemic effect of MMH in strictly fasted animals, we must assume that MMH accelerates glyconeogenesis.

The key glycolytic enzyme glucokinase is known to be depressed in diabetes, and the concentration of glucose-6-phosphate increases. In assays of the key glycolytic enzymes in liver homogenates from rats administered 0.036 mMole MMH/kg/hr for 13 hours, we find that glucokinase was inhibited by about 30%. Hexokinase and pyruvate kinase activity was decreased to an even greater extent. The substantial depression of pyruvate kinase in intoxicated animals may appear paradoxical in view of our contention that functional integrity of triose metabolism was not changed. We find that pyruvate kinase activity in the liver is much greater than that of glucokinase or phosphofructokinase, an observation previously reported by Weber et al., (1967). This relationship alone may be sufficient to explain that a roughly equivalent degree of inhibition would be most visible in the system with the lowest capacity. In addition, however, the relation of each key glycolytic enzyme to its gluconeogenic antagonist must be considered. According to Weber (1967), the gluconeogenic enzyme glucose-6-phosphatase possesses nearly seven times more activity in the liver than glucokinase, and similarly fructose 1,6-diphosphatase is 4 times more active than phosphofructokinase. In each case this relationship would accentuate or add to the inhibition of glycolysis. Conversely, the activity of pyruvate kinase is some 25 times greater than the two systems it is paired against, phosphoenol pyruvate carboxylase and pyruvate carboxylase. MMH added to liver homogenates from both treated and untreated animals caused no depression, and possibly increased glucokinase activity. This effect suggests that the observed inhibition is secondary to a regulatory change at some site removed from the liver.

The circumstantial evidence that MMH induced impaired insulin release is supported by the reasoning employed by Aleyassine and Lee (1971). They found that hydrazine directly interfered with insulin release from pancreas tissue in vitro, and that the monoamine oxidase inhibitors, phenelzine and pargyline (not hydrazine-derived), exerted a similar effect. Reference was made to the finding of Coore and Randle (1964) that adrenaline will also inhibit insulin release and that amine accumulation in pancreatic beta cells has been demonstrated histochemically following monoamine oxidase inhibition (Cegrell, et al., 1964). On the basis of that evidence, Aleyassine and Lee rationalize that intracellular accumulation of biogenic amines mediates the restraint of insulin release.

It must be remembered that the events we have observed are based on whole animal experiments. Some characteristics of individual tissues, especially with respect to insulin function, should also be considered.

Since liver cells are freely permeable to glucose a deficiency of insulin will not interfere with the accessibility of substrate, but will cause metabolic derangements, some of which have been identified above. The sum of these effects should result in return of re-formed glucose to the circulation. The brain may also possibly contribute to the process since it too can accept glucose without the transport-mediating effect of insulin. The predominant catabolic pathway of brain tissue is disputed but since alloxan diabetes causes more depression of hepatic than of intestinal glycolytic enzymes, (Tyrell and Anderson, 1971), we must then wonder whether glycolysis in the brain is more or less sensitive to loss of insulin. Insulin does not regulate metabolism in muscle as it does in liver, but the absence of insulin-facilitated glucose entry must contribute to the hyperglycemia.

To more firmly establish the suggested mechanism of intoxication, several steps should be undertaken. Direct measurement of insulin in circulation during these experiments is obligatory. Free fatty acids, which are known to rise in the absence of insulin, and known to influence both glycolytic and citric acid cycle activity, must also be measured. We have preliminary evidence that the respiratory quotient falls abruptly after acute MMH intoxication, suggesting an increased mobilization and utilization of free fatty acids, but there is no direct evidence in this area. Blood glucose specific activity should be determined at the peak of hyperglycemia after administration of glucose-1-, -2-, and -6-¹⁴C to test the assumptions about pentose cycle activity discussed earlier in this section. It also appears of considerable interest to evaluate the effects of alpha and beta adrenergic blockade, and of insulin releasers such as tolbutamide. If the premise of a greatly accelerated pentose phosphate pathway is to be accepted, an accounting must be made of the presumed excesses of NADPH, particularly at a time when the consumption, rather than the formation, of fatty acids must be increasing.

These experiments may also shed some light on the nature of the pentose phosphate pathway. Proposals have been made that glucose can ultimately undergo complete oxidation by this route, without entry into the citric acid cycle, but the accepted scheme does not account for randomization of carbons 4, 5 and 6 which could only emerge into the triose pool as glyceraldehyde-3-phosphate. This outlet accounts for only one-half mole of glucose for every three moles entering the cycle. Even though it is improbable that the collateral pathway can replace glycolysis, it may comprise a major safety valve, moving at least some carbohydrate based substrate into energy production and temporarily storing substrate as blood glucose and glycogen during the period in which fatty acid mobilization can be initiated. Unless other energy sources were established the investment of ATP for glucose phosphorylation would not be recovered at the level of the pentose phosphate pathway, and unless the NADPH formed could be efficiently coupled to ATP production, other energy requirements would not be met.

With all of these mechanistic questions a very fundamental additional question has to be asked: Is impairment in carbohydrate metabolism responsible for central nervous disturbance in hydrazine derivative intoxication, or does the disturbance result from a direct pharmacologic interaction?

FIGURE 1
EFFECT OF ACUTE PPH (0.15 MG/KG) ON OXIDATION OF
GLUCOSE- $U^{14}C$ INFUSED CONTINUOUSLY TO RATS AT 150 MG/HOUR

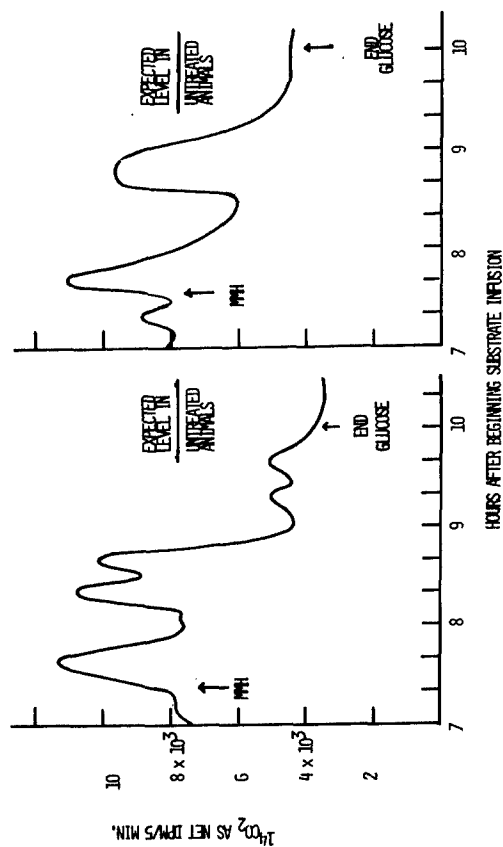


FIGURE 2
EFFECT OF 0.035 MG/KG/HOUR x 14 HOURS ON CATABOLISM OF
GLUCOSE- $6^{14}C$ INFUSED INTRAVENOUSLY TO RATS AT 150 MG/HOUR
DATA FROM INDIVIDUAL TREATED AND CONTROL
ANIMALS SHOWN IN FIGURE 3 AND FIGURE 4

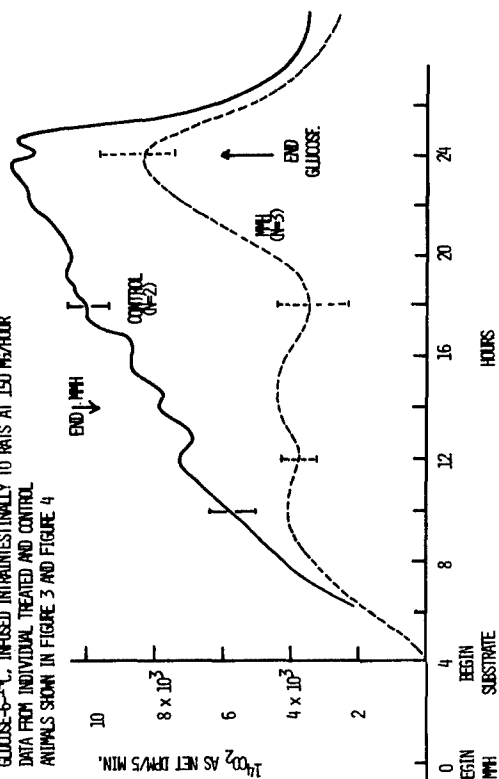


FIGURE 3
CATABOLISM OF GLUCOSE- $6^{14}C$ INFUSED INTRAVENOUSLY
AT 150 MG/HOUR TO TWO NON-INTOXICATED RATS

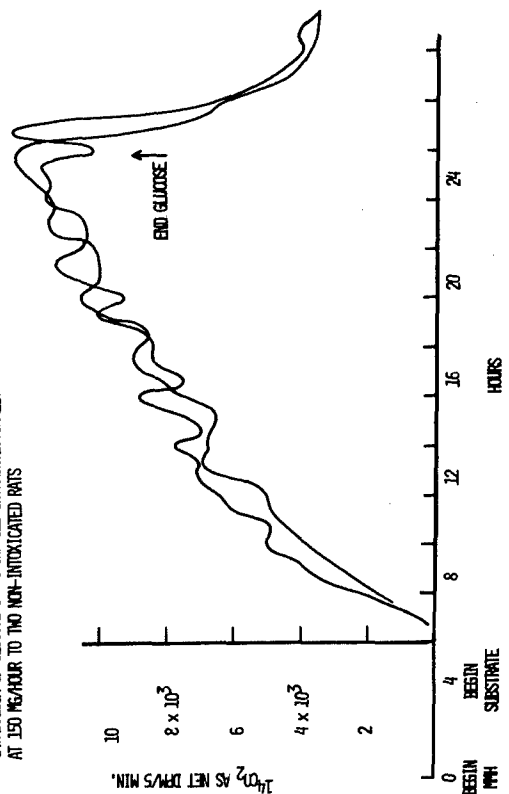


FIGURE 4
EFFECT OF 0.035 MG/KG/HOUR x 14 HOURS ON CATABOLISM
OF GLUCOSE- $6^{14}C$ INFUSED INTRAVENOUSLY AT 150 MG/HOUR

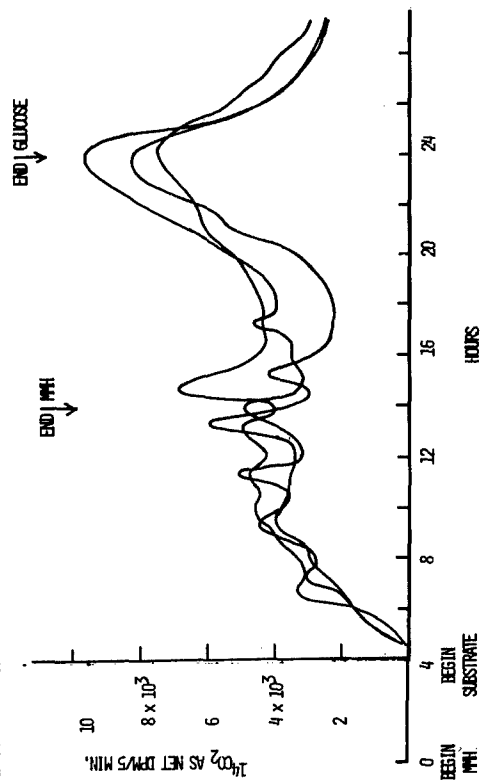


FIGURE 5
EFFECT OF 0.036 WHOLE MM/AG/HOUR x 14 HOURS ON CATABOLISM OF
GLUCOSE-2-¹⁴C, INFUSED INTRAPERITONEALLY TO RATS AT 150 MG/HOUR

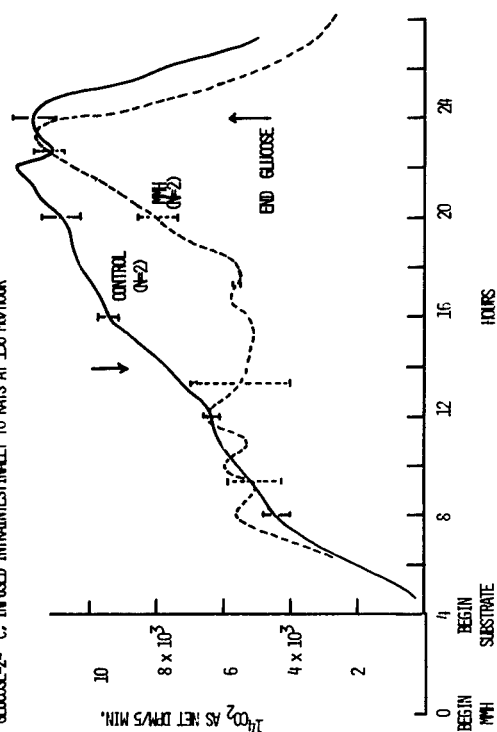


FIGURE 6
EFFECT OF 0.036 WHOLE MM/AG/HOUR x 14 HOURS ON CATABOLISM OF
GLUCOSE-3,4-¹⁴C, INFUSED INTRAPERITONEALLY AT 150 MG/HOUR

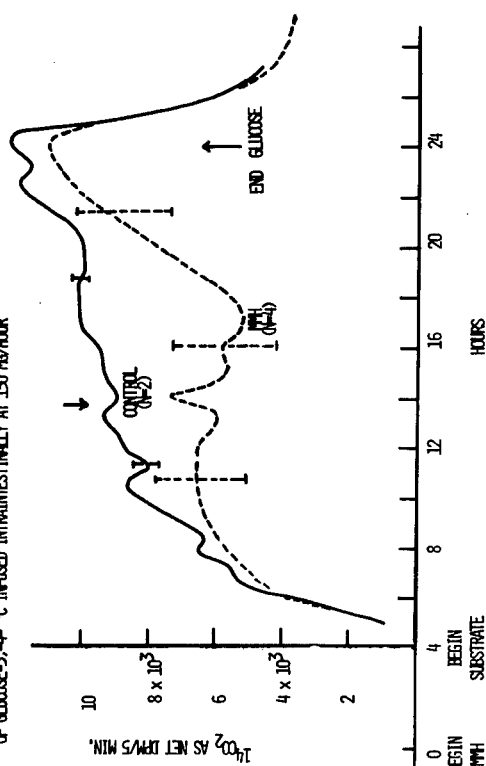


FIGURE 7
EFFECT OF 0.036 WHOLE MM/AG/HOUR x 14 HOURS ON CATABOLISM OF
GLUCOSE-1-¹⁴C, INFUSED INTRAPERITONEALLY TO RATS AT 150 MG/HOUR
DATA FROM INDIVIDUAL TREATED ANIMALS SHOWN IN FIGURE 8

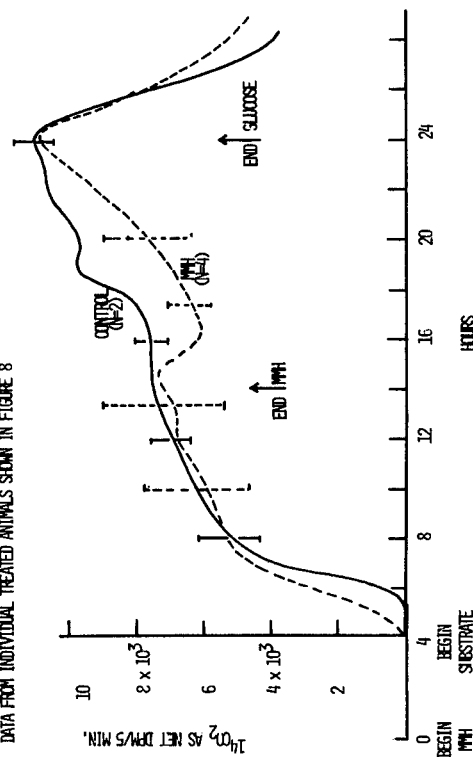


FIGURE 8
EFFECT OF 0.036 WHOLE MM/AG/HOUR x 14 HOURS ON CATABOLISM OF
GLUCOSE-1-¹⁴C, INFUSED INTRAPERITONEALLY AT 150 MG/HOUR
INDIVIDUAL DATA FROM FOUR ANIMALS

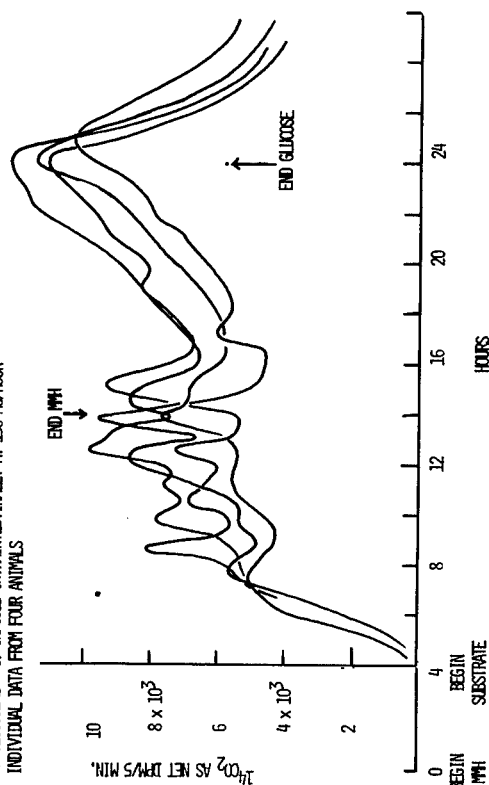


FIGURE 9
EFFECT OF 0.035 MOLE MMH/KG/HOUR x 24 HOURS ON CATABOLISM
OF TRACE AMOUNTS OF FRUCTOSE-6- 14 C INFUSED INTRAVENOUSLY
WITH 150 MG GLUCOSE/HOUR

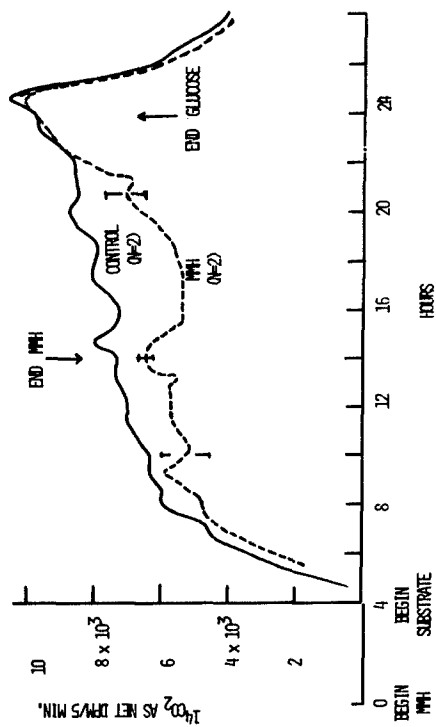


FIGURE 10
EFFECT OF 0.035 MOLE MMH/KG/HOUR x 24 HOURS ON CATABOLISM OF
GLYCEROL-2- 14 C, 10 MG/HR, INFUSED INTRAVENOUSLY WITH 150 MG GLUCOSE/HOUR

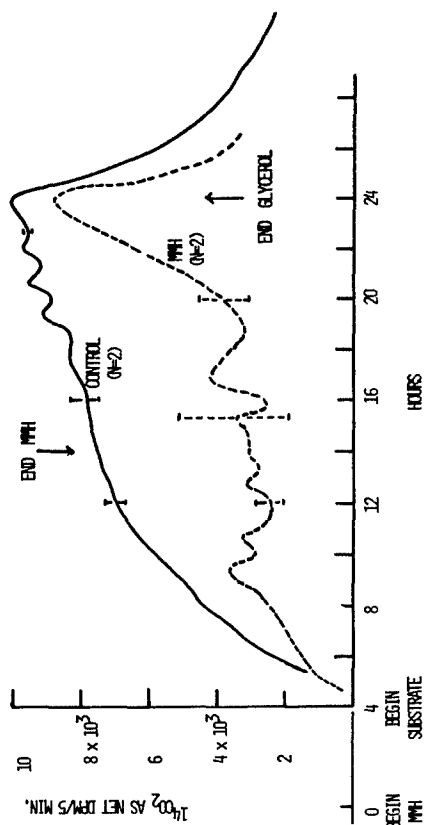


FIGURE 11
EFFECT OF 0.035 MOLE MMH/KG/HOUR x 24 HOURS ON CATABOLISM OF NA PRIMATE-2- 14 C,
10 MG/HR, INFUSED INTRAVENOUSLY WITH 150 MG GLUCOSE/HOUR

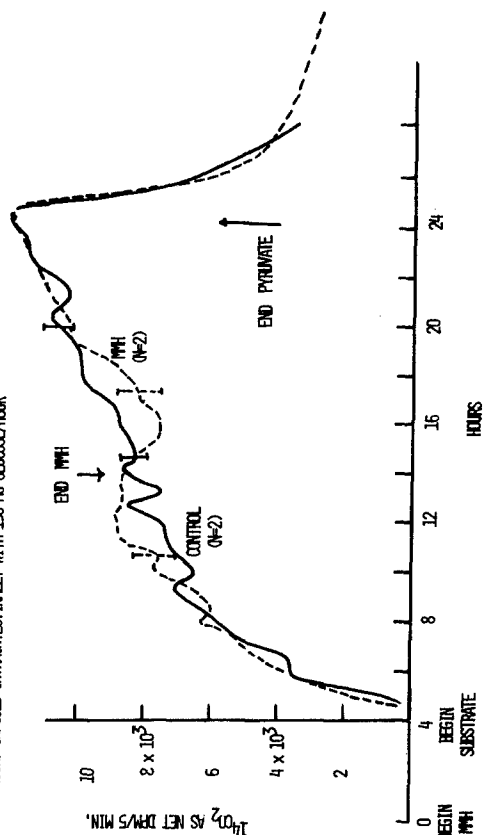


FIGURE 12

EFFECT OF 0.036 MOLE MMH/KG/HR $\times$ 14 HOURS ON CATABOLISM OF TRACE LEVELS OF Na ACETATE-1- 14 C, INFUSED INTRAIESTINALLY WITH 150 MG GLUCOSE/HOUR. EACH CURVE IS FITTED TO AVERAGED DATA FROM THREE ANIMALS. DATA FROM INDIVIDUAL TREATED ANIMALS IN FIGURE 13.

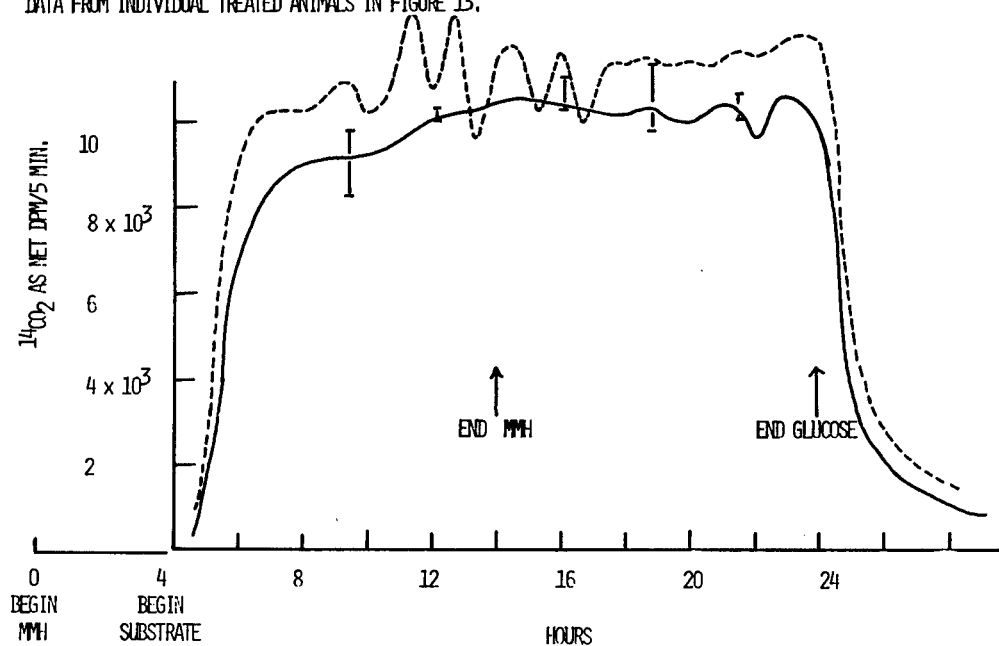


FIGURE 13

EFFECT OF 0.036 MOLE MMH/KG/HR $\times$ 14 HOURS ON CATABOLISM OF TRACE LEVELS OF Na ACETATE-1- 14 C, INFUSED INTRAIESTINALLY WITH 150 MG GLUCOSE/HOUR

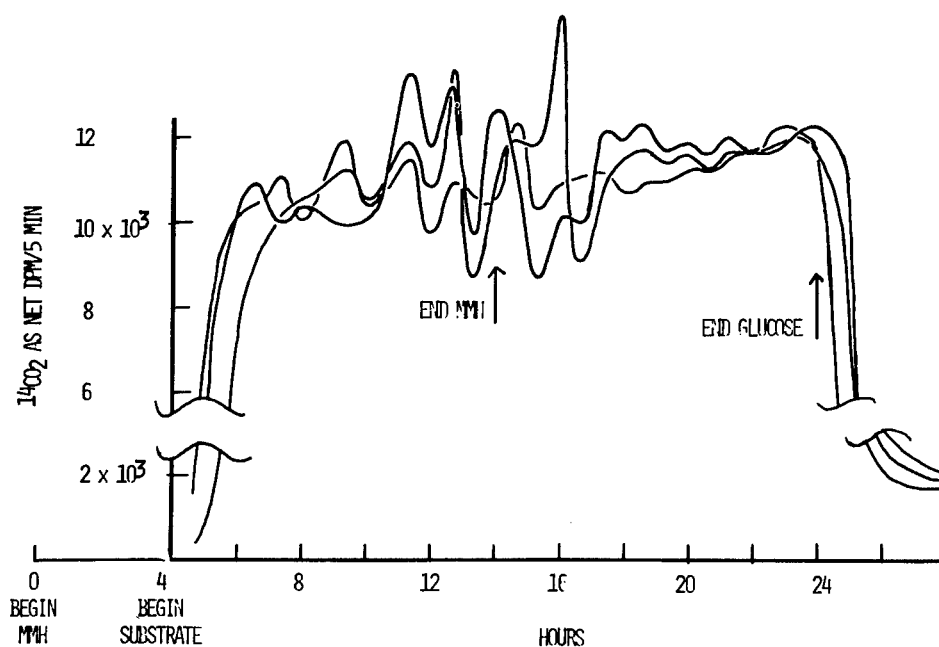


FIGURE 14
EFFECT OF 0.15 MILE MM/60/HR FOR 7 HOURS ON BLOOD GLUCOSE AND OXIDATION
OF GLUCOSE-6- 14 C INFUSED INTRAVENOUSLY TO RATS AT 150 MG/HR.
GLUCOSE AND RADIOISOTOPIC DATA FROM SEPARATE RATS IDENTICALLY TREATED
EACH POINT REPRESENTS A SINGLE BLOOD GLUCOSE DETERMINATION

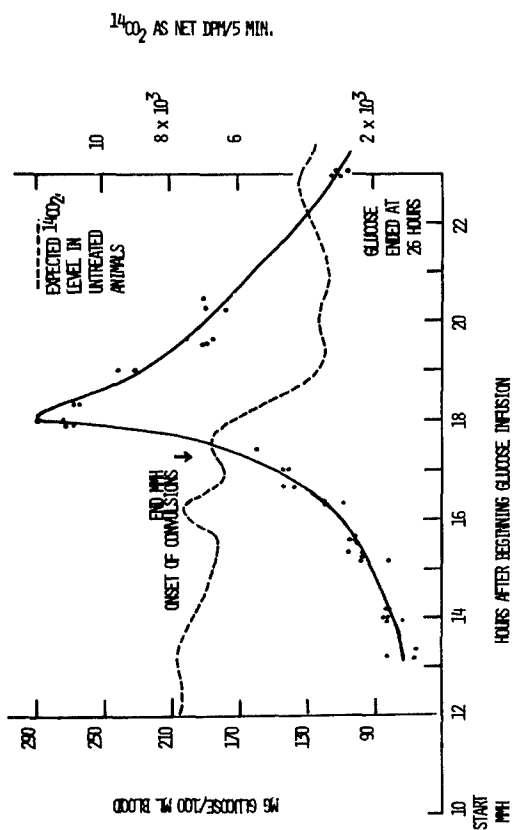


FIGURE 16
EFFECT OF 0.1 MILE MM/60/HR X 3 HRS ON BLOOD GLUCOSE
CONCENTRATION OF A 36 HOUR FASTED RAT.
A THREE LEVEL OF GLUCOSE- 14 C (LESS THAN 146 MG/HR) WAS
INFUSED CONTINUOUSLY FROM 0-18 HOURS

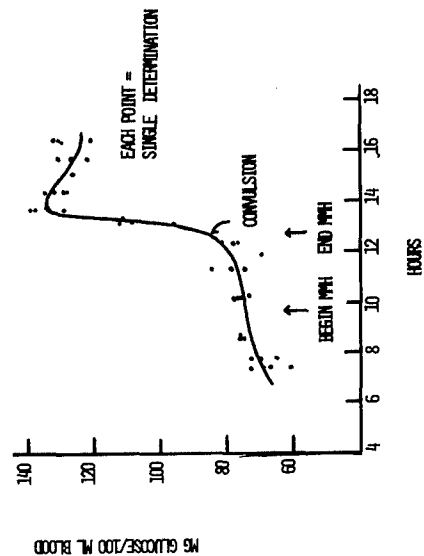


FIGURE 15
LOG PLOT OF BLOOD GLUCOSE DATA FROM FIGURE 14
RELATION BETWEEN APPARENT CHANGE OF SLOPE AND
ONSET OF CONVULSIONS
CURVE FITTED BY INSPECTION

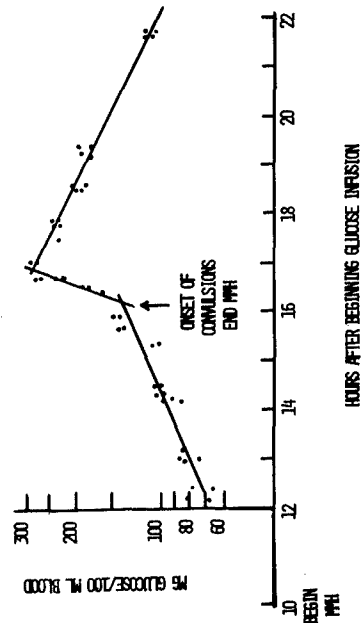


FIGURE 17
EFFECT OF PYRODOXINE HCL, 3 MG/KG/HR ON MMH INHIBITION OF GLUCOSE-6-¹⁴C
OXIDATION MMH 0.05 MOLE/KG/HR STARTED 4 HOURS PRIOR TO GLUCOSE INFUSION
A- PYRODOXINE INFUSED WITH GLUCOSE FROM HOUR 4
B- PYRODOXINE INFUSED WITH GLUCOSE FROM HOUR 8

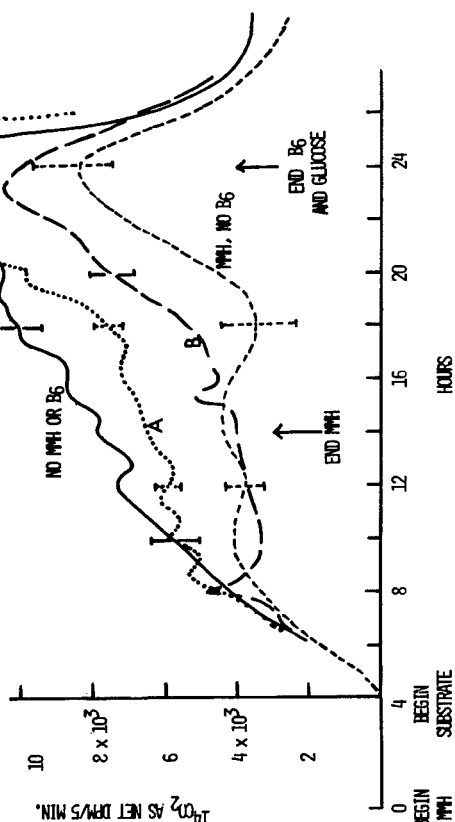


FIGURE 19
EFFECT OF SINGLE INJECTIONS OF 0.05 U
CRYSTALLINE INSULIN ON MMH INDUCED HYPERGLYCEMIA
ANIMALS CONTINUOUSLY INFUSED WITH 150 MG GLUCOSE/HR
MMH 0.1 MOLE/KG/HR
TOTAL DOSE 0.3 MOLE/KG
EACH POINT = SINGLE DETERMINATION

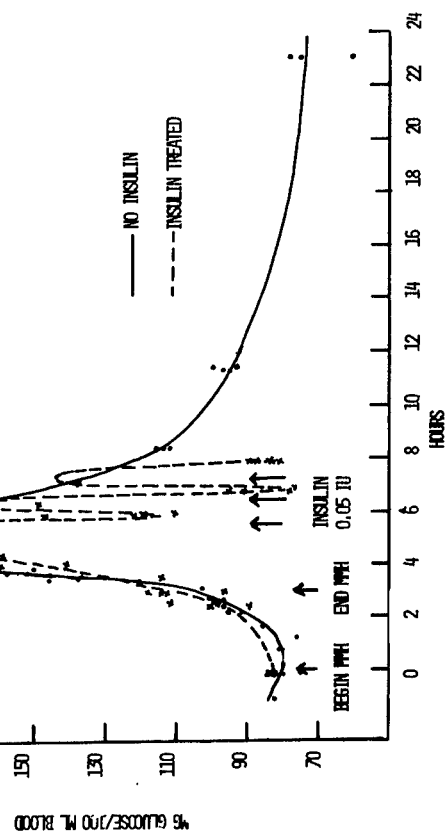
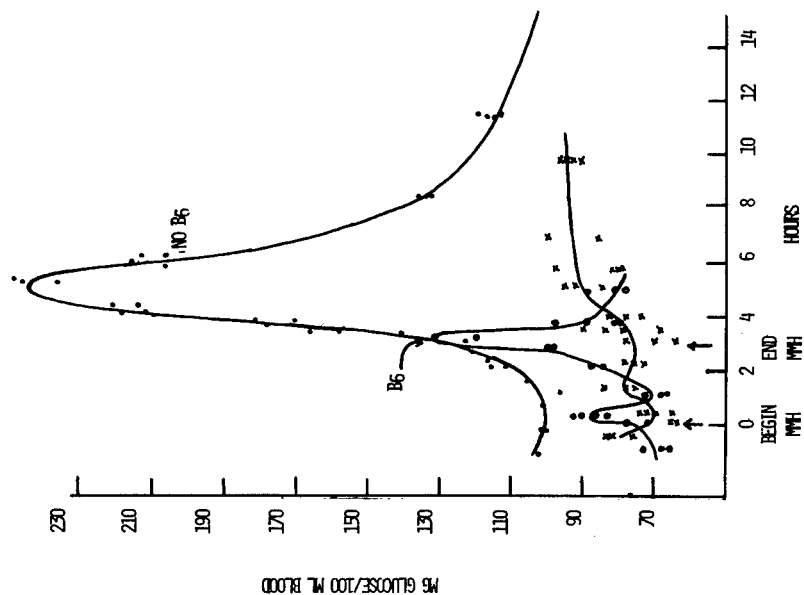


FIGURE 18
EFFECT OF A SINGLE INTRAPERITONEAL DOSE OF PYRODOXINE HCL
30 MG/KG ON MMH-INDUCED HYPERGLYCEMIA IN ANIMALS CONTINUOUSLY
INFUSED WITH 150 MG GLUCOSE/HR, MMH 0.1 MOLE/KG/HR x 3 HR



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